MICROBIAL INOCULANTS FOR ENHANCING DEGRADATION OF BIOSOLID WASTE IN AEROBIC COMPOSTING

By

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(2016-11-047)

THESIS

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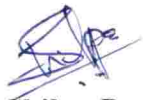
DECLARATION

I, hereby declare that this thesis entitled **'Microbial inoculants for enhancing degradation of biosolid waste in aerobic composting'** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.



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Certified that this thesis entitled **'Microbial inoculants for enhancing degradation of biosolid waste in aerobic composting'** is a record of research work done independently by **Ms. Shilpa P.** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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(Shilpa P.)

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LIST OF ABBREVIATIONS

µm	Micrometer
µg ml^{-1}	Micro gram per milli liter
BLASTn	Basic Local Alignment Search Tool
CD	Critical Difference
CESS	Centre for Earth Science Studies
cfu g^{-1}	Colony forming unit gram
cm	Centimeter
COH	College of Horticulture
CPCB	Central Pollution Control Board
DAI	Days After Inoculation
DNA	Deoxyribo Nucleic acid
EC	Electrical Conductivity
EM	Effective Microorganisms
FCO	Fertilizer Control Order
g	Gram
h	Hour
ITS	Internal Transcribed Spacer
K	Potassium
KSUDP	Kerala Sustainable Urban Development Project
kg	Kilogram
m	Meter
ml	Milliliter
mm	milli meter
mM	Millimolar
MSW	Municipal Solid Waste
N	Nitrogen

NCBI	National Centre for Biotechnology Information
No.	Number
P	Phosphorus
PCR	Polymerase chain reaction
PDA	Potato Dextrose Agar
ppm	parts per million
rDNA	ribosomal Deoxyribo Nucleic Acid
Sp	Species
U ml^{-1}	Units Per Millilitre

Introduction

1. INTRODUCTION

In recent years, waste management has emerged as a serious environmental and economic issue. Waste is usually dumped in oceans and open spaces, used as landfills, subjected to incineration or recycled. Though each of these methods has its own advantages as well as disadvantages, recycling is the best option, considering environmental pollution. Solid waste management mainly based on biological process like composting has gained importance because it involves resourceful and eco-friendly utilization of organic agro-industrial wastes. Composting of biosolid waste allows decomposition of waste materials into more compact form that can easily be managed (Chatterjee *et al.*, 2013). Collecting these organic residuals for municipal composting programme or for composting at home can conserve energy and natural resources, reduce air and water pollution, and save landfill space (Schwarz and Bonhotal, 2011). Composting process has always been commonly followed at farm and household levels for thousands of years for the effective recycling of organic matter and nutrients.

As microbes play a pivotal role in decomposition of cellulose, hemicelluloses and lignin, they can be manipulated for the effective management of biosolid wastes. Novel methods have been developed for rapid decomposition by utilizing microbial cultures as a starter for composting process instead of cow dung. Cow dung availability tends to be decreasing nowadays; hence the usage of microbial inoculums could be considered as the best substitute. Rapid decomposition of solid organic matter into compost can be achieved by adding microbial inoculum of decomposer microorganisms like *Trichoderma* and *Bacillus* (Balasundaram, 2009).

Recently, more importance has been given for microbial consortia, since they contain microorganisms with different physiological and biochemical characteristics that provide good degradation by their multiple mode of action. Apart from waste degradation, consortia also help in suppressing harmful pathogens and biochemical

contents in organic agro-wastes, thereby making the substrates suitable for improving the soil nutrient status and soil fertility.

Kerala Agricultural University has developed composting tonic that contains *Bacillus subtilis* isolated from cow dung and *Gongronella butleri* obtained from soil. The inoculum is being widely used for composting Municipal Solid Waste (MSW) and kitchen waste effectively under aerobic conditions using appropriate structures called Thumburmuzhi aerobic composting unit and KAU Smart Biobin (Girija *et al.*, 2011) respectively. This consortium is being widely used for waste management in several municipalities and households. However, a consortium containing more efficient microbes may work better in decomposing biosolid waste more rapidly. The present investigation was undertaken with an objective to explore the possibility of enhancing degradation of biosolid waste using microbial inoculants in aerobic composting.

Review of literature

2. REVIEW OF LITERATURE

2.1. Biosolid waste management

Solid waste management is a major challenge in urban areas due to rapid growth of population along with urbanization. Total annual waste generated in India including municipal solid wastes, agricultural wastes, cattle, poultry manures and agro industrial wastes was estimated to be 2500 million tons (Tandon, 1995). Seth (2010) reported that, annual municipal solid waste generated in the country was about 38 million tons. The per capita national municipal solid waste generation was reported to range between 200 and 600 g per day (CPCB, 2010). Waste disposal in low-lying areas without any operational control and protection cause environmental and health issues.

The average daily per capita generation of municipal solid waste in Kerala has been estimated as 0.178 kg with a very high variation from 0.034 kg for Koothuparamba to 0.707 kg for Thalassery (CESS, 2001; Padmalal *et al.*, 2002; Varma *et al.*, 2004). Reports also indicated that 79.8 per cent of the total waste generated is organic, compostable waste (KSUDP, 2006). According to Sasidharan and Muraleedharan (2009) biodegradable wastes from an ayurvedic pharmaceutical unit in Thrissur for disposal is about more than one ton per day.

Waste management involves the collection, transportation, processing, treatment, recycling or disposal of waste materials to decrease their adverse effects on environment and human health. Earlier, centralized management of biosolid waste, collected from households and MSW was being practiced. However, mixing of different types of waste makes it very difficult for recycling. Segregation of waste at source, into biodegradable and non-degradable is essential for further processing. Biodegradable waste can be recycled into energy in biogas plants or into compost.

Composting, landfill, incineration, pyrolysis and gasification are the major components in waste management technique (Adewale *et al.*, 2011).

Agricultural waste includes crop residues such as residual stalks, straw, leaves, shells, husks and animal wastes. Major portion of these wastes are disposed in to the environment or burn in open field (Sabiiti, 2004; Tumuhairwe *et al.* 2009). The municipal solid waste consist of more organic waste, composting of this organic waste would be the simple method to reduce the quantity to $1/4^{th}$ and the resulting product can be used for increasing the soil quality (Kalaivani *et al.*, 2011).

2.2. Composting

Composting is described as a biooxidative process including the mineralization and partial humification of complex organic molecules, leading to provide a stable final product, free of pathogens and phytotoxicity with certain humic characteristics (Zucconi and de Bertoldi, 1987; Haug, 1993; Bernal *et al.*, 1998).

According to Gaur (1999), composting is the biological degradation and stabilization of organic substrate under conditions that allow development of thermophilic temperature as a result of biologically produced heat. The final product is stable, free of pathogens and plant seeds and can be beneficially applied to land. Composting process follows four major stages such as initial mesophilic, a thermophilic then a second mesophilic and finally stabilization or maturation stage (Insam and de Bertoldi, 2003). Composting is one of the best methods for organic waste disposal, which is environmentally safe and involves low cost. It also generates useful products for improving soil fertility (Weber *et al.*, 2007; Bustamante *et al.*, 2008; Lu *et al.*, 2008).

The major end product of composting is humus. In order to get quality compost, the microorganisms in the compost pit should be provided with suitable environment such as temperature, moisture, oxygen content and substrate materials

(Pace *et al.*, 1995; Evans, 2001; Last, 2006). Ahmad *et al.* (2007) reported that composted products were known to enhance soil health, plant growth and plant disease management. Anbuselvi and Rebecca (2009) opined that, bio composting of organic wastes from the industrial and agricultural sectors can be successfully transformed in to valuable products such as soil conditioner.

2.3. Aerobic composting

Aerobic composting is a gentle method to degrade volatile organic matter through the involvement of microorganisms in terms of physical, chemical, biological, and transfer theories (Hamelers, 2005). The compost pile includes two kinds of microbes: one that requires oxygen, known as aerobes and the second kind that does not require oxygen, called anaerobe.

Gowda (1996) reported that carbon dioxide and water are the primary breakdown products under aerobic conditions and also forms water soluble, mineral forms of nutrients and humus which are non-toxic compounds. Under anaerobic conditions, organic acids are produced, some of these are foul smelling and phytotoxic as well. Aerobic composting utilizes large amounts of oxygen, especially during the initial stages. In the limited supply of oxygen, the process may turn anaerobic, which delay the composting process.

Hudson (1986) pointed out that, in aerobic decomposition process, succession of active microorganisms normally shifts from mesophilic in the early stages of thermogenesis to thermophilic at the peak of the thermo cycle. The biological decomposition and stabilization of organic materials under aerobic conditions allows the multiplication and activity of thermophilic microorganisms which leads to the production of heat, to form a final product that is stable, pest and pathogen free and useful in agriculture as manure (Balasundaram, 2009; Saravanan *et al.*, 2003).

Aerobic composting has been practiced from time immemorial for recycling of biosolid waste, using various processes and bins. Thumburmuzhi model aerobic composting unit is an eco-friendly technique for effective management of organic waste. This technology is now being practiced for municipal solid waste recycling in large scale at different parts of Kerala (Girija *et. al.*, 2011). KAU Smart Biobin developed at the Department of Agricultural Microbiology, College of Horticulture, Kerala Agricultural University, Thrissur, promotes decentralized management of household kitchen waste at source (Girija *et. al.*, 2016). It promotes aerobic degradation of kitchen waste, with the help of microbial consortia, impregnated in coir pith. This has been popularized by Thrissur Corporation in 2016 and 2017 and 1500 such units have been distributed.

2.4. Factors affecting composting process

In order to achieve successful composting, factors such as temperature, moisture content, aeration rate, pH, C:N ratio and particle size should be properly controlled (Pace *et al.* 1995).

2.4.1. C:N ratio

The microorganisms involved in the decomposition process requires the primary nutrients like carbon (C), nitrogen (N), phosphorous (P), and potassium (K). Microorganisms utilize carbon for both energy production, growth and as the basic building block, making up about 50 per cent mass of microbial cells. Nitrogen is crucial for protein synthesis and reproduction of microorganisms (Shilev *et al.* 2007).

The initial C:N ratio for better composting is 30-35 and above this value, the period of composting extends (Krishnamurthy, 1978). C:N ratio of the composting material is an important factor in decomposition process as well as properties of the end product. Slow degradation was found in substrate with wide C:N ratio and is gradually narrowed by the attack of microbes. Balancing of C:N ratio is essential

while mixing different kinds of substrates for composting. A C:N ratio of 40:1 increases the immobilization of available nitrogen in the compost. Many studies showed that the desirable range of C:N ratio was about 30:1 to 40:1 (Gaur, 1982; Zibiliske, 1998). Hardy *et al.* (1993) suggested that, C:N ratio in the range of 25 to 30:1 indicates the end of composting process.

Microorganisms involved in composting require 30 parts of carbon for every part of nitrogen they use up. If there is excess nitrogen, the microorganisms are unable to utilize and the process becomes inefficient and takes more time to complete the decomposition. For C:N ratios lower than 20:1, the available carbon is completely taken up without stabilizing the nitrogen, which in turn leads to the formation of excess smelly ammonia (Poincelot, 1974). During composting, the addition of easily degradable carbon source to nitrogen rich material increases the C:N ratio and reduces the nitrogen losses, as ammonia produced becomes immediately immobilized to new microbial biomass (Fassen and Dijk, 1979; Kirchman, 1985). According to Jakobsen (1994), relation between available carbon and nitrogen enables mineralization. If the available content of carbon related to nitrogen is high, the microbes absorb mineral nitrogen from the soil solution and cause nitrogen deficiency in plants.

Pan *et al.* (2012) developed a microbial consortium of two strains of *Bacillus subtilis* and a *Pseudomonas* sp. and applied to different organic substrates. They found a reduction in C:N ratio from 35:1 to 25-30:1 within 75-90 days. Hart *et al.* (2002) observed reduction in C:N ratio from 124:1 to 73.1:1 when celluloytic fungal isolates were inoculated to wheat straw. Balasundaram (2009) reported a reduction in C:N ratio during composting of ayurvedic herbal waste, weed waste, coir pith and saw dust inoculated with microbial consortium.

2.4.2. Aeration

Aeration influences the composting process and quality to a great extent. Aeration is considered as one of the key factors that affects the microbial activity, substrate degradation rate and temperature variation in the composting process (Kuter *et al.*, 1985). Various studies showed that, adequate aeration could increase the temperature in the compost pile to more than 60^0C, thereby accelerating the composting process and the final product was free from pathogens, parasites and weeds (Bishop and Godfrey, 1983; Parr *et al.*, 1994).

Poincelot (1974) reported that a minimum oxygen concentration of five per cent within the pore spaces of the compost is necessary for aerobic composting. Limited aeration might lead to anaerobic condition and too much aeration can prevent the thermophilic condition required for optimum rate of decomposition (Ahn *et al.*, 2007). Therefore, managing the amount of aeration at the appropriate level is considered as an important factor in efficient composting process. The optimal range of aeration rate is variable due to the dissimilarity of composting materials in different composting processes.

The complete conversion of carbohydrates into carbon dioxide and water is achieved through good aeration during degradation phase. An improper aeration may lead to the accumulation of acetic acid, which will affect plant growth, if the compost is incorporated in the soil (Jakobsen, 1994). Rasapoor *et al.* (2009) reported that, during the first two months of composting, an aeration rate of 0.6 L $(min)^{-1}$ (kg of $waste)^{-1}$ was observed, which was later reduced to 0.4 L $(min)^{-1}$ (kg of $waste)^{-1}$ until the final stage.

Karnchanawong and Suriyanon (2011) examined the efficiency of polyethylene bins having natural aeration for organic waste composting. Each bin was filled with 1.6 kg of food scraps and dry leaves until the bin was full. It was observed that the

bin provided with lateral and vertical systems of natural ventilation showed fastest degradation of waste compared to control.

2.4.3. Moisture

Moisture greatly influences the metabolic processes of microorganisms. Composting proceeds best at a moisture content of 40-60 per cent by weight and at low moisture levels, microbial activity is limited. The moisture content of less than 20 per cent will greatly inhibit biological processes due to a lack of available water, which in turn restricts the compost from further decomposition (Adhikari *et al.*, 2009). When the moisture content is high, the composting process is likely to be become anaerobic and this may lead to foul smell. Hence, optimum moisture content is necessary for composting process (Kumar *et al.*, 2010).

Gaur (1997) opined that, 40-60 per cent moisture content of substrate is desirable for enhancing the decomposition process. Moisture content varies from 50 to 85 per cent, depending upon the type of composting substrate (Zucconi and de Bertoldi, 1987). According to Haug (1980), for composting of vegetable trimmings, an initial moisture content as high as 85 per cent was optimum when using straw as bulking material and 76 per cent moisture content was needed when using paper as bulking material. Studies have shown that for effective composting of organic materials using microbial consortium the optimum moisture should be 60 per cent (Pointcelot, 1974; Goyal and Sindhu, 2011; Mohan and Ponnusamy, 2011).

2.4.4. Temperature

Temperature is considered as a good indicator of biological activity. High temperature builds up within the composting heap as a result of microbial activity in the waste, whereby heat is liberated through respiration of microorganisms (Finstein and Morris, 1975). According to Tiquia and Tam (2000) mesophilic (50-105^{0}F) and thermophilic (over 105^{0}F) stages are the most essential stages for composting.

Usually, the thermophilic temperature is attained after one to four days of composting. Goyal *et al.* (2005) observed gradual changes in temperature at various stages of decomposition of different organic wastes. An initial temperature of 28-30^{0}C was recorded at the starting stage of composting followed by an increase in temperature after 14 days and then later declined gradually. Nolan *et al.* (2011) noticed that the temperature reached above 60^{0}C on second day of composting, followed by one to two weeks of thermophilic stage and finally the temperature turned down in the cooling stage.

The regulation of temperature is essential since it significantly influences the activity of microorganisms. Change in temperature during composting determines the rate at which the biological process takes place and the succession of different microorganisms (Mustin, 1987). Peigne and Girardin (2004) reported that during the conversion of organic matter, heat is released by the oxidative action of microorganisms. Smith and Jasim (2009) suggested that, composting of domestic organic waste follows three temperature phases including a psychrophilic phase of 0-20^{0}C, mesophilic phase of 20-40^{0}C and a thermophilic phase of temperature above 45^{0}C. Adhikari *et al.* (2009) noted that temperature above 45°C (thermophilic phase) should be attained within a few days of composting process. Even though mesophilic temperature allows effective composting Pace *et al.*, (1995) suggested that maintaining thermophilic temperature at the initial stage, accelerates the decomposition of organic matter.

During waste decomposition, organic compounds are broken down by microorganisms utilizing the oxygen which releases heat energy, resulting in raise in temperature. According to Gowda (1996), the efficient decomposition proceeds at high temperatures of about 40-70^{0}C. Millner *et al.* (1987) noticed suppression of *Salmonella* sp. at 55^{0}C in compost. Prolonged high temperature of 55-60^{0}C for five to six weeks after degradation delays the maturation stage of composting (Zucconi and

de Bertoldi, 1987). The increase in temperature causes destruction of pathogens, weed seeds and fly larvae in the composting material (Pointcelot, 1974). The rise in the temperature helps in the destruction of pathogens more efficiently and makes the compost safer (Ryckeboer *et al.*, 2003).

2.4.5. pH

Composting process is highly influenced by pH. According to Ganapini *et al.* (1979) pH is an indicator of compost maturity. The range of pH value suitable for bacterial development is 6 to 7.5 while fungi prefer a pH of 5.5 to 8.0 (Verdonck, 1988). Kapetanios *et al.* (1993) noticed a stabilization in pH (7.3 to 8.0) at maturity stage of composting. Normally, composting process slows down at pH 6.0 or below and the pH should be prevented from rising above 8.5 to minimize the gaseous loss of nitrogen in the form of ammonia (Pandey, 1997).

The composting process is most effective at pH values between 6.5 and 8.0 (Christian *et al.*, 1997). At the maturity stage of composting pH declines due the nitrification process (Bernal *et al.*, 1998; Yu and Huang, 2009). Nakasaki *et al.* (2005) opined that during the thermophilic stage of organic waste decomposition, the pH value was about 8.7. Saidi *et al.* (2008) suggested that an alkaline pH could enhance the composting process by controlling pathogenic fungi that prefer acidic pH. The initial pH during composting was 5.0 because of the release of ammonia by proteolytic process and later pH continued to increase up to 7.6 during the final process (Gebeyehu and Kibert, 2013).

2.4.6. Particle size

Aerobic decomposition is highly influenced by particle size. The process of decomposition can be enhanced with smaller particle size. Smaller the particles, more the surface area available and microorganisms can work easily at the interface of particle surface and air. There is a direct proportion between the speed of biological

oxidation and the amount of surface exposed to the reactive agent (de Bertoldi *et al.*, 1983).

Particle size depends on the composting substrate, moisture content and total volume of composted organics. The compactness of compost increases with the fineness and wetness of the composted particles. The optimum particle size for composting is 25–75 mm. According to Gaur and Singh (1996) the optimum particle size should be 10 mm for composting system having forced aeration and 5 cm for naturally aerated system. Chopping of bulky organic material to 1-5 cm and mixing of bulking materials (wood chips or tree bark) for too small organic substrate are good for effective composting process. Inert materials such as bark and sawdust are needed if the composted organic matter has smaller size (Shilev *et al.* 2007).

2.5. Biochemical changes during composting

Substrates with primary components of plant materials such as cellulose, hemicelluloses and lignin are rather difficult to degrade and reduce the availability of other polymers by means of physical restriction (Ladisch *et al.*, 1983). Mesophilic and thermophilic microorganisms involved in cellulose, pectin, starch and lignin degradation were isolated by de Bertoldi and Zucconi (1980), and observed a gradual decline in the number of cellulolytic bacteria and increase in cellulolytic eumycetes towards the end of composting. Bacteria, fungi and actinomycetes utilize carbon and energy source from cellulose and hemicellulose present in decomposing substrates (Parr *et al.*, 2002).

Lu *et al.* (2005) noticed degradation of cellulose by four groups of mixed cultures of mesophilic bacteria. These four groups of bacterial cultures could degrade 23.5, 26.3, 19.4 and 24.5 per cent of filter paper cellulose respectively, when used as sole carbon source, after 7 days.

During aerobic decomposition, plant matter like lignin, cellulose, sugars, and proteins become oxidized to form molecules of lower molecular weight with more functional groups. The product of this decomposition of organic matter is called humus (Tiquia, 2005). Naik (2007) developed fungal consortium for the degradation of cellulose, hemicellulose and lignin content in the grape vine residues and reported a reduction in cellulose, hemicellulose and lignin from 38 to 21.2 per cent, 27 to 15.37 per cent and 35 to 13.11 per cent respectively after 90 days of composting. El-Hanafy (2008) observed degradation of 64 per cent and 59.2 per cent of synthetic lignins by *Bacillus subtilis* and *Bacillus* sp. respectively.

2.6. Microorganisms involved in composting

The predominant types of microorganisms present during composting are bacteria, fungi, and actinomycetes. Organic waste was transformed to plant nutrients by several naturally occurring microorganisms which were able to decrease the C:N ratio. They also enable the nutrient flow from one system to another and there by maintain the ecological balance (Novinsak *et al.* 2008; Umsakul, 2010; Kaushal and Bharti, 2015).

During the active composting and curing steps, there is a succession of different types of microorganisms. Hudson (1986) reported that the composition of active microflora of composting waste normally shifted from predominant mesophile in the early stages to thermophiles at the peak of the heating cycle. According to Kanotra and Mathur (1994) mixed cultures of fungi, bacteria and actinomycetes could enhance the rate of lignocelluloses decomposition. They also reported that the use of effective microorganisms reduces the composting period from 12 to 4 weeks. Ahmad *et al.* (2007) reported that bacteria, fungi and actinomycetes plays a crucial role in Municipal Solid Waste (MSW) degradation.

The dominant population during the entire composting process was bacteria, especially at the thermophilic stage of composting (Golueke, 1991; Rebollido *et. al.*, 2008; Zaved *et al.*, 2008). Ishii *et al.* (2000) carried out Density Gradient Gel Electrophoresis (DGGE) to examine the microorganisms present in compost and found that 87 per cent of the microorganisms present in thermophilic stage of compost belonged to genus *Bacillus*. Ryckebore *et al.* (2003) noticed that *Bacillus* was the most predominant bacteria during the thermophilic stage (55^0C) of decomposition of vegetable, fruit and garden wastes and it was also prevalent throughout the composting process.

Gaur (1982) opined that, composting occurs due to the bioconversion of organic material by mixed microbial population and the succession of bacteria, fungi and actinomycetes. He also reported that inoculation of cellulolytic *Aspergillus niger* and *Penicillium* sp. could reduce the time taken for composting as well as increase nitrogen, phosphorus and humus content in the final compost. Aerobic microorganisms are more suitable in the composting process because of their effectiveness in composting. Anaerobic microbes may releases odours and toxins in contrast with release of beneficial nutrients and odourless products by aerobic microbes (Jilani *et al.*, 2007). The specific types of microorganisms present at any given time depend upon the food sources available and the temperatures of the compost environment (David, 2013).

According to Hart *et al.* (2002) inoculation of wheat straw with different cellulolytic fungal isolates could increase the decomposition compared to uninoculated control. A rapid composting approach developed by the Institute of Biological Sciences in Philippines involved cellulolytic culture *Trichoderma harzianum* (Misra *et al.*, 2003). Gupta *et al.* (2004) evaluated CO_2 evolution during different stages of incubation to detect the degradation capacity of *Trichoderma viride, Bacillus polymyxa, Pseudomonas striata* and *Azospirillum* sp. *Bacillus*

polymyxa and Trichoderma viride NC-1 were more efficient in degradation of organic matter. Kerala Agricultural University has developed *Bacillus subtilis* isolated from cow dung, as inoculum for composting of MSW and kitchen waste (Girija *et al.*, 2011). Gupta *et al.* (2012) isolated eight cellulose degrading bacteria from different invertebrates (termite, snail, caterpillar, and bookworm and screened for degradation of cellulose based on the formation of clear zone on Congo red agar. Rajeswari (2017) isolated *Bacillus* sp., *Mucor* sp., and *Fusarium* sp., from fish waste compost piles and prepared microbial consortia for composting of fish waste.

2.7. Role of enzymes in composting

Enzymatic activity was considered as a potential tool for characterization of composting process. Complex carbohydrates were break down by bacterial enzyme in to simpler forms and use them as food source. To maintain earth carbon cycle, these have to be broken down and recycled. Microbes releases various hydrolytic enzymes to degrade these complex compounds (Hamdy, 2005).

Epstein, (1997) noted that the composting process involves degradation of sugars, organic acids, proteins and ligno-cellulose like complex polymers to simpler compounds by wide spectrum of endocellular and extracellular enzymes secreted by microbes. Depolymerization of different constituents of organic waste was carried out by hydrolytic enzymes which were released by microorganisms (Kandeler *et al.*, 1999). Similarly Verma (2002) reported that, complex polymers like cellulose and proteins in the organic waste were hydrolyzed to monomers like sugars and peptides by several hydrolytic enzymes such as lipase, protease, cellulase and amylase secreted by microbes. Microorganisms synthesize cellulase like inducible enzymes during their growth on cellulosic materials. Mondini *et al.* (2004) observed that, the complex macromolecules present in organic waste were hydrolyzed by various enzymes. As a result, water soluble compounds were released and consequently microbial growth was enhanced.

de Bertoldi and Zucconi (1980) isolated and characterized thermophilic and mesophilic bacteria based on their cellulolytic, amylolytic and lignolytic activity. Insoluble and polymeric carbon sources were degraded by the extracellular enzymes secreted by actinomycetes (McCarthy and Williams, 1992). According to Cai *et al.* (1999) *Volvariella volvacea*, an edible mushroom, produces a group of enzymes including endo-1,4-β-glucanase, cellobiohydrolase, and β-glucosidase for the conversion of cellulose to glucose.

Studies on municipal solid waste degradation indicated that, during the first 80 days of composting, activity of endo-cellulase and glucosidase was high and it indirectly reflected the microbial activity (Hermann and Schann, 1997). Arora and Rampal (2000) evaluated lignin degrading efficiency of *Daedalea flavida* and *Phlebia* sp. and the results revealed that the best producers of lignin peroxidase and laccase were *Daedalea flavida*, *Phlebia radiata* and *P. floridensis*. Lee and Koo (2001) noticed that *Trichoderma reesei* Rut C-30 produced large amounts of β-glucosidase, endo-β-glucosidase, endo-β-1,4-glucanase, and exo-β-1,4-glucanase.

In municipal solid waste and Horticultural waste piles, protease activity was found to be maximum during the bio-oxidative phase and final stage of composting (Garcia *et al.* 2009). Nurkanto (2010) isolated and characterized seven actinomycetes from soil and assessed growth and cellulase activities at different pH. *Streptomyces* and *Actinoplanes* showed significant correlation for cellulase activities, cell growth and pH.

Sarkar *et al.* (2011) selected seven potential microbial consortia for the degradation of organic kitchen waste, based on the production of enzymes such as amylase, protease, lipase and cellulose. Rawat and Tewari (2012) reported that *Bacillus subtilis* could secrete high levels of extracellular cellulolytic enzymes. Singh *et al.* (2014) isolated and characterized amylase producing bacteria from diverse

environmental samples and concluded that *Bacillus* sp. exhibited maximum enzyme production compared to other bacteria. Kazemi *et al.* (2017) stated that there was a significant correlation among enzyme activity and microbial population during MSW composting. Guo *et al.* (2017) isolated a novel strain of *Bacillus* sp. producing laccase, from forest soil.

2.8. Microbial consortium in composting

Though bacteria, fungi and actinomycetes play unique and important roles during composting, mixed cultures of microbes enhance the rate of lignocellulose degradation due to their synergistic activity through utilization of intermediate degradation products (Kanotra and Mathur, 1994). The microbial inoculants enhance decomposition process. The degree of humification process was found to be highest in composting with ligno-cellulolytic microorganisms (*Trichoderma* and white-rot fungi) followed by complex microorganisms (*Bacillus casei*, *Lactobacillus buchneri* and *Candida rugopelliculosa*) and uninoculated control (Wei *et al.*, 2007).

Shinde and Rote (1983) used different phosphate source and microbial cultures (*Aspergillus* sp., *Penicillium* sp. and *Trichurus spirulis*) in heap method of composting to prepare quality compost from sugarcane trash. The results indicated that the combination of phosphate sources and microbial cultures enhanced the composting of sugarcane trash (3 to 4 months) as compared with that of control in which decomposition process was not completed even after 5 months.

Nitrogen content was increased from 5 to 10 per cent in compost, when efficient celluloytic fungal cultures like *Trichuris spiralis*, *Paecilomyces fusisporus*, *Trichoderma viride* and *Aspergillus* sp. were inoculated to the organic waste (Gaur and Singh, 1996). Singh and Sharma (2002) found that the quality of compost was improved when different kinds of waste (mixed solid waste, municipal solid waste

and horticultural waste) were inoculated with consortia of *Pleurotus sajor*, *Trichoderma harzianum* and *Azotobacter chroococcum.*

Balasundaram (2009) reported that inoculation of microbial consortium in forest weeds, herbal waste and coir pith enhanced the speed of composting. Sarkar *et al.* (2011) suggested that bacterial consortium is highly significant for the degradation of organic waste. It brings down the time period of composting and reduces the foul smell during decomposition. Pan *et al.* (2012) studied composting of substrates such as organic waste, fruit waste, leaves, vegetable waste, wheat straw, newspaper, hay and rice husk by using microorganisms individually and as consortium. The C:N ratio of all these substrates was found to be decreased gradually to 25:1 from 30:1 within 75 - 90 days by using consortium of *B. subtilis* and *Pseudomonas* sp. The results of this study indicated that, consortium is more suitable for successful composting than individual isolate.

Inoculation of lingo-cellulolytic microbial consortium (*Aspergillus niger*, F44 and *Trichoderma viride*) was efficient in degradation of rice straw in comparison with single organism (Kausar *et al.* 2014). Game *et al.* (2017) evaluated microbial consortia containing *Bacillus* sp., *Aspergillus terreus* and *Streptomyces* sp. on rural and urban waste materials under *in vivo* composting experiments. Rajeswari (2017) composted fish solid waste using different microbial consortium, along with sugarcane bagasse and sawdust as bulking material. The results confirmed that microbial consortium of *Bacillus* sp., *Mucor* sp. and *Fusarium* sp. significantly reduced the composting period from 60 days to 40 days.

2.9. Microbial activity and compost stability

According to Iannotti *et al.* (1992) compost stability is the degree to which the organic fraction in compost has been decomposed. Immature compost may contain phytotoxic compounds which can affect the plant growth and the supply of

nutrients. Studies on nitrogen mineralization showed that nitrogen mineralization and stabilization of compost were dependent. Therefore, stable and mature compost can be considered as a good source of nitrogen (Bernal *et al.*, 1998).

For evaluating the level of compost activity and maturity, microbial ratio index may be used and it is referred as one of the criteria for compost quality (Kostov *et al.*, 1994). The degree of microbial activity is one of the potential parameter for compost stability (Richard and Zimmerman, 1995).

Tiquia (2005) observed that, microbial activities were linked to compost stability. Organic matter and nitrogenous compounds in pig manure were degraded by microorganisms and this increased microbial activity during the composting period. It was also noted that, towards the end of composting, C and N become stabilized and no more degradation was carried out by the activity of microorganisms. Steger *et al.* (2007) studied the changes in microbiological and physicochemical parameters during municipal organic waste composting and they found that compost maturity and microbial biomass were interrelated.

Garcia *et al.* (2009) carried out a biological comparison study using municipal solid waste, horticultural waste and sewage sludge and reported that the final product in MSW composting recorded significantly high mesophilic aerobic microbiota, fungi, actinomycetes and hemicellulolytic microorganisms. They concluded that nature of the starting material causes differences in biological parameters. Game *et al.* (2017) developed cellulolytic microbial consortium and tested its composting efficiency on rural and urban waste. The test consortium reduced the stabilization period of compost by 22.68 per cent over uninoculated control.

2.10. Quality of compost

Parameters like C:N ratio, composting temperature, pH, moisture content and the presence or absence of pathogens such as coli form bacteria are the major factors

which determine the quality of compost (Steger *et al.,* 2007; Erickson *et al.*, 2009; Al-Turki, 2010; Fourti *et al.,* 2011). Proper composting technology involves efficient conversion of organic waste in to quality compost without causing any environmental problems (de Guardia *et al.*, 2010).

Larney and Blackshow (2003) observed that during the thermophilic stage of composting, plant pathogens were killed by the heat and stable products were formed after four to six months. Taiwo and Oso (2004) opined that, type and succession of microorganisms during composting was directly related to the quality of compost. Stable and quality compost does not contain any pathogenic and phytotoxic substance. Quality compost can also be used as organic fertilizer and soil conditioner (Ahmad *et al.*, 2007). The heat generated during composting process kills the pathogens and seeds of weeds (Tweib *et al.*, 2012). Weil *et al.* (2013) found that the population of human pathogens (*Listeria, E. coli and Salmonella*) introduced in the substrate, was reduced to non-detectable levels during the second phase of composting. All the human pathogens disappeared beyond a temperature of 54.4^0C.

2.11. Benefits of compost

Self-heating activity of microorganisms results in the reduction of organic waste volume to 50 per cent of the original waste, which can be beneficially utilized to improve fertility, water holding capacity and tilth of soil (Finstein and Morris, 1975; Pare, 1999). Composting of municipal solid waste is an important part in sustainable agricultural and resource management, because compost plays multiple roles such as improving soil fertility and plant growth, amending the soil, decreasing the contaminants from soil and destroying pathogens (Zucconi and de Bertoldi, 1987; Reddy *et al.*, 2005).

Jagadeesh *et al.* (1996) composted red gram stalks with *Phanerochaete chrysosporium* and observed a reduction in composting period. The compost when

applied to soybean at 5 t ha^{-1} resulted in higher yield and nodulation. A field experiment conducted by Mamo *et al.* (1998) showed that maize grain yield was more in biowaste compost applied treatment than that of the control.

Mckeller and Nelson (2003) observed a suppression of cotton damping off by *Pythium* spp., when the crop was applied with compost of leaves and twigs from deciduous trees. Vallad *et al.* (2003) observed a reduction in bacterial streak symptoms caused by *Pseudomonas syringae* pv. tomato in *Arabidopsis* and tomato plants supplemented with paper mill residual compost. Application of commercial compost in cotton seedlings resulted in reduction of soil borne disease and increase in plant height (Mikhail *et al.,* 2005).

Ahmad *et al.* (2007) reported that enrichment of PGPR strain with fruit and vegetable waste compost resulted in improved growth and nutrient uptake in maize. Yang *et al.* (2012) combined two organic composts (maize powder and soya bean residue) and two biocontrol agents (*Bacillus subtilis* and *Bacillus megaterium*) and tested for the management of bacterial wilt in ginger. The results showed 3-30 per cent increase in biocontrol efficacy in combined application of bio agent compared to individual application. Application of the compost also improved colonization of biocontrol agent and nutrient availability.

Mrabet *et al.* (2012) evaluated the agronomic value of compost on maize and lettuce and found that, compost increased the yield of both crops. The increase in yield was directly related to the compost dose. Namasivayam and Bharani (2012) studied the growth promotion effect of fruit waste compost enriched with effective microorganisms on mung bean. Growth promotion and reduction in the incidence of plant diseases were observed in treated plots. Application of EM (Effective Microorganisms) compost amendments in calendula and marigold significantly improved the flower number and pigment content, moreover it enhanced the soil biological health by improving the soil enzyme activities (Sharma *et al.*, 2017).

Materials and methods

3. MATERIALS AND METHODS

The present study on "Microbial inoculants for enhancing degradation of biosolid waste in aerobic composting" was conducted in the Department of Agricultural Microbiology, College of Horticulture, Vellanikkara, during 2016- 2018. Details of materials used and the methods followed are presented below.

3.1. MATERIALS

3.1.1. Chemicals, glass ware and plastic ware

The chemicals required for the study were Analytical Grade (AR) and purchased from the agencies like HIMEDIA, Merck India Ltd., Sisco Research Laboratory (SRL). Molecular biology reagents and buffers needed for the experiment were obtained from HIMEDIA (Mumbai) and Sigma-Aldrich India Ltd. (Bangalore). The plastic ware was purchased from Tarson India Ltd. (Kolkata). Nutrient agar, potato dextrose agar and Kenknight's and Munaier's agar were used for isolation of bacteria, fungi and actinomycetes respectively, from compost samples. Selective media used for screening microorganisms for efficiency in degrading various carbon sources are given in Table 1. The composition of these media and different reagents used for the experiment are given in Appendix I and II.

3.1.2. Equipments and machinery

The equipment items required for the study were available at the Department of Agricultural Microbiology, College of Horticulture, Vellanikkara. Culture media were sterilized in autoclave Equitron-SLEFA (Eutech Instruments, Mumbai). pH meter (Eutech pH Tutor, Singapore) was used for checking pH of the culture media. Laminar air flow chamber (Rotek, Mumbai), was used for inoculation and streaking of microorganisms under sterile conditions. Water bath (Rotek, Mumbai) was used for maintaining the temperature during incubation of reaction mixture, during enzyme assay. Pure cultures of microbes were preserved in glycerol, in ultra-low temperature

deep freezer (Haier DW-86L90, Haier International Co. Ltd., China). For observing the morphology of microorganisms and for photomicrography, a compound binocular microscope (Leica-ICC50 from Leica, Germany) was used. *In vitro* DNA amplification was carried out in Eppendorf Mastercycler (Eppendorf, Germany). Table top high speed refrigerated centrifuge (Eppendorf-5804R, Eppendorf, Germany) was used for centrifugation. Visualization of DNA on agarose gel was carried out using UV transilluminator (UVP-Benchtop Transilluminator from UVP, USA). For enzyme assay, absorbance values were measured using Biomate 3SUV visible spectrophotometer (Thermoscientific, China). Pilot scale experiment with selected consortia was conducted in KAU Smart Biobins and for validation on large scale, Thumburmuzhi composting units were used. Temperature of compost was measured using Pen type digital thermometer (Chemik, Thrissur).

Two bacterial isolates (*Bacillus subtilis* and *B. niabensis*) and two fungal isolates (*Gongronella butleri* and *Trichoderma asperellum*) available in the repository of the Department of Agricultural Microbiology, College of Horticulture, Vellanikkara were used as reference cultures for screening.

Table 1. Selective media used for screening of different microorganisms

Carbon source	Selective media	Microorganism targeted
Cellulose	Carboxy methyl cellulose (CMC) agar	Cellulose degrader
Lignin	Lignin sulphonate media	Lignin degrader
Starch	Starch agar	Starch degrader
Protein	Calcium caseinate agar	Protein degrader
Pectin	Pectic agar	Pectin degrader
Chitin	Colloidal chitin media	Chitin degrader
Lipid	Spirit blue agar	Lipid degrader

3.2. METHODOLOGY

3.2.1. Collection of compost samples

Five hundred g samples were collected from four different sources *viz.,* KAU smart biobin compost from Beena Augustin, Vinpin Nagar, Paravattani, Thrissur, coir pith compost and Oushadhi ayurvedic waste compost from KAU Plant Propagation and Nursery Management Unit, Vellanikkara, Thrissur, and kitchen waste compost from Priya sabu, Vazhapilly House, Kalathode, Thrissur. These samples were stored in sealed poly bags under refrigerated condition for the isolation of decomposing microorganisms.

3.2.2. Isolation and enumeration of microorganisms

Serial dilution plate technique was adopted for the isolation of microorganisms from compost samples (Sutton, 2011). Three replicates for each compost sample was taken. Bacteria, fungi and actinomycetes were isolated using the respective media (Table 2) and the dilutions were standardized depending on the microbial load in the samples. Ten gram compost sample was added to 90 ml sterile water blank in a 250 ml conical flask and the contents were mixed by shaking for ten min. From this dilution, one ml of suspension was transferred to a test tube containing 9 ml sterile water blank and mixed thoroughly to obtain 10^{-2} dilution. Dilutions up to 10^{-6} were prepared by repeating the procedure. One ml suspension from 10^{-3}, 10^{-4} and 10^{-6} dilutions was taken and added to sterile Petri dish. Thereafter, molten and cooled culture media (nutrient agar, potato dextrose agar, Kenknight's and Munaier's agar) was poured in the Petri dish aseptically and rotated in clockwise and anticlockwise directions for uniform mixing of media with the suspension. After the solidification of media, the plates were incubated at ambient temperature in inverted position. Observations were made on the number of colonies in the respective media.

Table 2. Details of media and dilution used for isolation of microorganisms from compost samples

Microorganism	Media	Dilution	Incubation period (days)
Bacteria	Nutrient agar	10^{-6}	1
Fungi	Potato dextrose agar	10^{-4}	3-4
Actinomycetes	Kenknight and Munaier's agar	10^{-3}	5-7

After the incubation period, the microbial colonies were counted on the media of the respective dilution and expressed as colony forming units per gram of sample (cfu g^{-1}) (Goldman and Green, 2008).

3.2.4. Purification and maintenance of isolates

Isolates that were predominant in each sample were purified and maintained using standard procedure. The selected bacterial colonies and actinomycetes colonies were streaked on their respective agar media and single colonies were picked up. After obtaining pure cultures, bacterial and fungal isolates were maintained in agar slants at 4^0C. For long term storage, bacterial cultures were preserved in glycerol stock (broth culture containing 40 per cent glycerol) at -80^0 C (Angshumanjana *et al.*, 2016).

3.2.5. *In vitro* screening of microbial cultures for degradation of organic molecules

All the cultures were screened for their ability to degrade cellulose, lignin, starch, protein, chitin and pectin on selective media. Bacteria and actinomycetes were spotted and in the case of fungal isolates, five mm discs were placed at the centre of the selective medium contained in Petri dish. All the cultures were incubated at

ambient temperature. The diameter of clear zone was measured and degradation index was calculated (Hendricks *et al.*, 1995; Ribas *et al.*, 2009; Ahmad *et al.*, 2013; Cui *et al.*, 2015). The following formula was used to find the degradation index of microorganisms.

$$\text{Degrading index} = \frac{\text{Diameter of clear zone}}{\text{Diameter of colony}}$$

3.2.5.1. Cellulose decomposition

The screening for cellulose degradation was carried out on CMC agar. Plates were inoculated with microbial cultures and incubated for four days for bacteria and seven days for fungi and actinomycetes. At the end of incubation, the plates were flooded with Gram's iodine solution for bacteria or one per cent Congo red followed by 1 M NaCl for fungi (Kasana *et al.*, 2008). The cellulolytic isolates were detected by the presence of clear zone around the colonies after staining. Gram's iodine formed a bluish-black complex with cellulose but not with hydrolyzed cellulose, giving a sharp and distinct clear zone around the cellulose degrading bacterial colonies within 3 to 5 min. The hydrolyzing capacity of each isolate was estimated by measuring the diameter of the cellulolytic zone.

3.2.5.2. Lignin decomposition

Lignin sulphonate medium was used for testing the lignin degradation potential of selected microbes. Diameter of the clear zone was measured seven days after incubation.

3.2.5.3. Starch decomposition

For the detection of starch hydrolyzing microorganisms, starch agar (Srivastava and Baruah, 1986) was used. Plates with microbial colonies were

incubated for three days and then flooded with Gram's iodine. Diameter of the clear zone was measured.

3.2.5.4. Protein decomposition

Screening of protein degrading microorganisms was carried out on calcium caseinate agar (Frazier and Rupp, 1928). The isolates were spotted and the diameter of clear zone was noted after incubating the plates for two to four days.

3.2.5.5. Chitin decomposition

In vitro screening of chitin decomposing microorganisms was carried out using colloidal chitin agar medium (Hsu, 1975). The microorganisms were spotted and observation on the diameter of clear zone was recorded after seven days of incubation.

3.2.5.6. Pectin decomposition

Pectin degrading microorganisms were screened on pectic agar medium, where pectin served as the sole carbon source for the growth of the organisms. The bacterial, actinomycetes and fungal isolates were allowed to grow for seven days. The pectinolytic activity was determined based on the clear zone formation on pectic agar medium after it was flooded with 3.3 per cent Cetyltrimethyl ammonium bromide (Taieb *et al.*, 2011).

3.2.5.7. Lipid decomposition

For the detection and screening of lipolytic microorganisms, spirit blue agar was used along with a lipase reagent called tributyrin (Starr, 1941). The microorganisms were spotted and incubated for four days and then the diameter of clear zone around the colonies measured.

3.2.6. *In vitro* enzyme assay

The selected isolates were subjected to enzyme assay for quantitative estimation of enzyme activity for cellulase, β-1,3 glucanase, β-glucosidase, laccase, amylase, protease, pectinase and lipase.

3.2.6.1. Cellulase

The selected isolates were grown in CMC broth for 48 to 72 h. The cultures were centrifuged at 12,000 rpm for 10 min and the supernatant was collected as crude enzyme for quantitative assay. Cellulose activity was determined using 3,5 dinitrosalicylic acid (DNS) method (Sadasivam and Manickam, 1996). The reaction systems were prepared as follows: 0.5 ml enzyme extract added to 32 mg of Whatman No.1 filter paper (substrate). The mixture was incubated for 1 h at 50^0C in a water bath and 0.5 ml DNS reagent was added immediately after removing the enzyme substrate mixture from the water bath. The mixture was kept in a boiling water bath for five min and one ml of 40 per cent potassium sodium tartarate solution was added to this mixture followed by cooling to room temperature. Finally, the volume was made up to five ml and the absorbance was taken at 540 nm using spectrophotometer and compared with standard.

3.2.6.2. β-1, 3 glucanase

For testing the efficiency of isolates for *s*-1-3 glucanase activity, DNS method (Sadasivam and Manickam, 1996) was followed. The culture was centrifuged at 12,000 rpm for 10 min and 0.05 ml supernatant was added to 0.45 ml of one per cent CMC solution. The reaction mixture was incubated at 55^0C for 5 min and 0.5 ml DNS reagent was added immediately after removing the enzyme substrate mixture from the water bath. The mixture was kept in a boiling water bath for five min and one ml of 40 per cent potassium sodium tartarate solution was added to this mixture followed by cooling to room temperature. Finally, the volume was made up to five

ml. Absorbance was taken at 540 nm by employing spectrophotometer and compared with standard.

3.2.6.3. *β*-glucosidase

Enzyme assay was carried out to analyse the *β*-glucosidase activity according to the procedure of Breuil *et al.* (1985). The incubated microbial culture was centrifuged, one ml of supernatant was taken and one ml of 5 mM p-nitrophenyl-β-D-glucopyranoside (pNPG) solution was added. It was then incubated for 10 min at 45^0C and reaction was stopped by the adding one ml of 2 M sodium carbonate. The absorbance was measured at 410 nm using spectrophotometer and compared with standard.

3.2.6.4. Laccase

Guaiacol assay method was employed for laccase assay (Jhadav *et al.*, 2009). The substrate used was guaiacol (2 mM) in sodium acetate buffer (10 mM pH 5.0). The incubated culture was centrifuged at 10,000 rpm for 10 min and one ml supernatant that acted as the source of enzyme was mixed with three ml acetate buffer and one ml substrate formed the reaction mixture. The reaction mixture was incubated at 30^0C for 15 min and the laccase activity was estimated spectrophotometrically at 450 nm.

3.2.6.5. Amylase

Measuring the amount of reducing sugars produced from the enzymatic hydrolysis of starch was spectrophotometrically determined for quantification of amylase activity (Miller, 1959). Selected isolates were inoculated in starch broth and incubated at ambient temperature for 48 h. After the incubation, the culture was centrifuged at 10,000 rpm for five min to obtain the supernatant. The substrate was 0.5 ml of one per cent soluble starch and the reaction mixture was prepared by mixing

the substrate in 1.2 ml of 50 mM potassium phosphate buffer (pH 7) followed by the addition of 0.3 ml of supernatant. The reaction mixture was incubated at 40°C for 15 min and one ml of freshly prepared dinitrosalicylic acid was added to this reaction mixture and boiled at 100°C for five min. Absorbance was read at 540 nm and compared with standard.

3.2.6.6. Protease

The protease activity was determined by modified procedure of Tsuchida *et al.* (1986). Caseinate containing liquid media was inoculated with isolate and incubated at ambient temperature for 48 h. The culture media was then centrifuged at 7200 rpm for 15 min and the resulting supernatant was used as the enzyme source. The substrate used was two per cent casein in 0.2 M carbonate buffer. A mixture of 0.5 ml casein solution and an equal volume of enzyme was incubated at 55^0C for 10 min. The reaction was terminated by the addition of one ml of 10 per cent trichloroacetic acid followed by centrifugation. The reaction mixture was prepared by the addition of supernatant to 5 ml of 0.44 M sodium carbonate and one ml of two-folded diluted Folin and Ciocalteu's phenol reagent. This was kept for 30 min at room temperature and the optical densities of the solutions were observed at 660 nm against a reagent blank prepared in the same manner using tyrosine as the reference standard.

3.2.6.7. Lipase

The lipid degrading isolates were grown in trybutyrin broth medium at an ambient temperature for 48 h. The culture medium was centrifuged at 8000 rpm for 10 min and the clear supernatant obtained was taken for enzyme analysis. The lipase activity was determined on the basis of olive oil hydrolysis (Pinsirodom and Parkin, 2001). The substrate constituted 50 ml of five per cent olive oil-gum arabic emulsion pre-incubated for 15 min at 37^0C in water bath. To initiate lipolysis, 1.5 ml of enzyme

was added to it. A titration cocktail was prepared by the addition of 10 ml of 95 per cent ethanol and phenolphthalein was mixed with 5 ml phosphate buffered olive oil-gum arabic emulsion and titrated against 0.05 N sodium hydroxide until a pink colour appeared. Quantity of the fatty acid liberated in each sample was determined based on the equivalents of sodium hydroxide utilized to reach the end point of titration.

$$\mu\text{mol fatty acid/ml sample} = \frac{(\text{ml NaOH for sample} - \text{ml NaOH for blank}) \times N \times 1000}{\text{Volume of reaction mixture used}}$$

Where, N = Normality of the NaOH used

3.2.6.8. Pectinase

Pectinase activity was determined using the method illustrated by Miller (1959). The reaction mixture was prepared by the addition of one per cent pectin in citrate buffer and enzyme source in equal amounts. The enzyme source was obtained after centrifugation (3000 rpm for 30 min) of culture grown in basic liquid medium amended with pectin. The reaction mixture was incubated at 50^0C for 30 min in water bath, followed by the addition of three ml DNS solution to stop the reaction and again kept it in water bath for 10 min. The absorbance was read at 575 nm and the amount of released reduced sugar was determined using glucose standard.

3.2.7. Cultural characterization of isolates

Cultural characters of selected isolates were studied by visual observation. For this, the isolates were grown on respective growth medium and plates were incubated at ambient temperature.

3.2.7.1. Colony characters of bacterial isolates

Bacterial isolates were grown on solidified nutrient agar medium for 24 h. The colony characters *viz.,* size, surface, margin, elevation and colour were noted (Holt *et al.,* 1994). Colony characteristics of selected actinomycetes isolates grown on Kenknight and Munaier's agar media were examined. Colony characters such as size, surface, margin, elevation and colour were observed.

3.2.7.2. Colony characters of fungal isolates

Selected fungal isolates were grown on solidified potato dextrose agar medium and incubated for 3 to 4 days at 26±2^{0}C. Observations on colony colour, texture and growth of the fungal colonies were recorded (Funder, 1968).

3.2.8. Morphological characterization

3.2.8.1. Gram staining

Morphological studies of selected bacterial and actinomycetes isolates were carried out using Gram staining illustrated by Gram (1884). A clean sterile glass slide was taken and a drop of sterile water was added. For the preparation of a smear, a loopful of pure culture was mixed with the sterile water on the glass slide and heat fixed. The smear was flooded with primary stain crystal violet for one min and washed with tap water. Grams iodine was poured over the smear for one min, followed by 95 per cent ethanol (decolorizer). The smear was then washed in tap water and flooded with counter stain safranin for one min and washed. The colour, shape and arrangement of cells were examined under oil immersion objective of the compound microscope.

3.2.8.2. Endospore staining

Gram positive isolates were subjected to endospore staining (Cappuccino and Sherman, 1992). A thin smear of the pure culture was prepared on a clean glass slide and heat fixed. The glass slide was placed on a forceps above boiling water. The smear was then flooded with malachite green and heating was continued for 10-15 min. The slide was carefully washed with tap water and counter stained with safranin for 30 seconds. This was followed by washing with water and blot drying. The endospores were examined under oil immersion objective of the compound microscope.

3.2.8.3. Staining of fungal isolates

A drop of lactophenol cotton blue was placed on a sterile clean glass slide. A mycelial bit from actively growing fungal culture was taken using a sterilized loop and a coverslip placed on the thin preparation (Funder, 1968). After 5 min glass slide was observed under microscope.

3.2.9. Biochemical characterization of isolates

Biochemical characterization included indole test, methyl red test, catalase test and citrate test (Cappuccino and Sherman, 1992).

3.2.9.1. Indole production test

The isolates were inoculated in test tubes containing trypticase soy broth and an uninoculated broth was kept as control. After 24 to 48 h of incubation at ambient temperature, a few drops of Kovac's reagent was added to the broth and formation of cherry red ring was recorded.

3.2.9.2. Methyl red test

The bacterial isolates were inoculated in test tubes containing nutrient broth and an uninoculated control was maintained. After an incubation period of 24 to 48 h at ambient temperature, five drops of methyl red indicator was added to the test tubes and colour change was recorded.

3.2.9.3. Voges-Proskauer test

Voges-Proskauer medium was prepared in test tubes and autoclaved. The isolates were inoculated and kept for inubation at ambient temperature for 24 to 48 h. Ten drops of Baritt's reagent A followed by Baritt's reagent B were added. Observation on pink colour formation was examined after 15 min.

3.2.9.4. Catalase test

The bacterial isolates grown in nutrient broth was placed in a clean glass slide followed by addition of single drop of three per cent hydrogen peroxide. Observation on effervescence was recorded.

3.2.9.5. Citrate utilization test

Slants of Simmon's citrate agar were prepared and the bacterial isolates were streaked on the slants with an uninoculated control. The incubation period was 24 to 48 h at ambient temperature. Development of deep Prussian blue colour in the inoculated slants was examined.

3.2.10. Molecular characterization

3.2.10.1. 16S rRNA gene sequence analysis of selected isolates

The bacterial and actinomycetes isolates were subjected to molecular characterization by 16S rRNA gene sequencing to identify up to species level.

3.2.10.1. Amplification of 16S rRNA gene

Colony PCR was used for amplification of 16S rRNA gene (Woodman, 2008). Single isolated colony was taken using a micropipette and mixed with 10µl of sterile water. For amplification of 16S rRNA gene, 2µl of the suspension was used as template. The details about the primers (Siddapura *et al.,* 2010) used are given in Table 3.

Polymerase chain reaction was carried out in Eppendorf Master Cycler (Gradient) using 'Emerald Amp GT PCR' PCR master mix. The details of the composition of PCR reaction mixture are given in Table 4.

The reagents were mixed by a momentary spin and the PCR reaction set in master cycler. The details of master cycler programme are given in Table 3.

Table 3. Primers used for 16S rRNA gene amplification

Primer details	Sequence 5'- 3'	Length in bp
8 F	AGAGTTTGATCCTGGCTCAG	20
1522R	AAG GAG GTG ATC CAG CCG CA	20

Table 4. Composition of PCR reaction mixture

Component	Per reaction volume required (µl)
Master mix	12.5
Template	2.0
Forward primer	0.5
Reverse primer	0.5
dH_2O	9.5
Total	25.0

Table 5. Details of master cycle programme

No.	Step	Temperature (^{0}C)	Time (min)
1	Initial denaturation	95	3.00
2	Denaturation	94	1.30
3	Annealing	55	0.40
4	Primer extension	72	1.30
5	Steps 2-4	34 cycles	-
6	Final extension	72	20.00
7	Final hold	4	10.00

3.2.10.2. Agarose gel electrophoresis

Assessment of the quality of isolated DNA was carried out by agarose gel electrophoresis (Sambrook *et al.*, 1989). Hundred ml of 1X TAE buffer was prepared from 50X TAE stock solution (pH 8.0). Agarose gel was prepared by dissolving 1.0 g agarose (Genei, Low EEO) in 100 ml 1X TAE buffer and 0.5 µg ml^{-1} ethidium bromide prepared from a stock of 10 mg ml^{-1} was added to the cooled solution. The gel casting tray was set up with properly placed comb and agarose was poured into

the tray with care and allowed to solidify. The comb was carefully removed to obtain wells and1X TAE buffer was poured into the tank. The gel loading dye and 2 µl PCR product was mixed and loaded gently into the wells. The electrodes were connected and a constant voltage of 80 V was applied till the tracking dye reached at about 3 cm away from the anode end.

3.2.10.3. Gel documentation

After the separation of DNA bands using electrophoresis, they were visualized in the UV illuminator and photographed using gel documentation system.

3.2.10.4. Purification and sequencing of PCR product

The PCR product was then purified and sequenced at AgriGenome Labs Pvt Ltd., Kochi, using the primer 8F and 1522r. The sequencing of the PCR product was done using the sequencing machine ABI 3730 XL DNA analyser.

3.2.10.5. Nucleotide sequence analysis

Sequence analysis and nucleotide homology of each isolate were analyzed through the BLASTn (basic local alignment search tool) programme of NCBI (National Centre for Biotechnology Information) (http://www.ncbi.nlm.nih.gov). The accession sharing maximum homology with the query sequence was considered for the identification of the test organism.

Phylogenetic tree was constructed by aligning 16S rRNA gene sequences of the isolate with closely related accessions of bacteria or actinomycete in the NCBI GenBank databases. The tree was generated by using MEGA 7.0 software (Kumar *et al.*, 2016).

3.2.11. Compatibility of selected isolates

3.2.11.1. Compatibility of selected bacterial isolates

The selected bacterial isolates were tested for compatibility using cross streaking technique (Burlage *et al.*, 1998). Two different bacterial isolates were streaked vertically and horizontally in a Petri plate containing nutrient agar medium using a sterile loop. Control plates were maintained for each isolate. The cross streaked plates were incubated for 48 h at ambient temperature and observed for growth/ inhibition of growth.

3.2.11.2. Compatibility of selected bacterial isolates with actinomycete

The compatibility of the selected actinomycete with bacteria was tested by streaking the actinomycete vertically and bacteria horizontally, on nutrient agar contained in a Petri dish. The plate was incubated at ambient temperature for 48 h and observations on growth/ inhibition of growth were recorded.

3.2.11.3. Compatibility between fungi and bacteria/ actinomycetes

Compatibility of selected fungal isolate with bacteria/ actinomycetes was studied by dual culture method. A five mm agar disc of the respective fungal mycelia was inoculated on one side of a Petri dish containing PDA medium. The plates were then incubated at ambient temperature for 24 h. A loopful of 24 h old bacterial culture on nutrient agar was then streaked three cm away from the fungal disc on the same plate and incubated for 3-4 days at 26 ± 2^0 C. Three replicates were maintained and Petri plates inoculated only with fungus served as control. Seven days after incubation, the diameter of fungal growth from point of inoculation of the fungal disc to actively growing edges of fungus was measured in both inoculated and control plates. The following formula was used to find the per cent growth inhibition (PGI).

$$\textbf{PGI (\%)} = \frac{(C - T)}{C} \times 100$$

C - Diameter of fungal/ bacterial growth in control plate

T - Diameter of fungal/ bacterial growth in treated plate

3.2.11.4. Compatibility between fungal isolates

Dual culture method was followed (Dennis and Webster, 1971) for testing the compatibility between two fungal cultures. On PDA, five mm agar disc of both fungal cultures were placed at a distance of 2 cm from the periphery. Control plate and three replications were maintained at ambient temperature for 72 h. Diameter of the fungal isolates was measured when growth of the fungal isolate in control plate reached maximum and the per cent growth inhibition (PGI) was calculated.

3.2.12. Selection of microbial isolates for consortial formulation

Isolates were selected for testing their suitability for preparing the consortium. The selection of isolates was based on their ability to produce specific enzymes having definite role in biodegradation and the compatibility of the microorganisms.

3.2.13. Mass culturing of the selected isolates

The selected isolates were mass cultured in suitable culture broth. Nutrient broth was used for the multiplication of bacteria, starch casein broth for actinomycetes and potato dextrose broth for fungus. The broth cultures were kept for incubation for five (for bacteria and actinomycete) to 10 (for fungi) days at ambient temperature.

3.2.14. *In vitro* evaluation of efficiency of selected consortia

In vitro study was carried out to evaluate the efficiency of selected consortia for the degradation of vegetable waste. The microbial cultures were multiplied in nutrient broth, potato dextrose broth and starch casein broth for bacteria, fungi and actinomycetes respectively. Inoculated broth cultures were incubated for 2-5 days at

ambient temperature. Four different consortia were prepared by mixing equal amount of cultures (1:1:1) in a 500 ml conical flask aseptically. In a 250 ml conical flask, 100 g of steamed sterilized vegetable waste (containing carrot, potato, brinjal and bhindi) was taken and 10 ml of microbial culture was inoculated aseptically. Five treatments (four potential consortia and one uninoculated control) with four replications were maintained. All the flasks were incubated for 21 days at ambient temperature and observations were taken on weight reduction, consistency and enzyme activity. Visual observation on degradation of vegetable waste was also taken at regular intervals.

The treatment details of the experiment are given below

Design: CRD

Treatments: 5

T_1: Consortium I (*G. butleri* + *S. roseofulvus* AcOu-1 + *B. subtilis*)

T_2: Consortium II (*B. subtilis* BaBc-1 + *T. asperellum* + *Bacillus* sp. BaOu-1)

T_3: Consortium III (*B. subtilis* BaBc-1 + *G. butleri* + *Bacillus* sp. BaOu-1)

T_4: Consortium IV (*B. subtilis* + *G. butleri* + *B. subtilis* BaBc-1)

T_5: Uninoculated (Control)

Replications: 4

3.2.14.1. Visual observation on biosolid waste degradation

Conversion of biosolid waste in to slurry form was recorded as the visual indicator of biosolid waste degradation.

3.2.14.2. Weight reduction of biosolid waste

The per cent weight reduction of each treatment was calculated after 21 days of incubation by deducting final weight of vegetable waste from initial weight and expressing as per cent.

3.2.14.3. Enzyme activity of consortial formulations during degradation

After 21 days of inoculation, all the treatments were subjected to enzyme assay for cellulose, amylase and protease. Celluase activity was determined using the method suggested by Sadasivam and Manickam (1996), amylase activity as per Miller (1959) and protease assay as per Tsuchida *et al.* (1986).

3.2.14.4. Microbial population (cfu g^{-1})

Microbial population of compost was assessed by serial dilution and plating technique as described earlier.

3.2.15. Pilot scale experiment with selected consortia in KAU smart biobins

Best two treatments were evaluated for their efficiency in aerobic composting units designed for use in households (KAU smart biobins). This biobin was developed in the Department of Agricultural Microbiology, College of Horticulture (Girija *et. al.*, 2016). It is a cylindrical structure having 3¾ feet height and 1½ feet diameter and made of stainless steel mesh and with an inner ring of 3 feet height (Plate 1). The space between the two rings was filled with dry leaves before application of biosolid waste.

Bacterial and fungal cultures were mass multiplied in their respective broth and equal amount of culture broth mixed together (1:1) under aseptic conditions. Coir pith based inoculum was prepared by mixing the coir pith, water and consortium in the ratio 4.5 kg: 6 liter: 0.5 liter. The mixture was incubated for 24 h at ambient temperature and then air dried to 20 per cent. This coir pith based inoculum was applied between the layers of waste for composting.

The vegetable and fruit waste required for the experiment was collected from Sakthan market, Thrissur. In each biobin, initially 12 kg of biosolid waste was added

KAU smart biobin

Coir pith based inoculum

Plate 1: Pilot scale experiment in KAU smart biobin

and after 15 days, 20 kg waste was added (Plate 2). Each layer consisted of 4 kg biosolid waste and 0.25 kg coir pith based inoculum. In positive control, 2 kg of cow dung was added in slurry form instead of coir pith based inoculum. The negative control consisted of waste and dry leaves in all the layers, without any added cow dung or inoculum. Observations on volume reduction of biosolid waste, daily variation in temperature and composting period were recorded. Yield and quality of compost were analyzed 30 days after inoculation.

The treatment details of the experiment are given below

Design: CRD

Treatments: 4

T_1: Consortium I (*B. subtilis* BaBc-1 + *T. asperellum* + *Bacillus* sp. BaOu-1)

T_2: Consortium II (*B. subtilis* + *G. butleri* + *B. subtilis* BaBc-1)

T_3: Cow dung slurry

T_4: Uninoculated (Control)

Replications: 5

3.2.16. Evaluation of selected consortium in Thumburmuzhi composting unit

For the validation of the results obtained in previous experiment, large scale replicated trial of Thumburmuzhi composting model was laid out in the composting shed of the Department of Agricultural Microbiology, COH Vellanikkara. Thumburmuzhi composting model is a ferrocement model of 4 ft x 4 ft x 4 ft dimensions. It consists of four pillars with side bars and these side bars can be easily fixed and inserted through the grooves on the pillars (Plate 3).

Evaluation of selected consortium in Thumburmuzhi composting units was conducted according to the procedure experimented by Girija *et al.* (2011). Microbial cultures were mass produced in respective culture broth (nutrient broth and potato dextrose broth) and kept for incubation at ambient temperature. After incubation

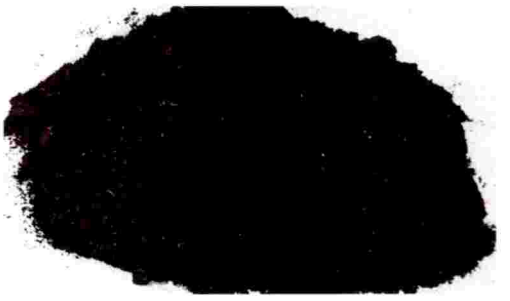
Segregation of waste

Addition of waste

Addition of coir pith based inoculum

Completely filled KAU smart biobin

Collection of compost



Final compost

Plate 2: Steps involved in composting of biosolid waste using coir pith based inoculum in KAU smart biobin

period, the mass produced cultures were mixed together in the ratio of 1:1 to make the inoculum. Vegetable and fruit waste required for the experiment were collected from Sakthan market, Thrissur.

Six inch layers, each of dry leaves and biosolid waste were layered in each Thumburmuzhi unit. For inoculated treatment, 250 ml inoculum (50 ml inoculum was diluted with 200 ml of water) was sprayed over the layer of dry leaves (Plate 4). About 700 kg of different vegetable and fruit waste was added to each unit and 100 kg of fresh cow dung was added in positive control. The uninoculated unit served as negative control. Observations on variation in temperature and volume reduction of biosolid waste were recorded (daily up to 30 days and then once every week). Yield and quality of compost were analyzed after the maturation period.

The treatment details of the experiment are given below

Design: CRD

Treatments: 3

T_1: Consortium I (*B. subtilis* BaBc-1 + *T. asperellum* + *Bacillus* sp. BaOu-1)

T_2: Cow dung

T_3: Uninoculated

Replications: 5

3.2.17. Physical parameters of composting

Physical parameters of composting such as volume reduction, variation in temperature, duration of composting process (KAU biobin) and yield of compost were recorded.

a. Composting area

b. Thumburmuzhi composting units

c. Individual Thumburmuzhi composting unit

Plate 3: Thumburmuzhi composting units

Addition of dry leaves

Spraying of microbial consortium

Addition of biosolid waste

Thumburmuzhi composting unit filled with biosolid waste

Plate 4: Steps involved in composting of biosolid waste using microbial consortium in Thumburmuzhi composting unit

3.2.17.1. Volume reduction of biosolid waste

The height was recorded at regular intervals during the period of composting. The change in volume from initial volume was calculated as volume reduction. The final volume reduction was determined in per cent with respect to the initial volume.

3.2.17.2. Variation in temperature during composting

The temperature was recorded daily using digital thermometer and the maximum temperature in each treatment was noted. The data were recorded in degree centigrade.

3.2.17.3. Composting period

The day when compost collected from biobin was indicated as composting period in the present investigation. Number of days taken for initiation of compost formation from each treatment was recorded.

3.2.17.4. Yield of compost

The total yield of each treatment was assessed by deducting the residue weight from the total quantity of compost obtained.

3.2.18. Moisture content of compost

Moisture content in compost was recorded after maturation period by gravimetric method (FCO, 1985).

3.2.18.1. Bulk density of compost

Bulk density of compost determined using measuring cylinder (FCO, 1985).

3.2.18.2. Colour and odour of compost

The colour and odour of matured compost was monitored.

3.2.19. Chemical parameters

Chemical analysis of the compost samples was carried out at the Radiotracer Lab, College of Horticulture, Vellanikkara. Compost samples were collected after the maturation period and analyzed for chemical parameters according to the standard procedure. Methods of compost analysis are given in Table 6.

Table 6. Methods of compost analysis

Sl. No.	Particulars	Method	Reference
1	Particle size	Sieving	FCO, 1985
2	Organic carbon	Ashing	
3	Total N	Microkjeldahl digestion and distillation	Jackson, 1958
4	Total P	Ashing- 25% HCl extract- Bray 1 extractant-spectrophotometry	FCO, 1985
5	Total K	Ashing- 25% HCl extract- Bray 1 extractant-spectrophotometry	
6	Available Cu and Zn	Ashing- 25% HCl extract- Bray 1 extractant-spectrophotometry	
7	pH	pH meter 1:10 suspension (Organic waste:water)	
8	EC	Conductivity meter	Jackson, 1958
9	Heavy metals (As, Cr, Pb, Cd)	Ashing- 25% HCl extract- Bray 1 extractant-spectrophotometry	FCO, 1985

3.2.20. Biological parameters

The collected compost samples were subjected to phytotoxicity studies, presence or absence of human pathogen and microbial population analysis.

3.2.20.1. Microbial population (cfu g^{-1})

Microbial population of compost was assessed by serial dilution and plating technique after the maturation of compost. The microbial population of the compost was then compared to that of cow dung.

3.2.20.2. Phytotoxicity

For testing the phytotoxicity of the compost, a modified method of Zucconi *et al.* (1981) was used. Anagha variety of tomato seeds were used for the seed germination test in compost extract. In 90 mm UV sterilized Petri plates, Whatman No.2 filter paper was placed and wetted with three ml of 1:10 compost: water extract and 15 tomato seeds were placed on the paper. The control plates contained distilled water instead of compost extract and all the treatments were run in replicate. To minimize the moisture loss, the plates were sealed with parafilm. All the plates were kept in the dark for four days at room temperature and after four days seed germination in compost extract and control was noted and compared.

$$\text{Per cent germination} = \frac{\text{No of seeds germinated}}{\text{Total no. of seeds}} \times 100$$

3.2.20.3. Human pathogens

Serial dilution and pour plate method was used for the detection of *Escherichia coli*, *Salmonella* sp. and *Shigella* sp. in compost samples (Cappuccino and Sherman, 1992). Ten gram compost sample was transferred to 90 ml sterile water

blank and the contents were homogenized by shaking. One ml suspension from this dilution was transferred to 9 ml sterile water blank and mixed thoroughly to obtain 10^{-2} dilution. Dilutions up to 10^{-3} were prepared by repeating the procedure. One ml of 10^{-3} dilution was poured to the sterile Petri plates thereafter melted cooled Eosin methylene blue agar (EMB) and *Salmonella Shigella* (SS) agar media was separately added in respective plates. Control plates and three replicates for each sample were maintained. Petri plates were then incubated for 48 h at ambient temperature. The number of microbial colonies in each plate was enumerated.

3.2.21. Economics of composting in Thumburmuzhi composting unit

The economics of composting in Thumburmuzhi composting unit was determined based on the benefit cost ratio. The BC ratio was calculated as per the formula given below.

$$BCR = \frac{\text{Total returns}}{\text{Total cost}}$$

3.2.22. Statistical analysis

Analysis of Variance (ANOVA) was carried out by employing Web Agri Stat Package (WASP 2.0). Appropriate transformation was done according to the method suggested by Gomez and Gomez (1984). Duncan's Multiple Range Test (DMRT) was used for the multiple comparison between the treatment means.

Results

4. RESULTS

The results of the study on 'Microbial inoculants for enhancing degradation of biosolid waste in aerobic composting' conducted during the period 2016-2018 at Department of Agricultural Microbiology, College of Horticulture, Vellanikkara are presented in this chapter.

4.1. Enumeration of microorganisms from different compost samples

In order to obtain microorganisms capable of enhancing degradation of biosolid waste, compost samples were collected from four different sources *viz.,* KAU Smart biobin, coir pith, Oushadhi ayurvedic and kitchen waste composting units. The population of bacteria, fungi and actinomycetes was enumerated in all these samples.

Population of bacteria showed wide variation from 1.67 to 215.67 x 10^6 cfu g^{-1} (Table 7 and Plate 5). Compost from KAU biobin recorded highest bacterial population (215.67 x 10^6 cfu g^{-1}) followed by kitchen waste compost (9.67 x 10^6 cfu g^{-1}) and Oushadhi ayurvedic waste compost (8.00 x 10^6 cfu g^{-1}). Bacterial populations of these two samples were found to be on par. Coir pith compost recorded the lowest bacterial population (1.67 x 10^6 cfu g^{-1}).

Population of fungi was less when compared to that of bacteria, and it ranged from 3.00 to 42.67 x 10^4 cfu g^{-1}. Compost sample from kitchen waste recorded significantly higher population of 42.67 x 10^4 cfu g^{-1} followed by coir pith compost (19 x 10^4 cfu g^{-1}), which was on par with KAU biobin compost (4.67 x 10^4 cfu g^{-1}). Minimum population (3.00 x 10^4 cfu g^{-1}) of fungi was found in Oushadhi ayurvedic waste compost.

Actinomycetes population could not be detected in KAU biobin compost, coir pith compost as well as kitchen waste compost. However, Oushadhi ayurvedic waste

compost was found to be good source of actinomycetes (with a population of 129 x 10^3 cfu g^{-1}), compared to other compost samples.

Table 7. Enumeration of microorganisms from different compost samples

Source of compost	*Population of microorganisms (cfu g^{-1} of compost)		
	Bacteria ($x10^6$)	**Fungi ($x10^4$)**	**Actinomycetes ($x10^3$)**
KAU biobin	215.67 (2.29)[a]	4.67 (0.64)[c]	0.00
Coir pith	1.67 (0.20)[c]	19.00 (1.27)[b]	0.00
Oushadhi ayurvedic waste	8.00 (0.89)[b]	3.00 (0.46)[c]	129.00 (2.11)[a]
Kitchen waste	9.67 (0.97)[b]	42.67 (1.60)[a]	0.00
CD (0.05)	0.323	0.321	0.070

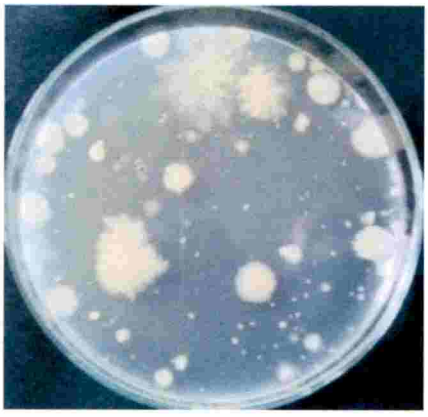
*Mean of three replications

The values followed by same letter in each column do not differ significantly according to DMRT

Log transformed values are given in parentheses.

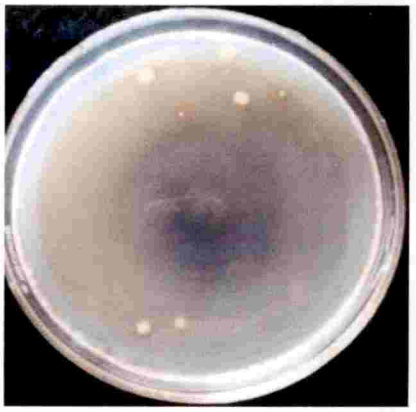
4.1.1. Isolation of microorganisms from compost samples

After enumeration, the predominant microorganisms obtained were selected for further studies. A total of 14 microorganisms were isolated from different compost samples including eight bacteria (two each from KAU biobin compost, coir pith compost, Oushadhi ayurvedic waste compost and kitchen waste compost), four fungi (two each from coir pith compost and kitchen waste compost) and two actinomycetes (from Oushadhi ayurvedic waste compost) (Table 8). All the isolates were assigned

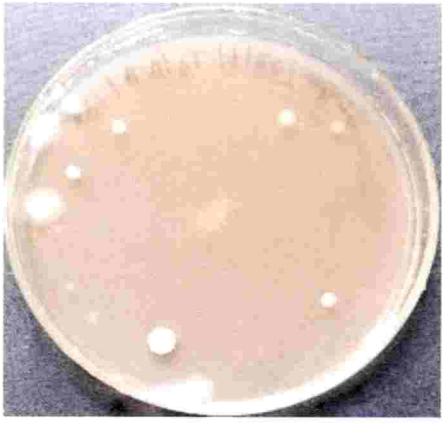


KAU biobin compost

Coir pith

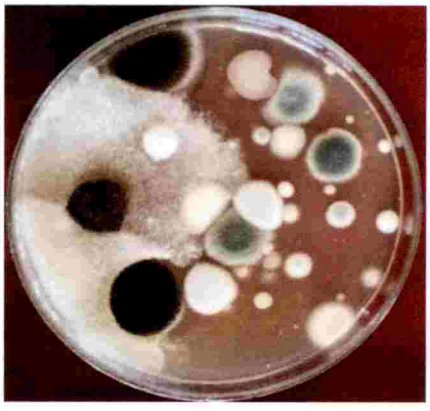


Oushadhi ayurvedic waste compost

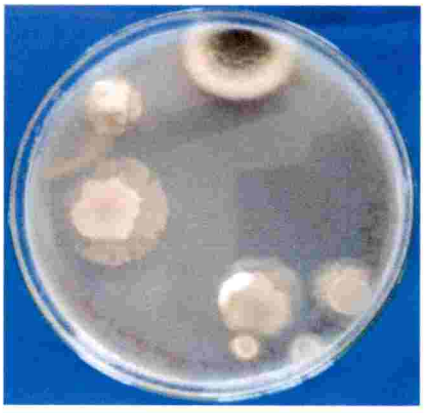


Kitchen waste compost

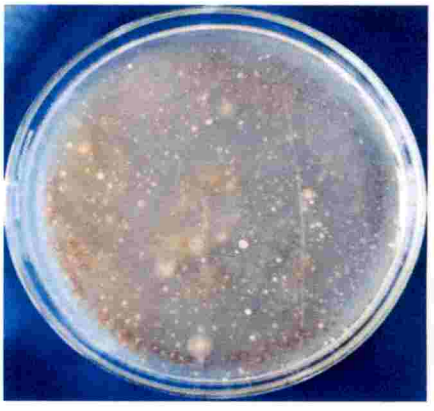
A: Bacteria



Coir pith compost



Kitchen waste compost



Oushadhi ayurvedic waste compost

B: Fungi

C: Actinomycetes

Plate 5: Microorganisms isolated from different compost samples

names based on the type of microorganism and source. The eight bacterial isolates were designated as BaBc-1 (bacteria from biobin compost), BaBc-2 (bacteria from biobin compost), BaCp-1 (bacteria from coir pith compost), BaCp-2 (bacteria from coir pith compost), BaOu-1 (bacteria from Oushadhi ayurvedic waste compost), BaOu-2 (bacteria from Oushadhi ayurvedic waste compost), BaKi-1 (Bacteria from kitchen waste compost) and BaKi-2 (Bacteria from kitchen waste compost). Fungal isolates were designated as FCp-1 (fungus from coir pith compost), FCp-2 (fungus from coir pith compost), FKi-1 (fungus from kitchen waste compost) and FKi-2 (fungus from kitchen waste compost). The actinomycetes isolates were designated as AcOu-1 (actinomycete from Oushadi ayurvedic waste compost) and AcOu-2 (actinomycete from Oushdi ayurvedic waste compost). The details of microbial isolates obtained from compost samples are presented in Table 8.

4.2. Purification and maintenance of isolates

Individual colonies from the mixed cultures were purified by streaking on respective agar medium and purified colonies were transferred to agar slants. Pure cultures of all the isolates were stored in refrigerator (4^0C) and used for further studies. The bacterial isolates were also preserved in 40% glycerol at -80^0C in deep freezer.

4.3. *In vitro* screening of isolates for degradation of components of biosolid waste

All the microbial isolates obtained from different sources were tested *in vitro* for degradation of cellulose, lignin, starch, protein, chitin and pectin. Apart from this, two bacterial isolates (*Bacillus subtilis* and *B. niabensis*) and two fungal isolates (*Gongronella butleri* and *Trichoderma asperellum*) available in the repository of the Department of Agricultural Microbiology, College of Horticulture, Vellanikkara were also used as reference cultures for screening. Ten isolates which showed growth on selective media were only selected for *in vitro* screening.

The degrading ability of these microbes was assessed by measuring the diameter of clear zones produced on the respective selective medium and the degradation efficiency of isolates was determined by calculating the degradation index, as detailed in Materials and Methods. Data on decomposition of organic substrates by microbial isolates are presented in Table 9.

Table 8. Microorganisms isolated from different compost samples

Microorganism	Source of compost	Number of isolates	Code for isolates
Bacteria	KAU biobin	Two	BaBc-1 BaBc-2
	Coir pith	Two	BaCp-1 BaCp-2
	Oushadhi ayurvedic waste	Two	BaOu-1 BaOu-2
	Kitchen waste	Two	BaKi-1 BaKi-2
Fungi	Coir pith	Two	FCp-1 FCp-2
	Kitchen waste	Two	FKi-1 FKi-2
Actinomycetes	Oushadhi ayurvedic waste	Two	AcOu-1 AcOu-2

4.3.1. Cellulose decomposition

Among the different microbes, fungal isolates recorded maximum diameter of clear zone as compared to bacteria (Plate 6). *G. butleri* recorded maximum clear zone diameter (52 mm) on CMC agar, which was on par with *T. asperellum* (50 mm).

Among the bacterial isolates, BaOu-1 and BaBc-1 produced maximum clear zone of 30 mm. AcOu-2 recorded smallest clear zone with a diameter of 3 mm, and no clear zone was produced by AcOu-1 on CMC agar medium. All the isolates except BaKi-1 and AcOu-2 exhibited degradation index more than one (>1). The cellulose degradation index was highest in fungal isolates and the maximum degradation index was noted in *G. butleri* (1.7) followed by *T. asperellum* (1.6).

4.3.2. Lignin decomposition

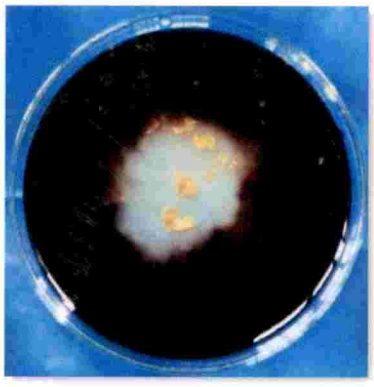
The ability of the isolates to degrade lignin was evaluated using lignin sulphonate medium. Though all the isolates showed growth on the medium, none of the bacterial, fungal and actinomycete isolates developed any clear zone around the colonies.

4.3.3. Starch decomposition

In the case of starch degradation, the bacterial isolate BaCp-1 produced the maximum clear zone of 18 mm (Plate 7). This was followed by *B. niabensis* (KAU isolate), which was on par with *B. subtilis* with a clear zone of 15 mm and 12.5 mm respectively. None of the fungal isolates produced any clear zone, indicating their inability to degrade starch. Actinomycete AcOu-2 produced a clear zone of 9.33 mm diameter. The highest degradation index (1.6) was recorded in BaCp-1. The lowest value for degradation index (1.1) was recorded in two actinomycete isolates (AcOu-1 and AcOu-2). Two bacterial isolates (BaOu-1 and BaKi-1) and two reference cultures of fungi (*G. butleri* and *T. asperellum*) recorded zero degradation index.

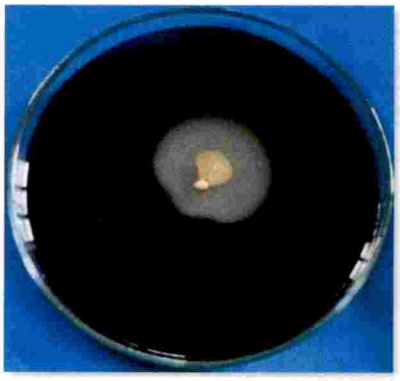
4.3.4. Protein decomposition

The clear zone of protein degraders on the selective medium ranged from zero to 20 mm (Plate 8). The isolate BaOu-1 produced maximum zone of 20 mm dimeter, followed by BaBc-1 and *B. subtilis* (10 mm). None of the fungal and actinomycete



BaBc-1

BaCp-1



BaOu-1

BaKi

B. subtilis **(KAU isolate)**

B. niabensis **(KAU isolate)**

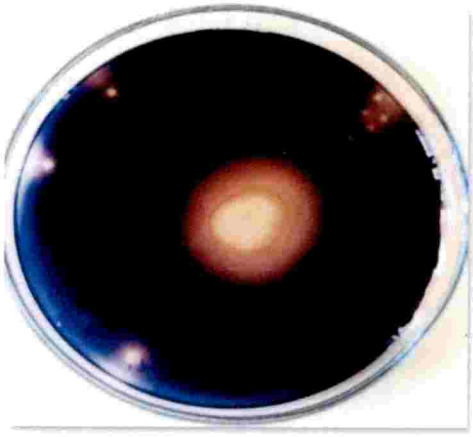
AcOu-1

G. butleri **(KAU isolate)**

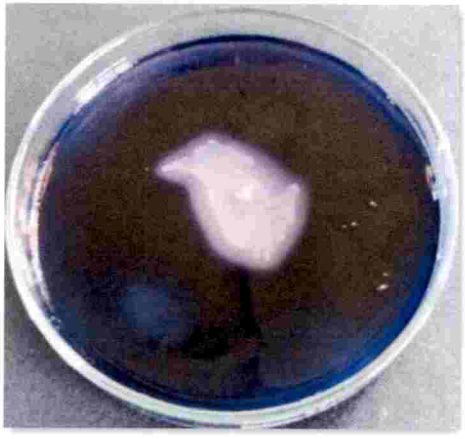
T. asperellum **(KAU isolate)**

Plate 6: Cellulose degradation by microbial isolates

BaBc-1

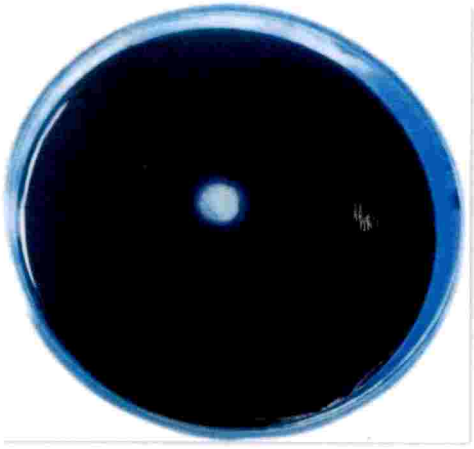


BaCp-1



***B. subtilis* (KAU isolate)**

***B. niabensis* (KAU isolate)**



AcOu-1

AcOu-2

Plate 7: Starch degradation by microbial isolates

isolates were able to degrade protein under *in vitro* condition. The protein degradation index ranged from 1.3 to 1.6 and the highest degradation index was in BaOu-1 (1.6) followed by BaCp-1 (1.5). The lowest protein degradation index (1.3) was noted in BaBc-1 and zero degradation index was recorded in BaKi-1, *B. niabensis*, AcOu-1, AcOu-2, *G. butleri* and *T. asperellum*.

4.3.5. Pectin decomposition

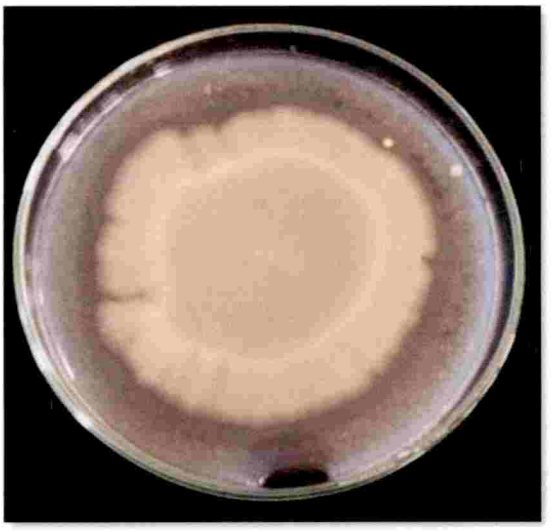
Pectin degradation potential of the isolates was tested on pectic agar and none of the bacterial, fungal and actinomycetes isolates produced clear zone. Hence the pectin degradation index of all the selected isolates was recorded as zero.

4.3.6. Chitin decomposition

In vitro screening of chitin degradation carried out on colloidal chitin media. None of the bacterial isolates, fungal isolates and actinomycete isolates were able to degrade chitin and the respective degradation efficiency was recorded as zero.

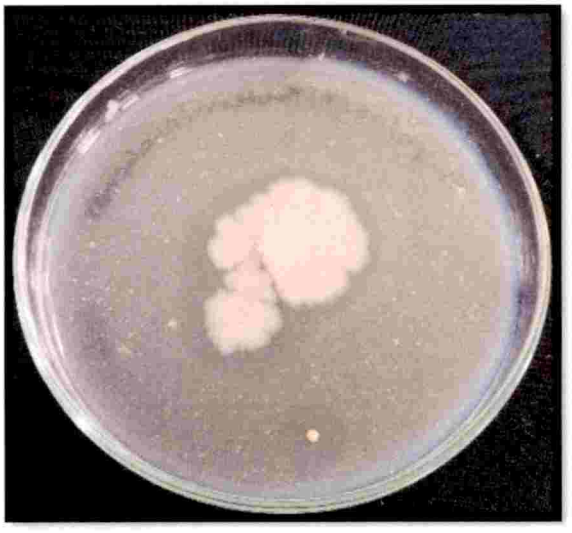
4.3.7. Lipid decomposition

The ability of the isolates to degrade lipid were assessed and only two bacterial isolates produced blue colour around the colony on spirit blue agar (plate 7). Blue colour zone of 10 and 8 mm diameter were produced by BaBc-1 and *B. subtilis* respectively. Other bacterial, fungal and actinomycetes isolates tested did not produce any zone and hence the lipid degradation index was recorded as zero.

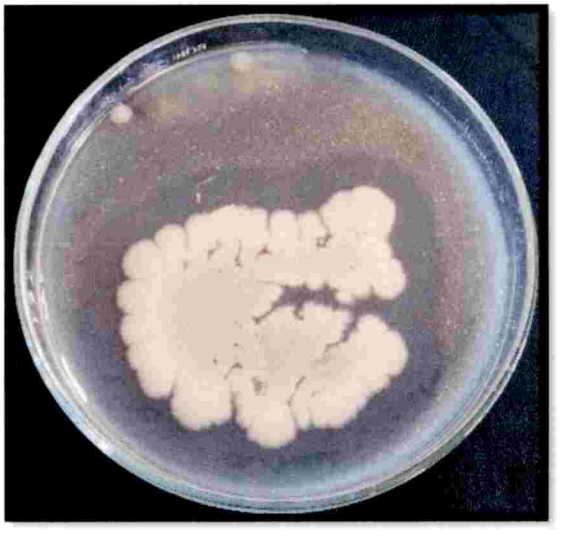


BaBc-1

BaCp-1

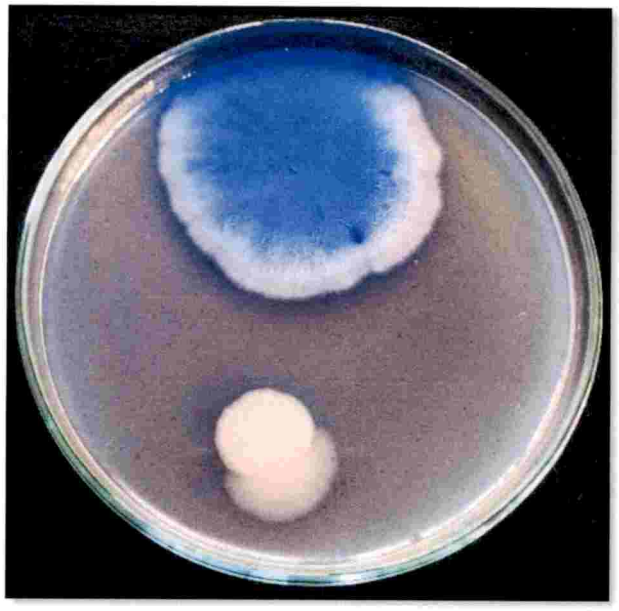


BaOu-1

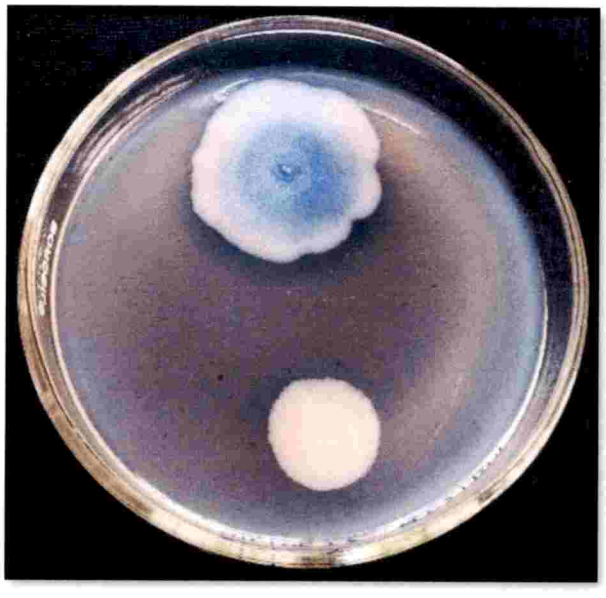


***B. subtilis* (KAU isolate)**

Plate 8: Protein degradation by microbial isolates



BaBc-1



B. subtilis **(KAU isolate)**

Plate 9: Lipid degradation by microbial isolates

Table 9. Decomposition of organic substrates by microbial isolates

Isolates	*Diameter of clear zone (mm)							Degradation index (Diameter of clear zone/ Diameter of colony)						
	Cellulose	Lignin	Starch	Protein	Pectin	Chitin	Lipid	Cellulose	Lignin	Starch	Protein	Pectin	Chitin	Lipid
BaBc-1	30.00^{b}	0	12.00^{c}	10.00^{b}	0	0	30^{a}	1.5	0	1.2	1.3	0	0	1.3
BaCp-1	2.00^{d}	0	18.00^{a}	12.00^{b}	0	0	0	1.3	0	1.6	1.5	0	0	0
BaOu-1	30.00^{b}	0	0	20.00^{a}	0	0	0	1.5	0	0	1.6	0	0	0
BaKi-1	10.00^{d}	0	0	0	0	0	0	1	0	0	0	0	0	0
B. subtilis	12.00^{d}	0	12.50^{bc}	10.00^{b}	0	0	20^{b}	1.3	0	1.3	1.4	0	0	1.2
B. niabensis	22.00^{c}	0	15.00^{b}	0	0	0	0	1.4	0	1.4	0	0	0	0
AcOu-1	3.00^{e}	0	10.30^{cd}	0	0	0	0	1.3	0	1.1	0	0	0	0
AcOu-2	0	0	9.33^{d}	0	0	0	0	0	0	1.1	0	0	0	0
G. butleri	52.00^{a}	0	0	0	0	0	0	1.7	0	0	0	0	0	0
T. asperellum	50.00^{a}	0	0	0	0	0	0	1.6	0	0	0	0	0	0
CD (0.05)	3.24	-	2.52	3.14	-	-	0.76	-	-	-	-	-	-	-

*Mean of three replications

In each column values followed by same letter do not differ significantly according to DMRT

Based on *in vitro* assay for degradation of organic molecules on selected media, indicated by clear zone formation, eight isolates were selected for further enzyme assay. The bacterial isolate BaKi-1 and the actinomycete AcOu-2 were eliminated from enzyme assay, since they produced very small zones around the colonies and hence, recorded very low degradation indices.

4.4. *In vitro* enzyme assay

All the eight selected isolates (Plate 10) were subjected to enzyme assay for quantitative estimation of enzyme activity including cellulase, β-1,3 glucanase, β-glucosidase, laccase, amylase, protease, pectinase and lipase activity. Data on enzyme activity are given in Table 10.

4.4.1. Cellulase

All the isolates exhibited cellulase activity, which ranged from 0.13 to 1.20 U ml^{-1} (Fig. 1). Fungi recorded higher cellulase activity as compared to bacteria. Significantly higher activity (1.20 U ml^{-1}) was recorded by *G. butleri* and this was followed by BaBc-1 (0.865 U ml^{-1}). BaOu-1 recorded the minimum cellulase activity (0.13 U ml^{-1}).

4.4.2. β-1,3 glucanase

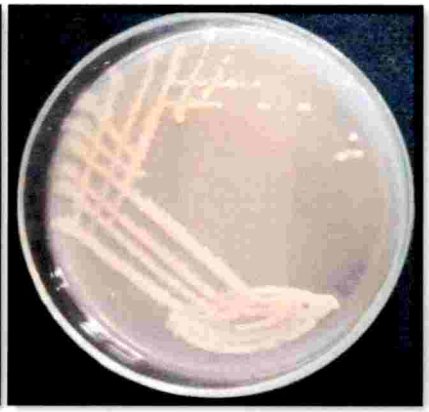
All the isolates exhibited β-1,3 glucanase activity, which ranged between 17.03 to 22.24 U ml^{-1} (Fig. 2). *B. subtilis* (KAU isolate) showed significantly higher β-glucosidase activity of 22.24 U ml^{-1}, which was on par with BaBc-1 (22.90 U ml^{-1}) and BaOu-1 (20.97 U ml^{-1}). β-1,3 glucanase production by BaOu-1 was also on par

BaBc-1

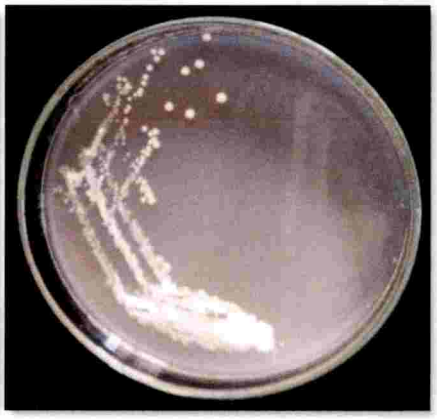
BaCp-1

BaOu-1

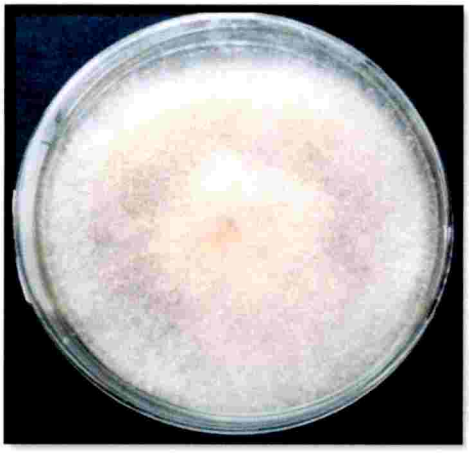
B. subtilis (KAU isolate)



B. niabensis (KAU isolate)



AcOu-1



G. butleri (KAU isolate)

T. asperellum (KAU isolate)

Plate 10: Isolates selected for quantitative enzyme assay

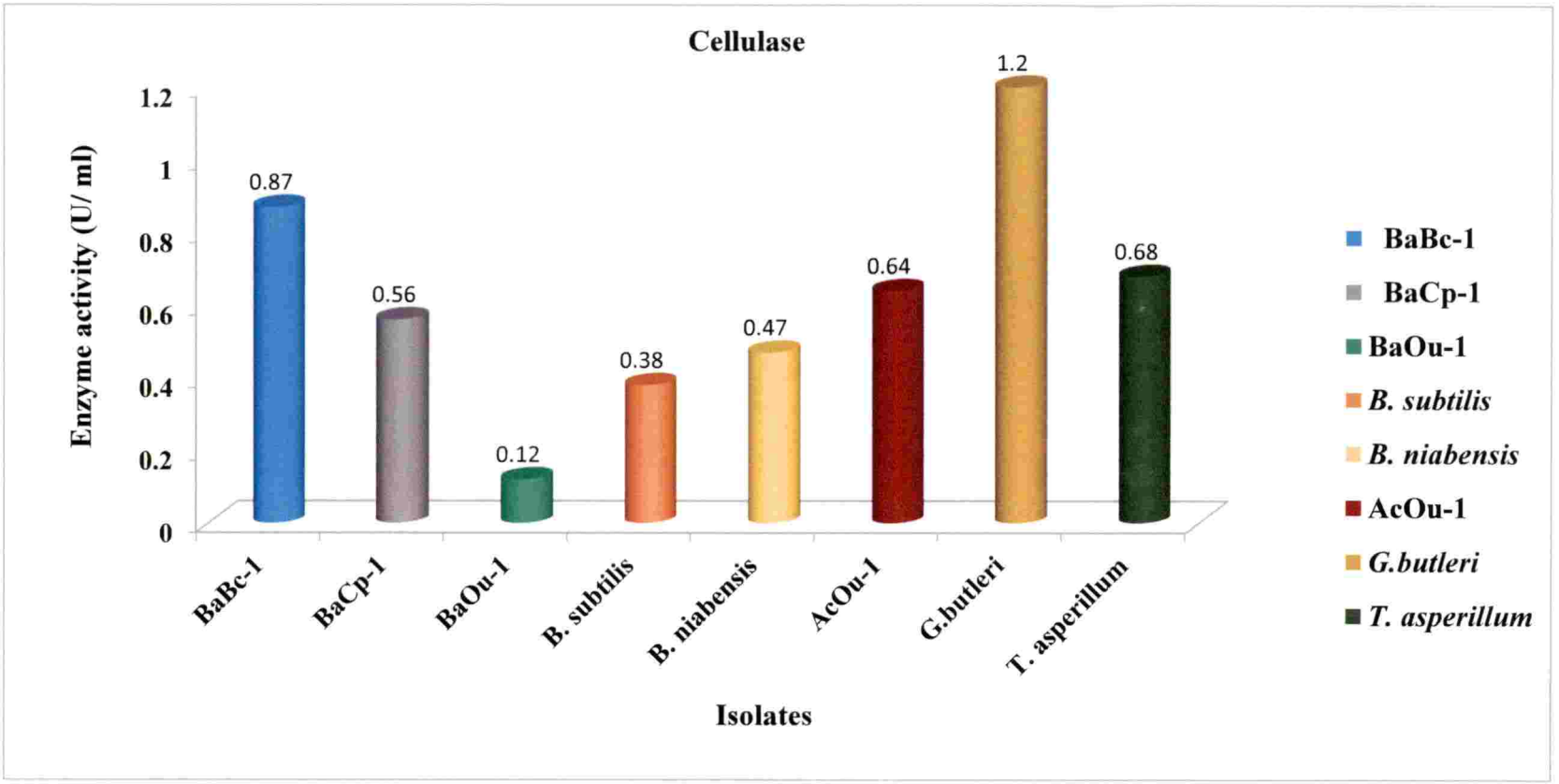


Fig. 1. Cellulase activity of selected isolates

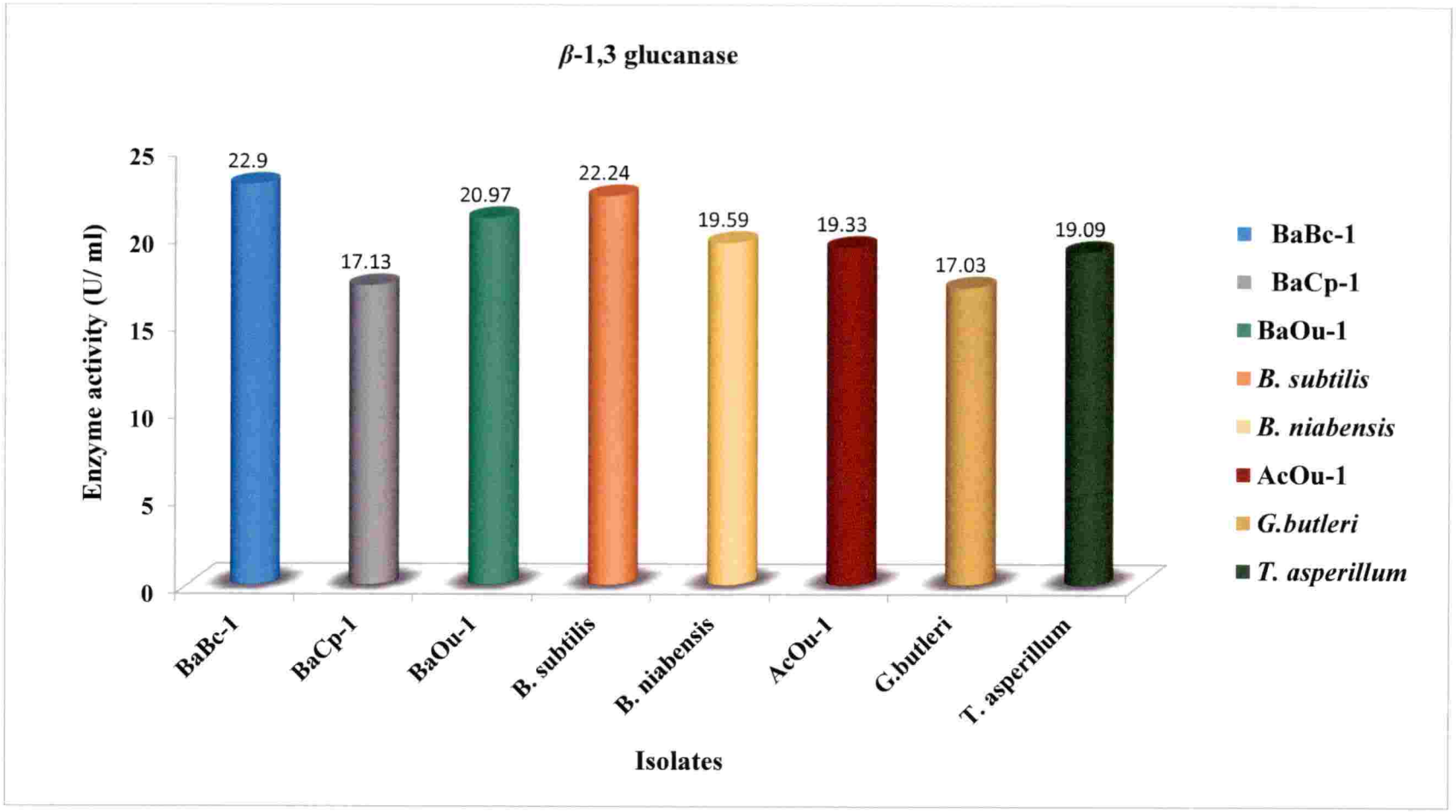


Fig. 2. β-1,3 glucanase activity of selected isolates

with *B. niabensis* (19.59 U ml^{-1}) and AcOu-1 (19.33 U ml^{-1}) respectively. The fungal isolate *G. butleri* recorded significantly lower β-1,3 glucanase activity (17.03 U ml^{-1}).

4.4.3. β- glucosidase

All the selected isolates exhibited β-glucosidase activity and in general, higher activity was recorded by fungal isolates (Fig. 3). The isolate *G. butleri* recorded significantly superior β-glucosidase activity (0.42 U ml^{-1}) which was on par with *T. asperellum* (0.39 U ml^{-1}), BaBc-1 (0.28 U ml^{-1}) and *B. subtilis* (KAU isolate) (0.27 U ml^{-1}). The least β-glucosidase activity of 0.17 U ml^{-1} was found in *B. niabensis* (KAU isolate).

4.4.4. Laccase

All the eight isolates exhibited laccase activity and there was a significant difference observed among the isolates (Fig. 4). Significantly higher activity (6.33 U ml^{-1}) was recorded in BaOu-1 followed by *G. butleri* (4.85 U ml^{-1}) which was on par with *T. asperellum* (3.87 U ml^{-1}). The isolate BaBc-1 showed the least laccase activity of 1.57 U ml^{-1}.

4.4.5. Amylase

Amylase activity was exhibited by all the selected isolates, which ranged from 0.05 to 0.25 U ml^{-1} (Fig. 5). The isolate AcOu-1 recorded significantly higher amylase activity (0.25 U ml^{-1}) followed by BaOu-1 (0.22 U ml^{-1}). The least activity of 0.05 U ml^{-1} was observed in the isolate *B. subtilis* (KAU isolate).

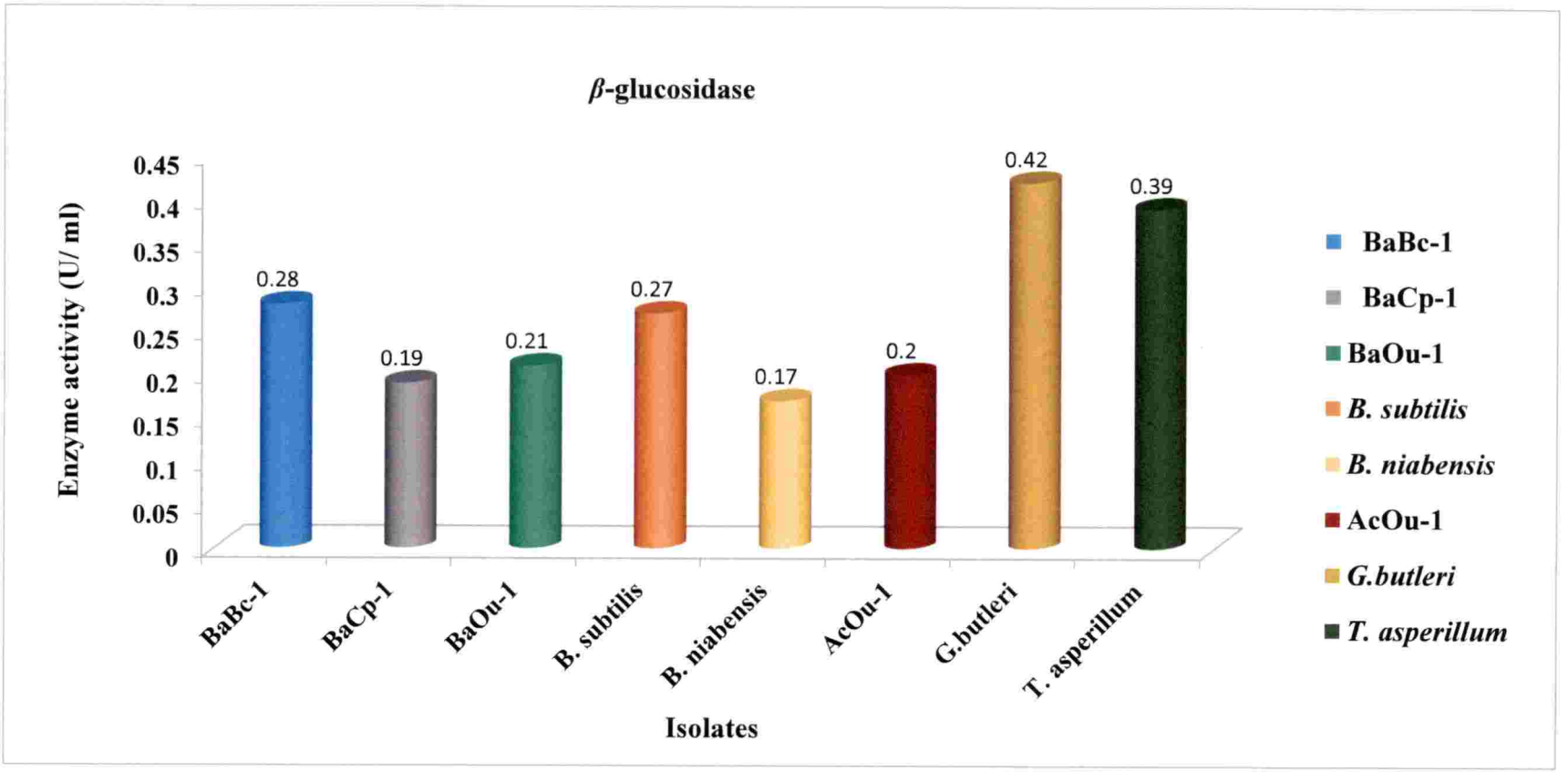


Fig. 3. β- glucosidase activity of selected isolates

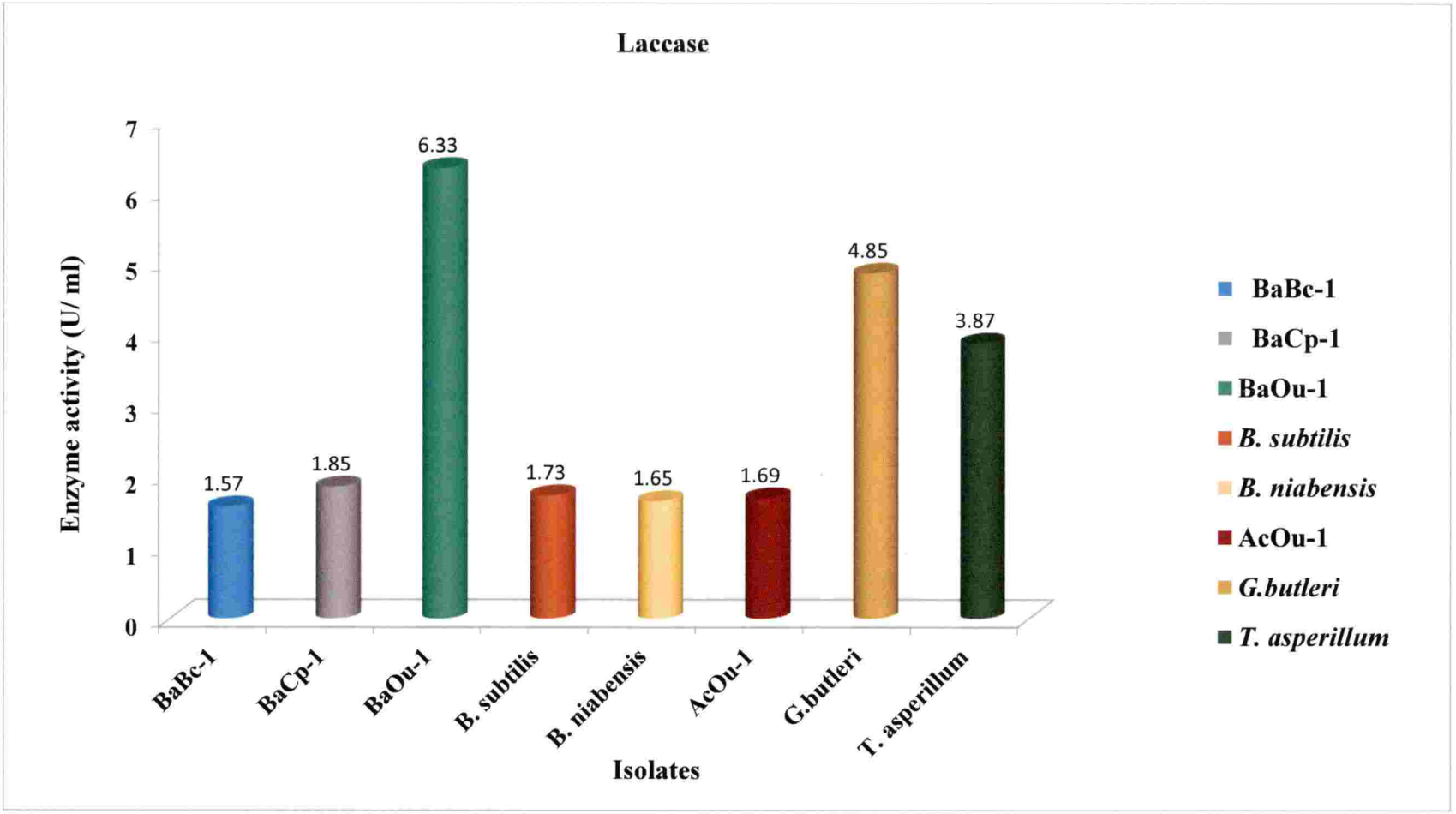


Fig. 4. Laccase activity of selected isolates

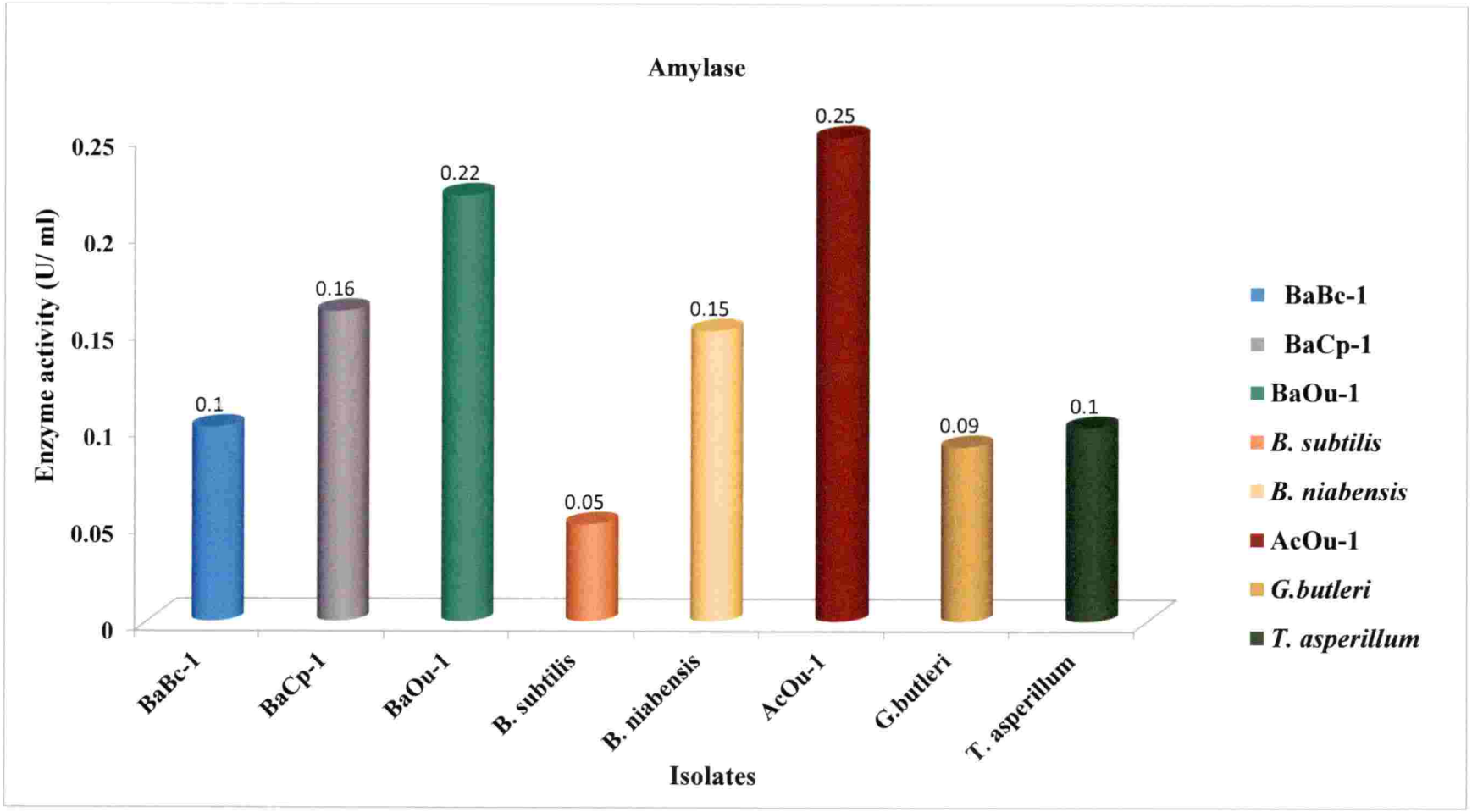


Fig. 5. Amylase activity of selected isolates

4.4.6. Protease

All the selected isolates exhibited protease activity and the fungal isolates showed the highest activity compared to the bacterial isolates (Fig. 6). The isolate *G. butleri* recorded significantly higher activity of 216.70 U ml^{-1}, which was on par with *T. asperellum* (210.07 U ml^{-1}). This was followed by AcOu-1 (189.92 U ml^{-1}), BaBc-1 (188.13 U ml^{-1}), *B. subtilis* (KAU isolate) (187.04 U ml^{-1}), BaOu-1 (184.45 U ml^{-1}), *B. niabensis* (KAU isolate) (182.72 U ml^{-1}) and BaCp-1 (176.09 U ml^{-1}).

4.4.7. Pectinase

Out of eight isolates tested, all except AcOu-1 exhibited pectinase activity. Significantly higher pectinase activity (19.85 U ml^{-1}) was recorded by the isolate BaBc-1 followed by *B. subtilis* (KAU isolate) with 13.74 U ml^{-1} (Fig. 7).

4.4.8. Lipase

Lipase activity of selected isolates was determined by titration method. Maximum activity of the enzyme (3.57 U ml^{-1}) was observed in *Bacillus subtilis* (KAU isolate) and the minimum activity (0.63 U ml^{-1}) in AcOu-1.

Based on the degradation efficiency of various organic substrates enzyme activity, three isolates obtained in the present investigation (BaBc-1, BaOu-1, AcOu-1) and three reference cultures (*B. subtilis*, *G. butleri* and *T. asperellum*) were selected for further experiments. Details of the isolates are given in Table 11.

4.5. Characterization of selected isolates

The eight isolates including five bacteria (BaBc-1, BaCp-1, BaOu-1, *B. subtilis* and *B. niabensis*), one actinomycete (AcOu-1) and two fungi (*G. butleri* and *T. asperellum*) were selected for further characterization using cultural, morphological and biochemical tests.

4.5.1. Cultural characterization of selected isolates

Colony characters (size, form, elevation, margin and colour) of selected bacteria, actinomycete and fungi were recorded on nutrient agar, Kenknight's and Munaier's agar agar and PDA respectively.

4.5.1.1. Colony characters of bacterial isolates

All the colonies appeared creamy white, except *B. niabensis* which was yellowish white. All bacterial isolates produced large circular colonies. Surface of colonies was flat, with lobate margin (Table 12). The actinomycete colony on Kenknight and Munaier's agar media was small, white and circular, with entire margin and raised elevation.

4.5.1.2. Colony characters of fungal isolates

The colony of *G. butleri* appeared as white fluffy growth. *T. asperellum* produced sparse, white mycelia initially and after four days of incubation it turned light green due to sporulation (Table 13).

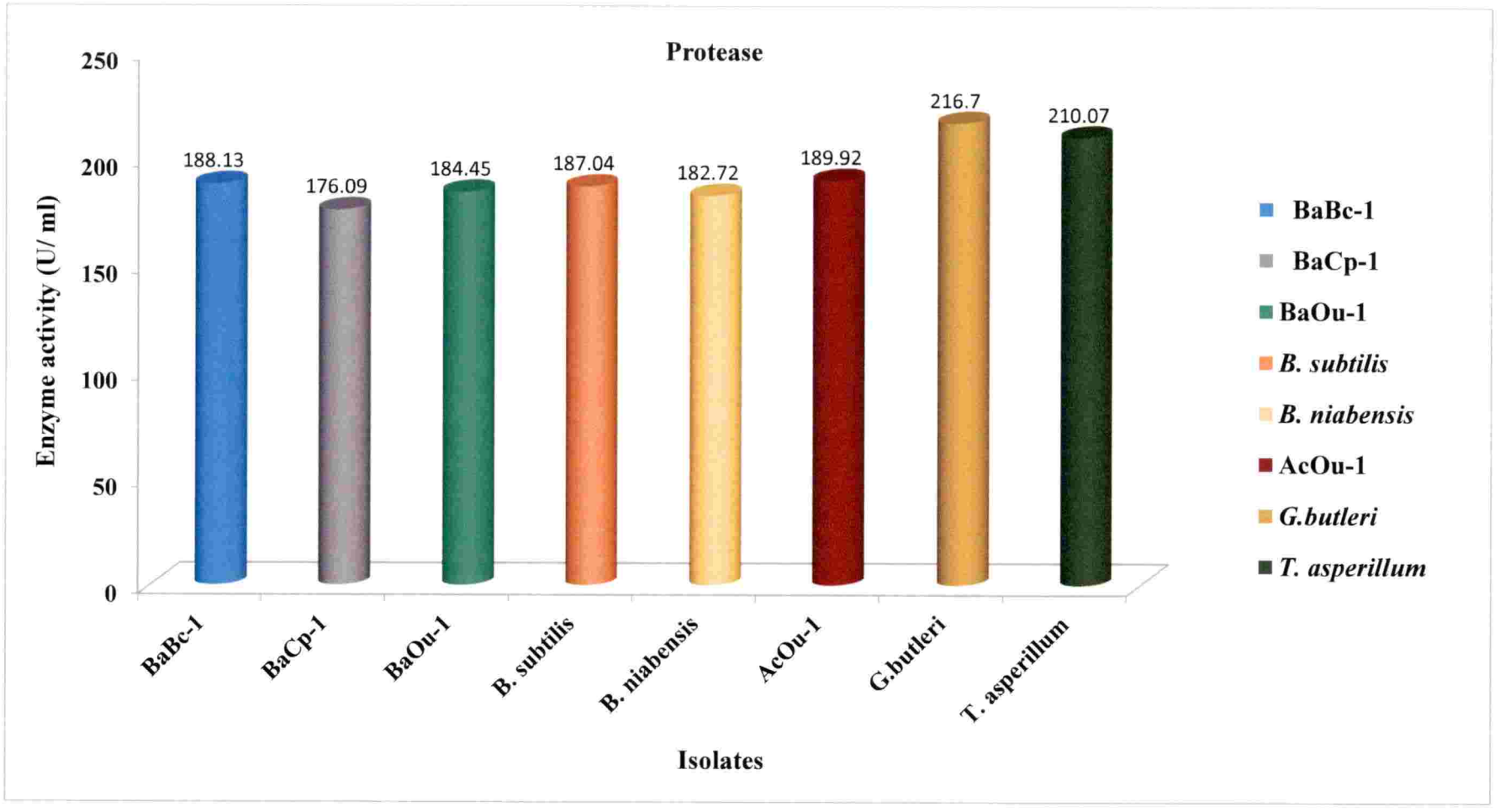


Fig. 6. Protease activity of selected isolates

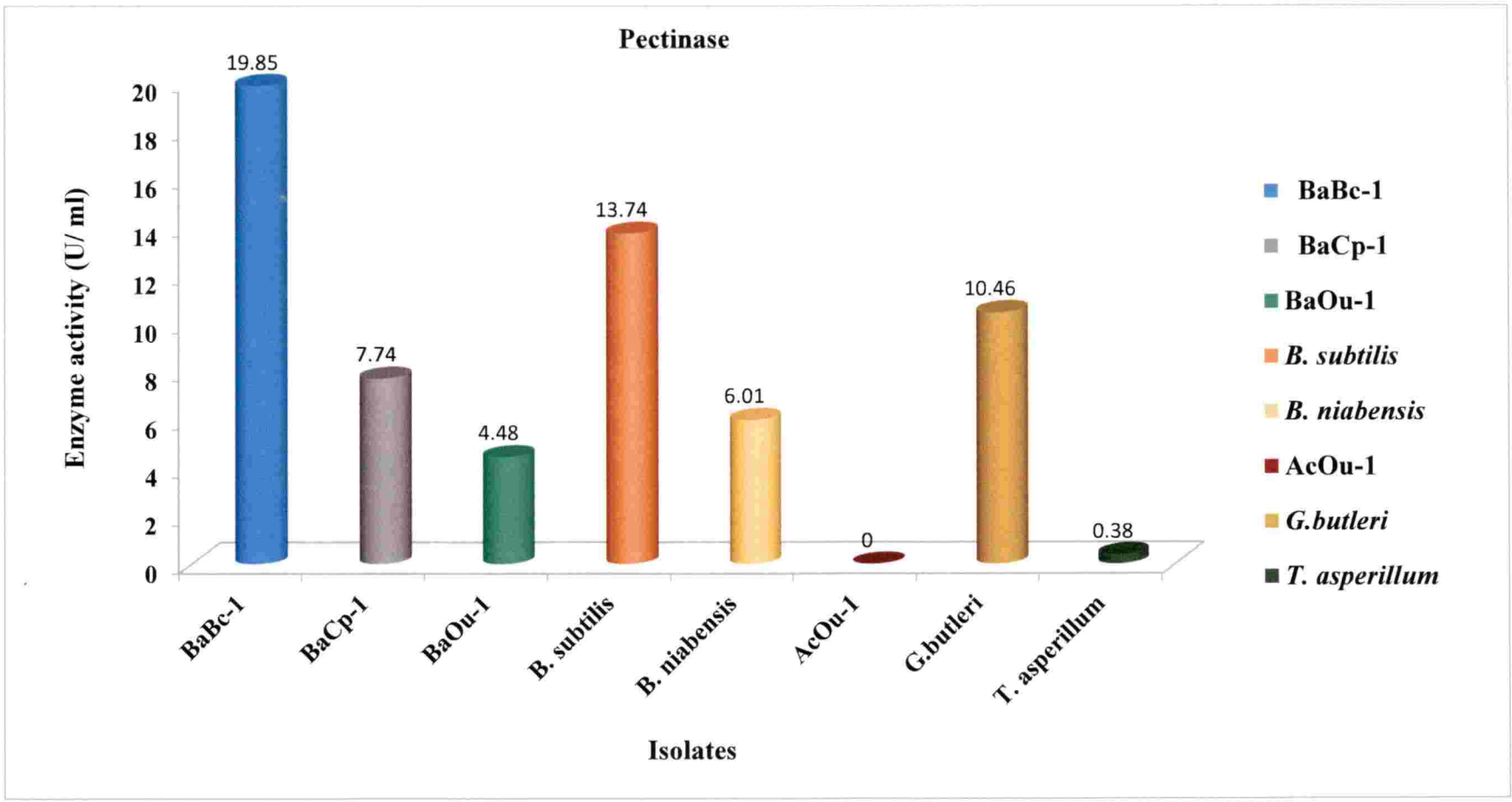


Fig. 7. Pectinase activity of selected isolates

4.6. Morphological characterization

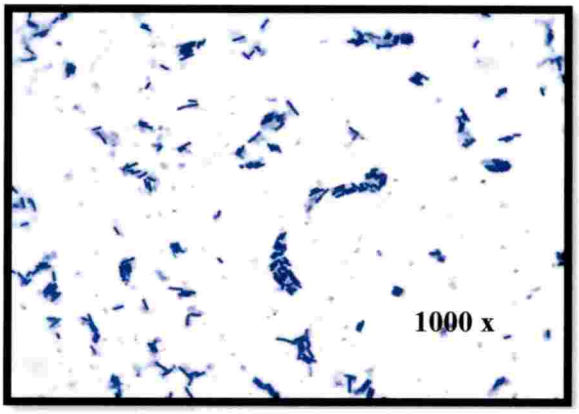
The morphological characters of all eight isolates were studied under *in vitro* conditions by Gram staining for bacteria and actinomycete isolates (Plate 11) and lactophenol cotton blue staining for fungal isolates (Plate 12).

4.6.1. Gram staining

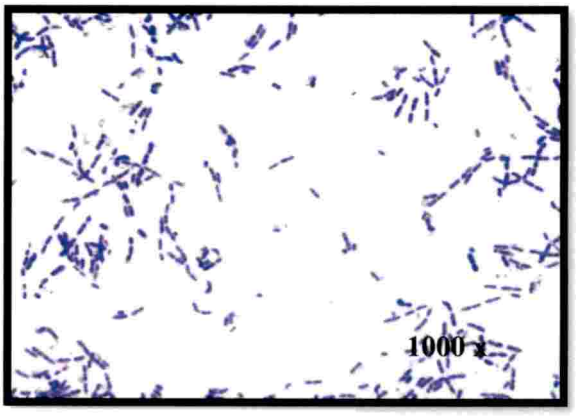
When the Gram stained cultures were viewed under microscope, isolates showed purple colour, indicating they were Gram positive, with rod shaped cells arranged singly and in chains (Table 14).

4.6.2. Endospore staining

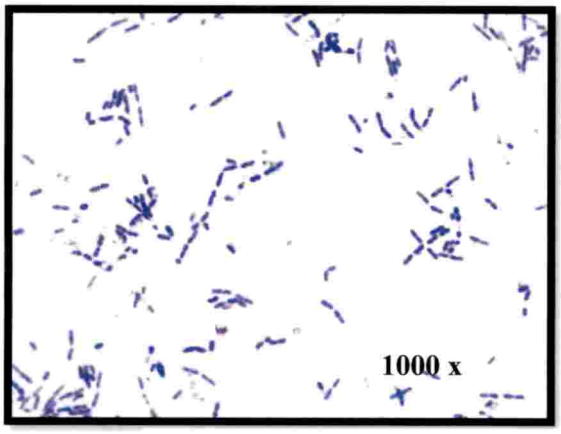
Endospore staining revealed the presence of endospores in all the bacterial isolates (Table 14). Vegetative cells of all the isolates appeared pink in colour and endospores appeared green in colour.



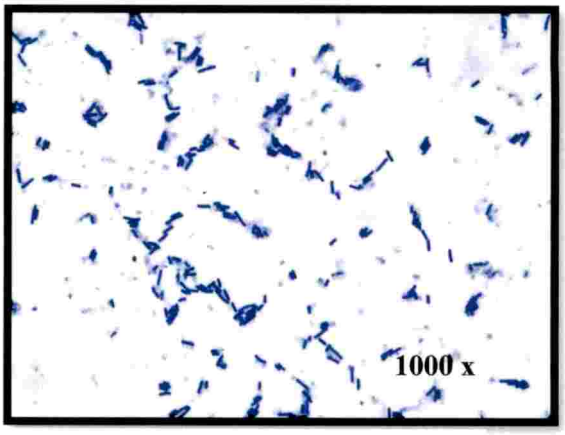
BaBc-1



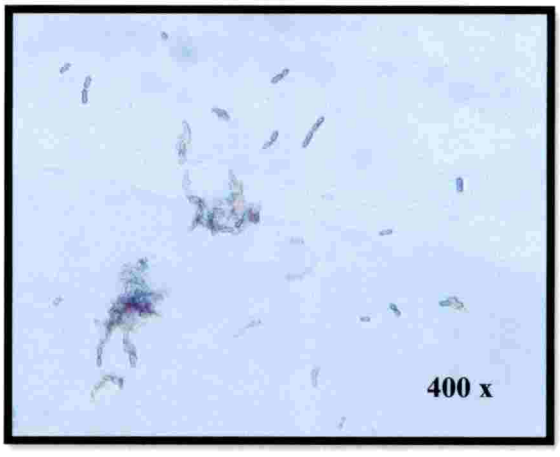
BaCp-1



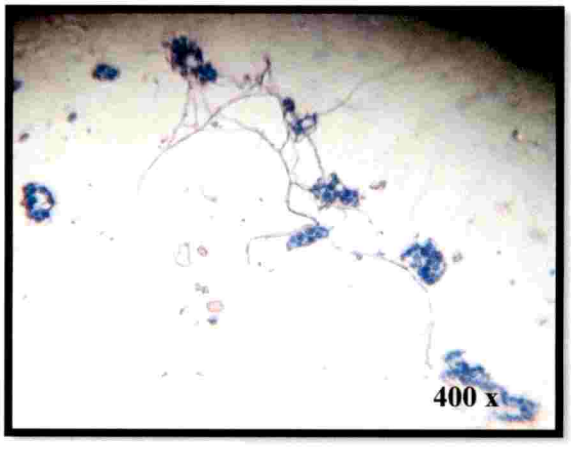
BaOu-1



B. subtilis (KAU isolate)

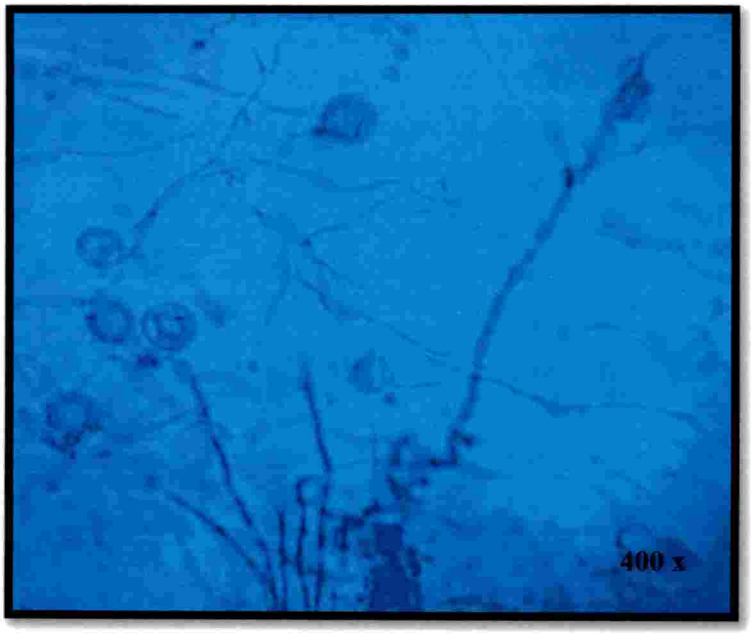


B. niabensis (KAU isolate)

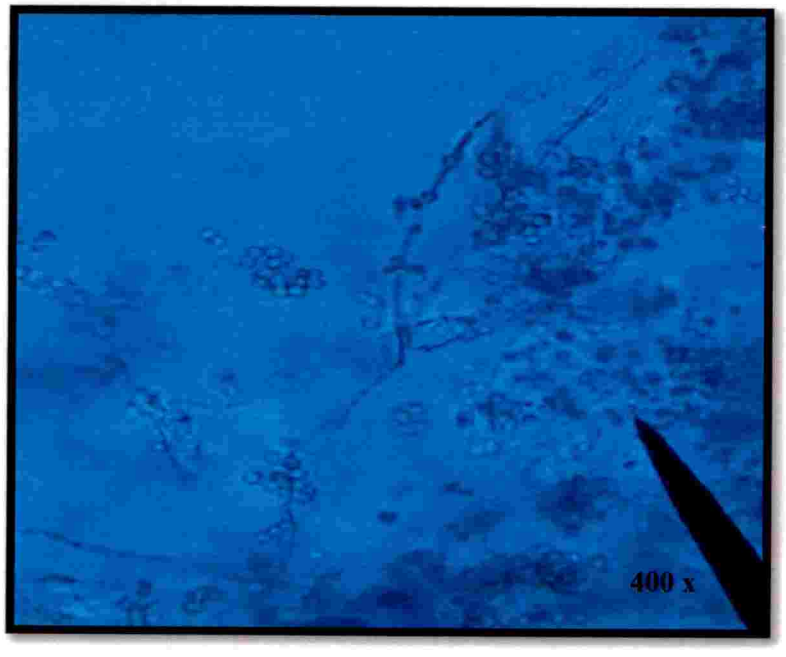


AcOu-1

Plate 11: Microscopic view of selected bacterial isolates



G. butleri (KAU isolate)



T. asperellum (KAU isolate)

Plate 12: Microscopic view of selected fungal isolates

Table 10. Enzyme activity of selected isolates

Isolates	*Enzyme activity (U ml^{-1})							
	Cellulase	β-1,3 glucanase	β-glucosidase	Laccase	Amylase	Protease	Pectinase	Lipase
BaBc-1	0.87^{b}	22.90^{a}	0.28^{ab}	1.57^{c}	0.10^{d}	188.13^{b}	19.85^{a}	2.67
BaCp-1	0.56^{cd}	17.13^{cd}	0.19^{b}	1.85^{c}	0.16^{c}	176.09^{b}	7.74^{cd}	1.63
BaOu-1	0.12^{f}	20.97^{ab}	0.21^{b}	6.33^{a}	0.22^{b}	184.45^{b}	4.48^{d}	2.40
B. subtilis (KAU isolate)	0.38^{e}	22.24^{a}	0.27^{ab}	1.73^{c}	0.05^{f}	187.04^{b}	13.74^{b}	3.57
B. niabensis (KAU isolate)	0.47^{de}	19.59^{b}	0.17^{b}	1.65^{c}	0.15^{c}	182.72^{b}	6.01^{d}	2.00
AcOu-1	0.64^{c}	19.33^{b}	0.20^{b}	1.69^{c}	0.25^{a}	189.92^{b}	0	0.63
G. butleri (KAU isolate)	1.20^{a}	17.03^{d}	0.42^{a}	4.85^{b}	0.09^{e}	216.70^{a}	10.46^{c}	0.97
T. asperellum (KAU isolate)	0.68^{c}	19.09^{bc}	0.39^{a}	3.87^{b}	0.10^{de}	210.07^{a}	0.38^{e}	2.37
CD (0.05)	0.161	1.973	0.177	0.306	0.010	17.674	3.262	NS

*Mean of three replications

The values followed by same letter in each column do not differ significantly according to DMRT

Table 11. Details of isolates selected for cultural, morphological and biochemical characterization

Sl. No	Isolates	Enzymes produced by the isolates
1	BaBc- 1	β-1,3 glucanase β-glucosidase Pectinase Lipase
2	BaOu-1	β-1,3 glucanase Laccase Lipase
3	*B. subtilis* (KAU isolate)	β-1,3 glucanase β-glucosidase Lipase
4	AcOu-1	Amylase Lipase
5	*G. butleri* (KAU isolate)	Cellulase β-glucosidase Protease Lipase
6	*T. asperellum* (KAU isolate)	β-glucosidase Protease Lipase

Table 12. Colony characters of selected bacterial isolates

Isolates	Colony size	Colony form	Colony elevation	Colony margin	Colony colour
BaBc-1	Large	Circular	Flat	Lobate	Creamy white
BaCp-1	Large	Circular	Flat	Lobate	Creamy white
BaOu1	Large	Circular	Flat	Lobate	Creamy white
B. subtilis (KAU isolate)	Large	Circular	Flat	Lobate	Creamy white
B. niabensis (KAU isolate)	Large	Circular	Flat	Lobate	Yellowish white
AcOu-1	Small	Circular	Raised	Entire	White

Small: colony diameter <0.2 mm, Medium: colony diameter 0.2 mm-0.4 mm, Large: colony diameter 0.5 mm-0.7 mm

Table 13. Colony characters of selected fungal isolates

Isolates	Colony characters			Sporulation
	Colour	Elevation	Margin	
G. butleri (KAU isolate)	White mycelium	Raised	Circular	White spores
T. asperellum (KAU isolate)	White mycelium	Raised	Circular	Light green sporulation

Table 14. Morphological characterization of selected bacterial isolates

Isolates	Gram reaction	Cell shape	Presence/ absence of endospore
BaBc-1	Positive	Rod	Present
BaCp-1	Positive	Rod	Present
BaOu-1	Positive	Rod	Present
B. subtilis (KAU isolate)	Positive	Rod	Present
B. niabensis (KAU isolate)	Positive	Rod	Present
AcOu-1	Positive	Filamentous	Absent

4.7. Biochemical characterization of selected bacterial isolates

Selected bacterial isolates were subjected to biochemical tests and the results of biochemical characterization are presented in Table 15. All the isolates showed positive reaction for Voges-Proskauer test, catalase and citrate utilization test. For indole production and methyl red test, all the isolates showed negative reaction.

Table 15. Biochemical characters of selected bacterial isolates

Biochemical test	BaBc-1	BaCp-1	BaOu-1	*B. subtilis*	*B. niabensis*	AcOu-1
Indoleproduction	-	-	-	-	-	-
Methyl red test	-	-	-	-	-	-
Voges-Proskauer	+	+	+	+	+	+
Catalase	+	+	+	+	+	+
Citrate utilization	+	+	+	+	+	+

\+ Positive - Negative

4.8. Molecular characterization

The molecular characterization of selected bacterial isolates and actinomycete were carried out by 16S rRNA sequencing. PCR amplified products on one per cent gel appeared as crisp bands of 1500bp size (Plate 13). Nucleotide sequences obtained from Agrigenome, Kochi were used for homology search using BLASTn (Plates 14, 15, 16 and 17). The sequence analysis of the isolate BaBc-1 showed 98 per cent query coverage and 99 per cent identity to *Bacillus subtilis* strain JK1216S (NCBI accession No. KF135464.1).

The isolate BaCp-1 showed 96 per cent query coverage and 98 per cent identity with *Bacillus cereus* strain APK (NCBI accession No. JF938537.1). The sequence analysis of the isolate BaOu-1 showed 96 per cent query coverage and 98 per cent identity to *Bacillus* sp. strain PVR16 (NCBI accession No. KF648911.1). The isolate AcOu-1 showed 98 per cent query coverage and 98 per cent identity to *Streptomyces roseofulvus* strain FMR2 (NCBI accession No. KF254735.1).

Based on 16S rRNA gene sequencing, the isolates were tentatively identified and the sequences deposited in NCBI databank, as BaBc-1: *Bacillus subtilis* (NCBI accession No. MK254686), BaCp-1: *Bacillus cereus* (NCBI accession

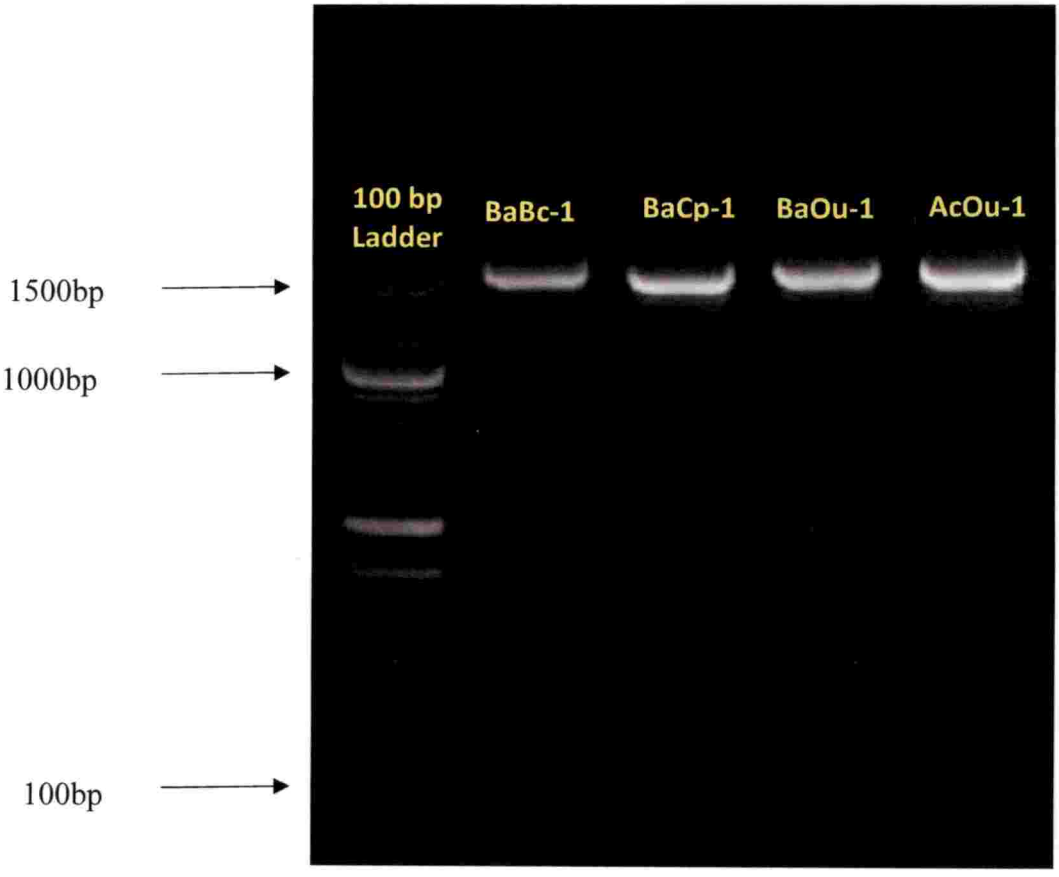


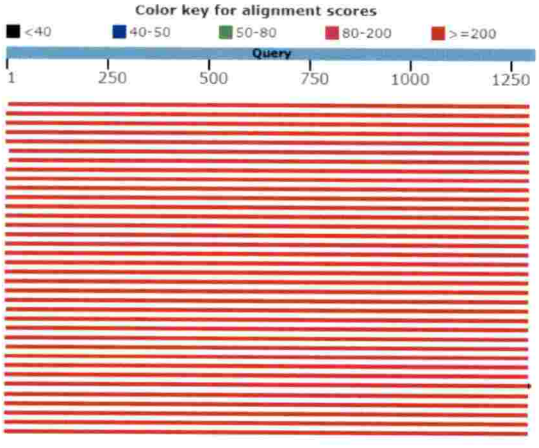
Plate 13: PCR Amplification of 16S rRNA gene of selected isolates

AGGGGACTTGCTCCTGATGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCG
GGAAACCGGGGCTAATACCGGATGGTTGTTTGAACCGCATGGTTCAAACATAAAAGGTGGCTTCGGCTACCACTTACAG
ATGGACCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTG
ATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAA
GTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTACCGT
TCGAATAGGGCGGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAG
GTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCT
CAACCGGGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAAAT
GCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGACGCTGAGGAGCGAAAGCGTGGG
GAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAG
TGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCC
CGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAATCC
TAGAGATAGGACGTCCCCTTCGGGGGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGG
TTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCATTCAGTTGGGCACTCTAAGGTGACTGCCGGTGACA
AACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACAGA
ACAAAGGGCAGCGAAACCGCGAGGTTAAGCCAATCCCACAAATCTGTTCTCAGTTCGGATCGCAGTCTGCAACTCGACT
GCGTGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCG
TCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGAGGTAACCTCTAGAGCAGCCGCCGAAA

Merged sequence

Description	Max. score	Query coverage (%)	Accession	Identity (%)	E value
Bacillus subtilis strain JK1216S	2289	98	KF135464.1	99	0.0
Bacillus subtilis strain SASCBT01	2287	98	KY921596.1	99	0.0
Bacillus subtilis strain MML5327	2287	98	MF687954.1	99	0.0
Bacillus subtilis strain NAB37	2287	98	MF663661.1	99	0.0

Sequences showing homology



BLASTn output

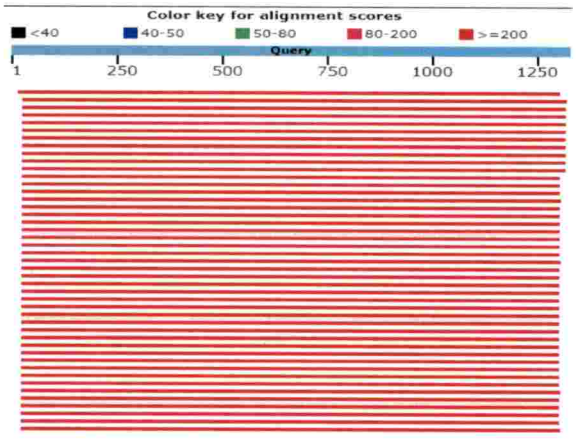
Plate 14: 16S rRNA sequence analysis of BaBc-1

TGCTTCTTATTGGAAGTTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAAC
CGGGGCTAATACCGGATAACATTTTGAACCGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGATGGACCCGC
GTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGG
GACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCC
GCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGAC
GGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATT
GGGCGTAAAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGG
AGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGGAACACCAGTGGCGA
AGGCGACTTTCTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGC
CGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGAGTA
CGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGA
AGAACCTTACCAGGTCTTGACATCCTCTGACAACCCTAGAGATAGGGCTTCTCCTTCGGGAGCAGAGTGACAGGTGGTGCATG
GTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCATCATTTAGTTG
GGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCT
ACACACGTGCTACAATGGACGGTACAAAGAGCTGCAAGACCGCGAGGTGGAGCTAATCTCATAAAACCGTTCTCAGTTCGGA
TTGTAGGCTGCAACTCGCCTACATGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCC
TTTTGATACCACATCCCGCCCAAT

Merged sequence

Description	Max. score	Query coverage (%)	Accession	Identity (%)	E value
Bacillus cereus strain APK	2250	96	JF938537.1	98	0.0
Bacillus cereus strain SJ 27	2248	97	KF731614.1	98	0.0
Bacillus sp. L1 (2016)	2244	97	KU500623.1	98	0.0
Bacillus sp. QS1	2244	97	KT783531.1	98	0.0

Sequences showing homology



BLASTn output

Plate 15: 16S rRNA sequence analysis of BaCp-1

GTGCTATTGCGCAGCTATAATGCAGTCGAGCGAATGGATTAAGAGCTTGCTCTTATGAAGTTAGCGGCGGAC
GGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGAT
AACATTTTGAACCGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGATGGACCCGCGTCGCA
TTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCAC
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CTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAG
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GCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTTTCTTAAGT
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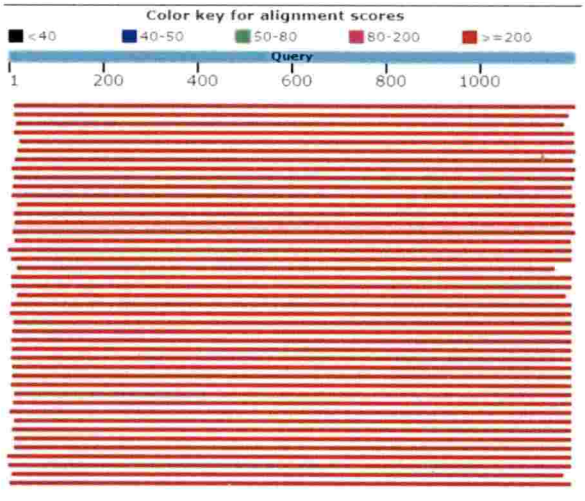
GTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCACTCCGC
CTGGGGGAGTACGGCCGCAAGGCTGAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGT
GGTTTAATTCGAAGCAACGCGAAGACCCTTACCAGGTCTTGACATCCTCTGACACCCTAGAGATAGGGCTTC
TCCTTTCGGAGCAGAGTGACAGTGTGCATCTGTCGTCAGCTCGTGTCGTGAGATGTGGTAAGTCCCCGCAAC
GAGCGCCATCGTGATCTAGTGCCATCATTAGTTGGGCACTCTAGGTGACTGCGGTGACCACGAGAGGTGGG
ATGACGTCATCATCATGCTCTATGACTGGCTACCACGGTGCTCACATTGAAC

Merged sequence

Description	Max. score	Query coverage (%)	Accession	Identity (%)	E value
Bacillus sp. strain PVR16	1991	96	KF648911.1	98	0.0
Uncultured *Bacillus* sp. Clone W2	1995	97	FJ863099.1	97	0.0
Bacillus sp. Strain TA1-204	2006	98	KY476269.1	97	0.0
Bacillus sp. Strain TC2-30	1978	98	KY673671.1	97	0.0

Sequences showing homology



BLASTn output

Plate 16: 16S rRNA sequence analysis of BaOu-1

TGCAAGTCGAACGATGAAGCCCTTCGGGGTGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTTCACTCTGGGACAAGC
CCTGGAAACGGGGTCTAATACCGGATACGAGACGGGGAGGCATCTCCTCGTCTGGAAAGCTCCGGCGGTGAAGGATGAGCCCGCGGCCT
ATCAGCTTGTTGGTGGGGTAATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACG
GCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCT
TCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCG
GTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGGTGTGAAAGCCCGGGGCT
TAACCCCGGGTCTGCATCCGATACGGGCAGGCTAGAGTGTGGTAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCA
GGAGGAACACCGGTGGCGAAGGCGGATCTCTGGGCCATTACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCT
GGTAGTCCACGCCGTAAACGTTGGGAACTAGGTGTTGGCGACATTCCACGTCGTCGGTGCCGCAGCTAACGCATTAAGTTCCCCGCCTGGG
GAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCAGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAG
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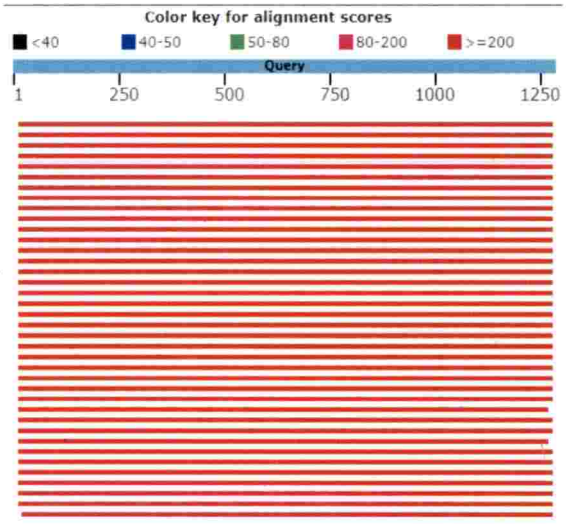
CTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCTGTGTTGCCAGCATGCCCTTCGGGGTGATGGGGACTCAC
AGGAGACCGCCGGGGTCAACTCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTACAATG
GCCGGTACAAAGAGCTGCGATGCCGTGAGGCGGAGCGAATCTCAAAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATG
AAGTCGGAGTTGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACGTCACGAAAGTC
GGTAACACCCGAAGCCGGTGGCCCCTTGTGGGAGGGAGCTGT

Merged sequence

Description	Max. score	Query coverage (%)	Accession	Identity (%)	E value
Streptomyces roseofulvus strain FMR2	2198	98	KF011477.1	98	0.0
Streptomyces sp. X7-8	2198	98	KT581326.1	98	0.0
Streptomyces roseofulvus strain 125 (br9)	2198	98	KF254735.1	98	0.0
Streptomyces roseofulvus strain NBRC 15816	2198	98	NR 112579.1	98	0.0

Sequences showing homology



BLASTn output

Plate 17: 16S rRNA sequence analysis of AcOu-1

No.MK254688), BaOu-1: *Bacillus* sp. (NCBI accession No. MK253288) and the actinomycete isolate AcOu-1: *Streptomyces roseofulvus* (NCBI accession No. MK254687). This was also in conformity with cultural, morphological and biochemical characterization.

Evolutionary analysis of BaBc-1, BaCp-1, BaOu-1 and AcOu-1 was conducted by constructing the phylogenetic trees (Plates 18, 19, 20 and 21). The bacterial isolate *B. subtilis* strain BaBc-1 was clustered together with *Bacillus subtilis* strain JK1216S (Accession No. KF135464.1) with 74 per cent bootstrap confidence value. *B. cereus* strain BaCp-1 was closely related to *Bacillus cereus* strain APK (Accession No. JF938537.1) with a bootstrap confidence value of 100 per cent and *Bacillus* sp. strain BaOu-1 was related to *Bacillus* sp. PVR16 (Accession No. KF648911.1) with a bootstrap confidence value of 86 per cent. *Streptomyces roseofulvus* strain AcOu-1 shared close affinity with *Streptomyces roseofulvus* strain FMR2 (Accession No. KF254735.1) and other four *Streptomyces roseofulvus* strains and one *Streptomyces* sp. (bootstrap confidence value of 98 per cent).

4.9. Compatibility of selected isolates

The mutual compatibility between selected isolates was tested by cross streak or dual culture method, as indicated in 3.2.10 under Materials and Methods. The results of compatibility test are given in Table 16 and Plate 22-23.

4.9.1. Compatibility of selected bacterial isolates

The compatibility test between bacterial isolates revealed that, *B. subtilis* BaBc-1 and *Bacillus* sp. BaOu-1 were compatible with each other, as no inhibition zone was observed. No inhibition was observed when *B. subtilis* BaBc-1 was cross streaked with *B. subtilis*. Inhibition was observed at the juncture of the isolates *Bacillus* sp. BaOu-1 and *B. subtilis*, which indicated the incompatibility of these two isolates. The growth of *B. subtilis* was inhibited by *Bacillus* sp. BaOu-1.

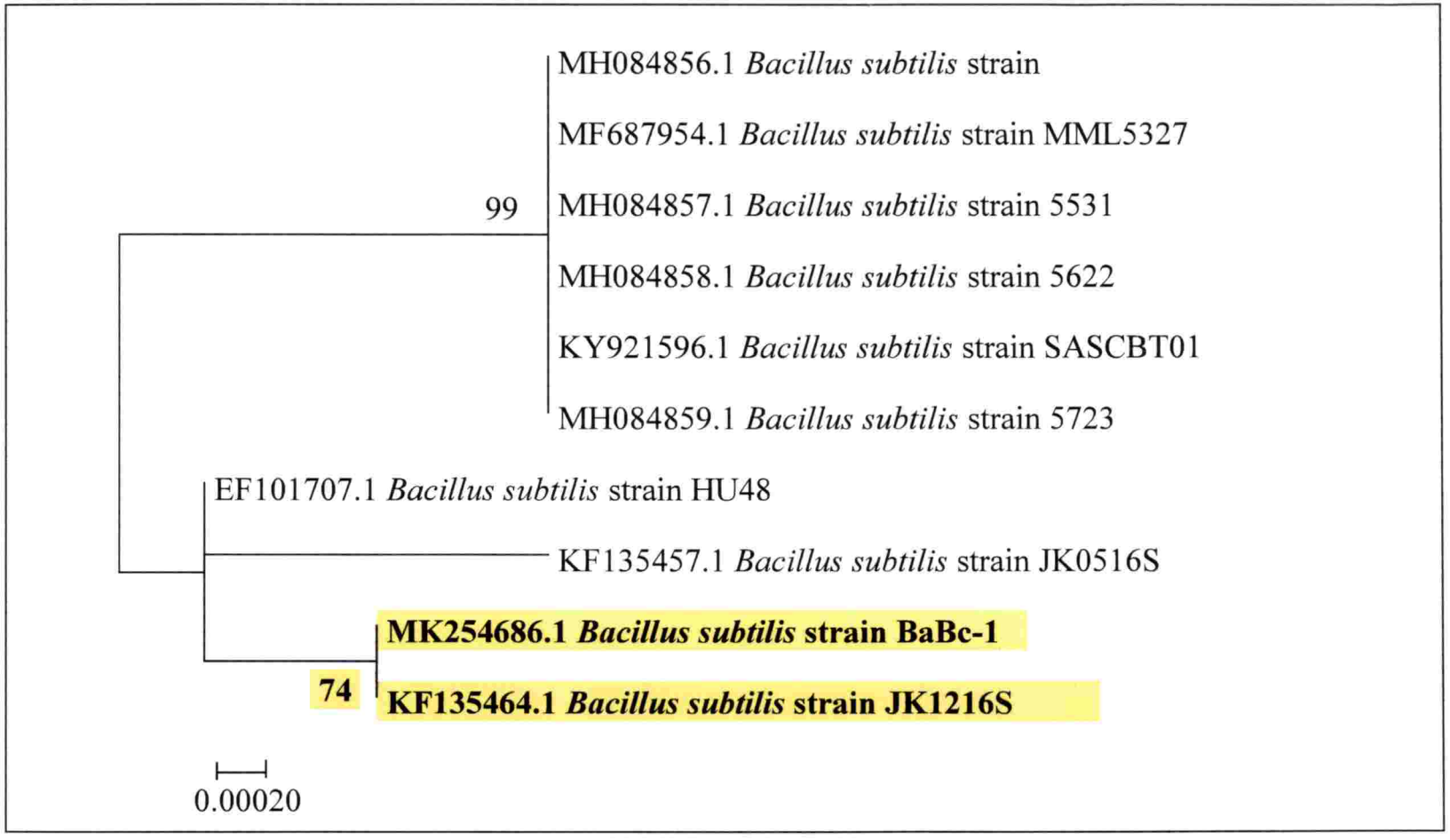


Plate 18: Phylogenetic tree of *B. subtilis* strain BaBc-1 with other members of *B. subtilis* on the basis of 16S rRNA gene sequences

Isolate with maximum bootstrap confidence value is highlighted

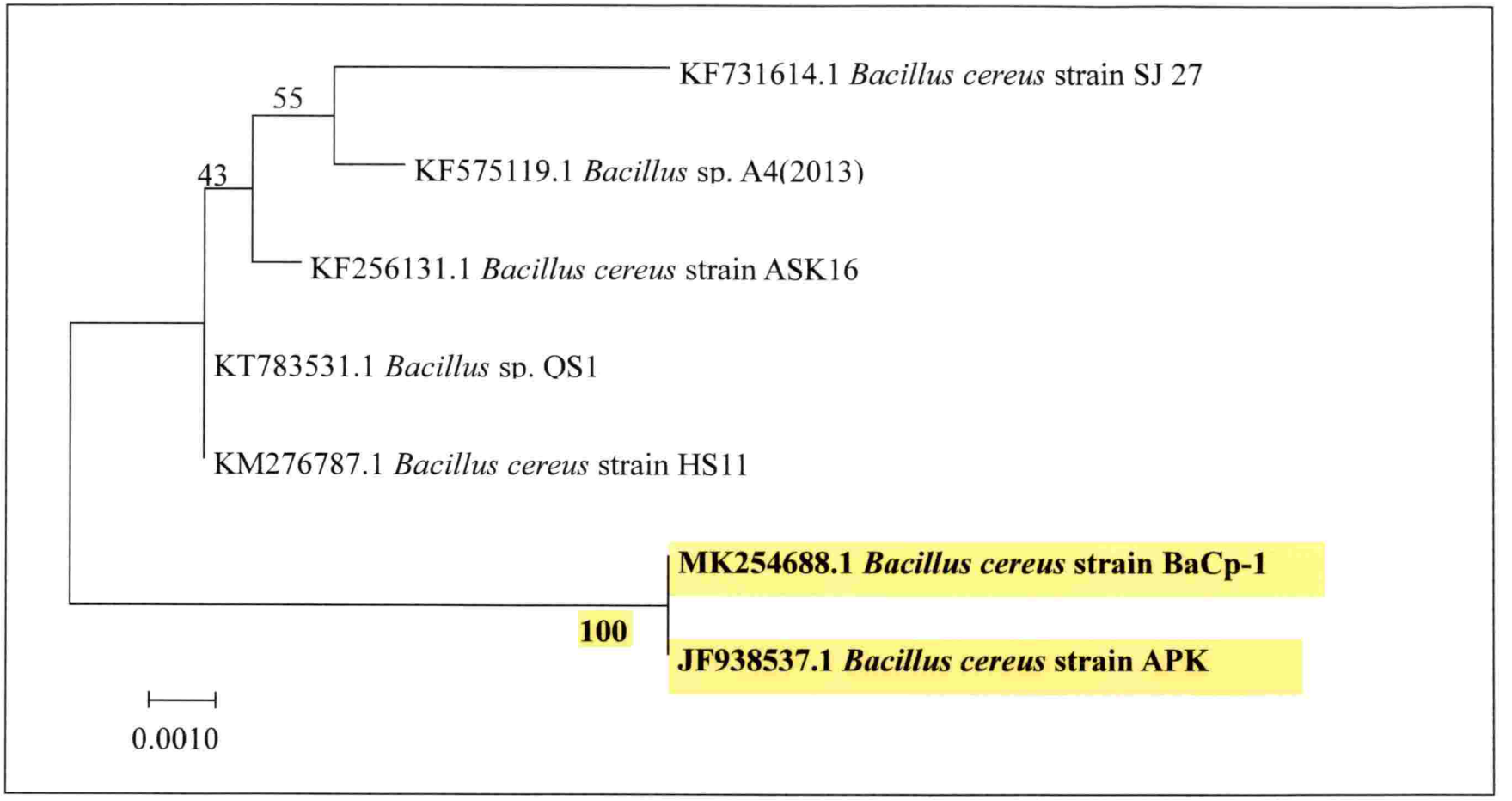


Plate 19: Phylogenetic tree of *B. cereus* strain BaCp-1 with other members of *Bacillus* on the basis of 16S rRNA gene sequences

Isolate with maximum bootstrap confidence value is highlighted

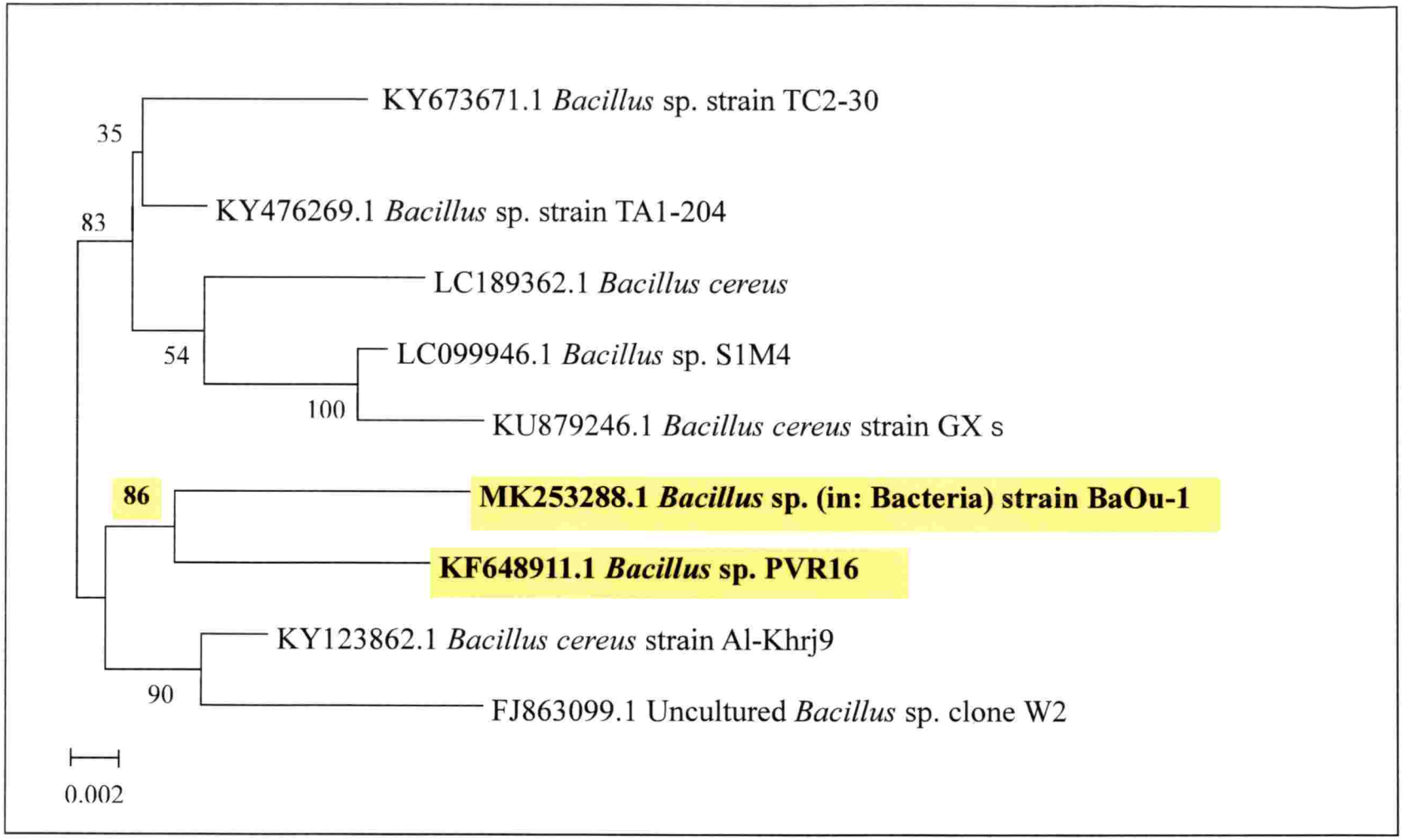


Plate 20: Phylogenetic tree of *Bacillus* sp. strain BaOu-1 with other members of *Bacillus* on the basis of 16S rRNA gene sequences

Isolate with maximum bootstrap confidence value is highlighted

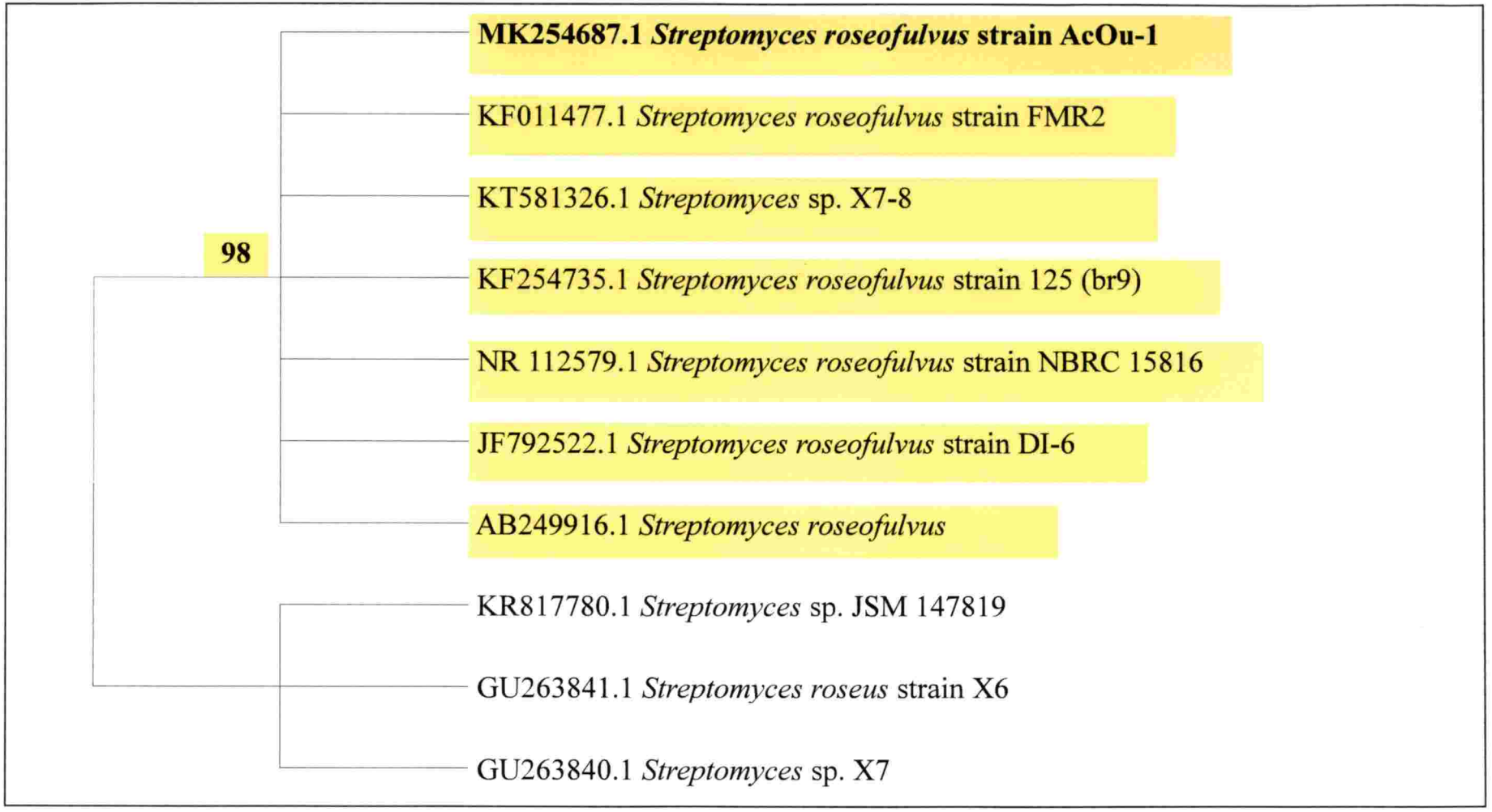


Plate 21: Phylogenetic tree of *Streptomyces roseofulvus* strain AcOu-1 with other members of *Streptomyces* on the basis of 16S rRNA gene sequences

Isolate with maximum bootstrap confidence value is highlighted

4.9.2. Compatibility of bacteria with actinomycete

Three selected bacterial isolates were cross streaked with *S. roseofulvus* AcOu-1 separately. Results showed that *S. roseofulvus* AcOu-1 was compatible with *Bacillus* sp. BaOu-1 and *Bacillus subtilis*. Incompatibility was noticed between *B. subtilis* BaBc-1 as inhibition was observed at the juncture. The bacterial isolate *Bacillus subtilis* inhibited the growth of actinomycete AcOu-1.

4.9.3. Compatibility between fungi and bacteria/actinomycete

All the bacterial isolates including actinomycete showed compatibility with *G. butleri* and *T. asperellum*.

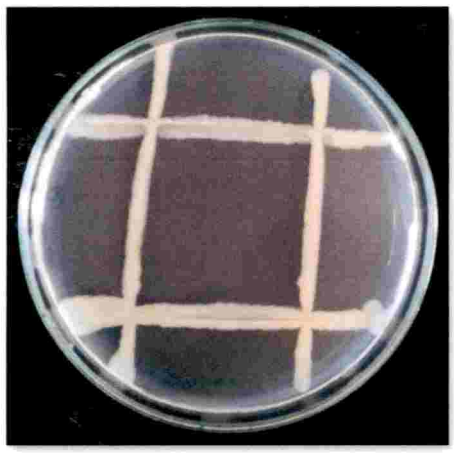
4.9.4. Compatibility between fungal isolates

Compatibility reaction between *G. butleri* and *T. asperellum* showed that, *T. asperellum* inhibited the growth of *G. butleri*. The per cent inhibition of *G. butleri* was 50 per cent (Table 17).

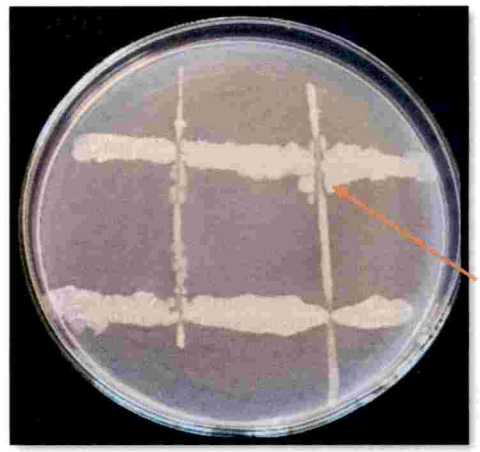
Table 16. Compatibility of selected isolates

Isolates	*B. subtilis* BaBc-1	*Bacillus* sp. BaOu-1	*B. subtilis* (KAU isolate)	*S. roseofulvus* AcOu-1	*G. butleri* (KAU isolate)	*T. asperellum* (KAU isolate)
B. subtilis BaBc-1		+	+	-	+	+
Bacillus sp. BaOu-1	+		-	+	+	+
B. subtilis (KAU isolate)	+	-		+	+	+
S. roseofulvus AcOu-1	-	+	+		+	+
G. butleri (KAU isolate)	+	+	+	+		-
T. asperellum (KAU isolate)	+	+	+	+	-	

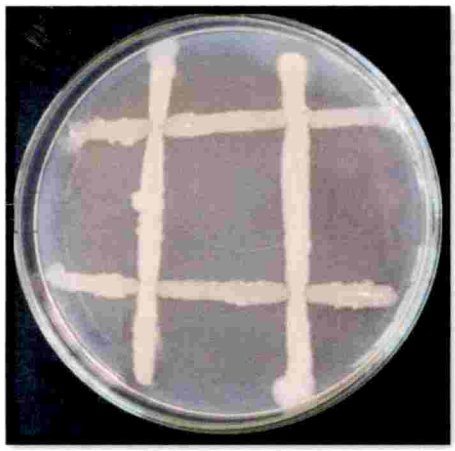
+ Compatible, - Incompatible



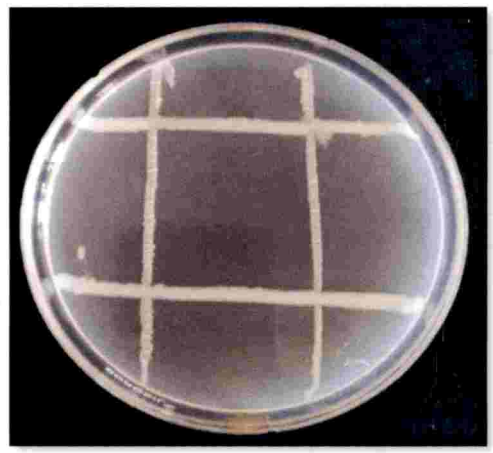
BaBc-1 & BaOu-1



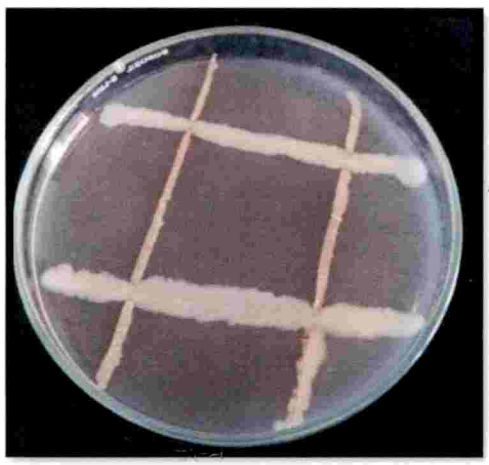
BaOu-1 & *B. subtilis*



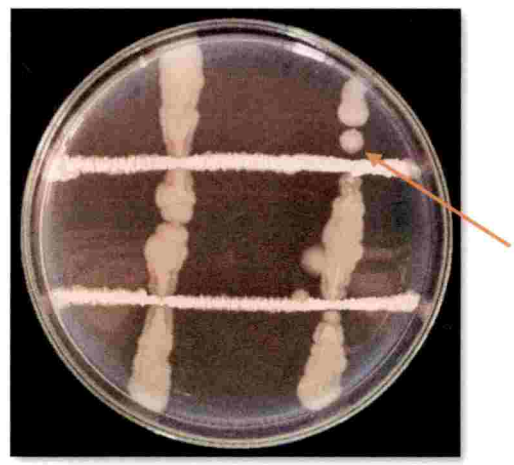
BaBc-1 & *B. subtilis*



BaOu-1 & AcOu-1



B. subtilis & AcOu-1



BaBc-1 & AcOu-1

Plate 22: Compatibility of selected bacterial isolates

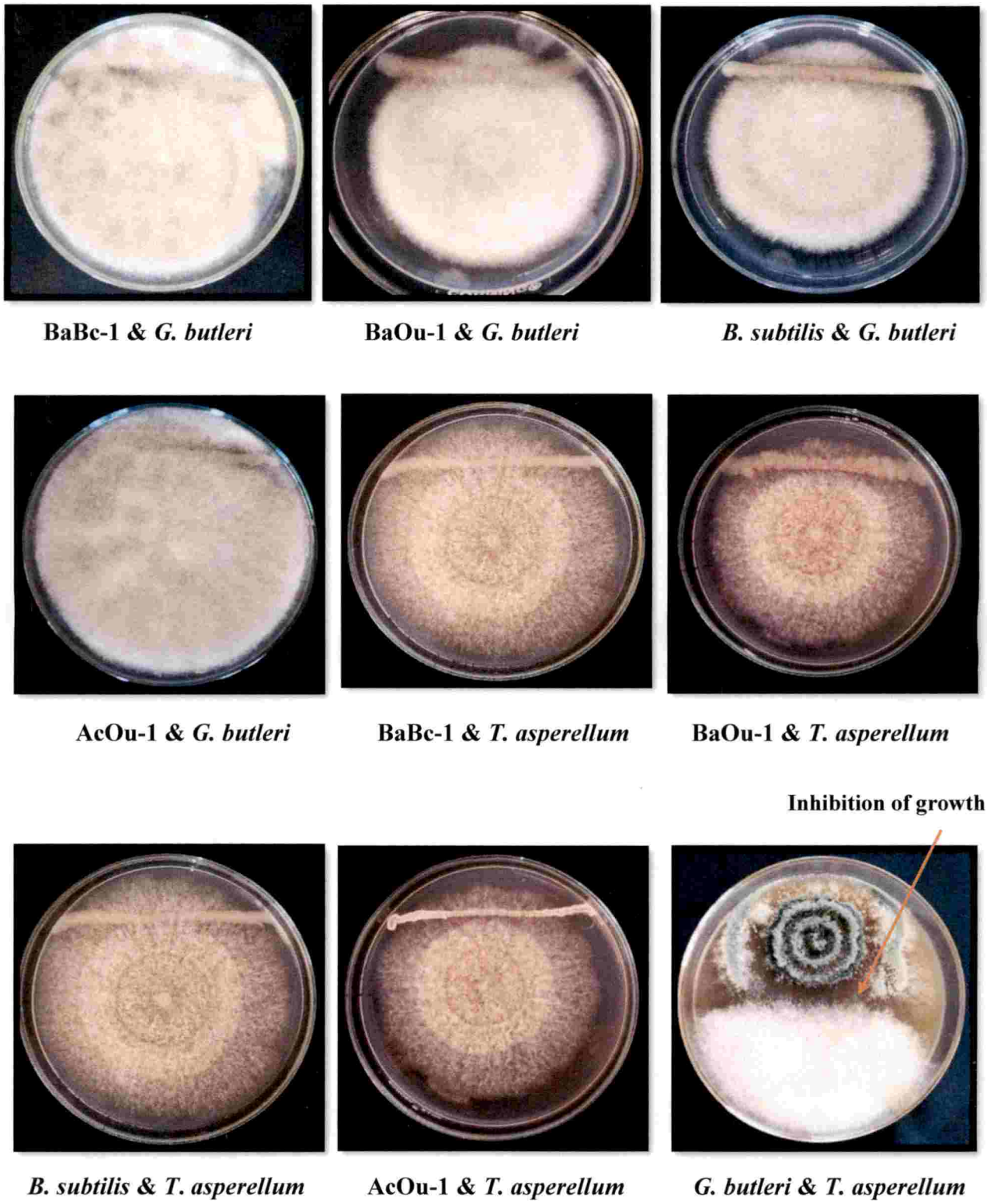


Plate 23: Compatibility of selected fungal isolates

Table 17. Per cent inhibition of the growth of *G. butleri* by *T. asperellum*

Isolate	Colony growth in diameter (cm)		Inhibition (Per cent)
	Control	Treatment	
G. butleri	9	4.5	50

4.10. Selection of microbial isolates for consortial formulation

Based on the enzyme activity and compatibility, suitable combinations of six potential isolates including three isolates, *B. subtilis* BaBc-1, *Bacillus* sp. BaOu-1and *S. roseofulvus* AcOu-1and three reference cultures, *B. subtilis*, *G. butleri* and *T. asperellum* were selected for different microbial consortium (Table 18).

Table 18. Details of consortial formulations of selected microorganisms

Consortium	Combination of microbial isolates
I	*B. subtilis* (KAU isolate) *G. butleri* (KAU isolate) *S. roseofulvus* AcOu-1
II	*B. subtilis* BaBc-1 *Bacillus* sp. BaOu-1 *T. asperellum* (KAU isolate)
III	*B. subtilis* BaBc-1 *Bacillus* sp. BaOu-1 *G. butleri* (KAU isolate)
IV	*B. subtilis* (KAU isolate) *B. subtilis* BaBc-1 *G. butleri* (KAU isolate)

4.11. *In vitro* evaluation of efficiency of selected consortia

Consortium I, consortium II, consortium III, consortium IV and uninoculated control were T_1, T_2, T_3, T_4 and T_5 respectively. All the consortia were evaluated for their efficacy to degrade the biosolid waste under *in vitro* condition for three weeks.

4.11.1. Visual observation on biosolid waste degradation

In treatments with microbial consortia, degradation was initiated on 3rd day, as indicated by formation of slurry. Initiation of degradation was noticed in uninoculated control only after six days. Seventy five per cent degradation was observed in all the inoculated treatments, on 17th day after inoculation, whereas in uninoculated control, only 50 per cent degradation was observed. Visual observation on degradation of biosolid waste proved that, a period of 21 days of decomposition resulted in complete degradation of biosolid waste in T_2 (*B. subtilis* BaBc-1 + *T. asperellum* + *Bacillus* sp. BaOu-1) and T_4 (*B. subtilis*+ *G. butleri* + *B. subtilis* BaBc-1). In the uninoculated control, only 75 per cent degradation was noticed even after 21 days. Data on biosolid waste degradation in flask culture are presented in Table 19 and Plate 24.

4.11.2. Effect of microbial consortia on weight reduction of biosolid waste

Results of per cent weight reduction 21 days after inoculation showed that, there was no significant difference among treatments. However, maximum weight reduction was noticed in T_2 (*B. subtilis* BaBc-1 + *T. asperellum* + *Bacillus* sp. BaOu-1) with 10 per cent reduction and T_4 (*B. subtilis* + *G. butleri* + *B. subtilis* BaBc-1) with 10 per cent reduction. The minimum weight reduction (6.25 per cent) was found to be in uninoculated treatment. The effect of microbial consortia on weight reduction of biosolid waste is presented in Table 20.

T_1 T_2 T_3 T_4 T_5

1st DAI

T_1 T_2 T_3 T_4 T_5

6th DAI

T_1 T_2 T_3 T_4 T_5

21st DAI

T_1- *G. butleri* + *S. roseofulvus* AcOu-1 + *B. subtilis*
T_2- *B. subtilis* BaBc-1 + *T. asperellum* + *Bacillus* sp. BaOu-1
T_3- *B. subtilis* BaBc-1 + *G. butleri* + *Bacillus* sp. BaOu-1
T_4- *B. subtilis* + *G. butleri* + *B. subtilis* BaBc-1
T_5- Uninoculated control

Plate 24: *In vitro* degradation of vegetable waste

4.11.3. Enzyme activity of consortial formulations during degradation

After three weeks of degradation, all the treatments were subjected to enzyme assay in order to determine the activity of cellulase, amylase and protease (Table 21).

Table 19. Visual changes during biosolid waste degradation in flask culture

Treatments	Visual observation on biosolid waste degradation			
	3rd day	6th day	17th day	21st day
T_1 (*G. butleri* + *S. roseofulvus* AcOu-1 + *B. subtilis*)	Initiated	Degradation continues	75% Degraded	90% Degraded
T_2 (*B. subtilis* BaBc-1 + *T. asperellum* + *Bacillus* sp. BaOu-1)	Initiated	Degradation continues	75% Degraded	Completely degraded
T_3 (*B. subtilis* BaBc-1 + *G. butleri* + *Bacillus* sp. BaOu-1)	Initiated	Degradation continues	75% Degraded	90% degraded
T_4 (*B. subtilis* + *G. butleri* + *B. subtilis* BaBc-1)	Initiated	Degradation continues	75% Degraded	Completely Degraded
T_5 (Control)	Not initiated	Degradation initiated	50% Degraded	75% degraded

4.11.3.1. Cellulase

All the treatments exhibited cellulase activity, which ranged from 0.07 to 1.53 U ml^{-1}, T_4 (*B. subtilis* + *G. butleri* + *B. subtilis* BaBc-1) recorded significantly superior cellulase activity of 1.53 U ml^{-1} followed by T_2 (*B. subtilis* BaBc-1 + *T. asperellum* + *Bacillus* sp. BaOu-1) with 0.96 U ml^{-1}. Cellulase activity was minimum (0.07 U ml^{-1}) in T_5 (control).

4.11.3.2. Amylase

Amylase activity was detected in all the five treatments. Significantly higher production (5.33 U ml^{-1}) was observed in T_2 (*B. subtilis* BaBc-1 + *T. asperellum* + *Bacillus* sp. BaOu-1). This was on par with T_1 (*G. butleri* + *S. roseofulvus* AcOu-1 + *B. subtilis*), which recorded an activity of 4.88 U ml^{-1}. Significantly lower amylase production was recorded in uninoculated control, which was 1.77 U ml^{-1}.

4.11.3.3. Protease

All the treatments exhibited protease activity, which ranged from 36.976 to 107.50 U ml^{-1}. Significantly high protease activity of 107.50 U ml^{-1} was recorded in T_2 (*B. subtilis* BaBc-1 + *T. asperellum* + *Bacillus* sp. BaOu-1). Minimum protease activity was recorded in T_3 and T_5 (36.98 U ml^{-1} and 41.55 U ml^{-1} respectively), which were statistically on par.

4.11.3.4. Microbial population in biosolid waste inoculated with consortia

The microbial population of each treatment was estimated by serial dilution plating technique, on 21st day of composting.

The total number of bacteria was found to be higher as compared to the fungal isolates (Table 22), T_4 (*B. subtilis* + *G. butleri* + *B. subtilis* BaBc-1) recorded significantly high bacterial population (90 x 10^6 cfu g^{-1}) followed by T_2 (*B. subtilis* BaBc-1 + *T. asperellum* + *Bacillus* sp. BaOu-1) with a population of 49.33 x 10^6 cfu g^{-1}. Fungal population ranged from 1.67 to 9 x 10^4 cfu g^{-1}. The treatment T_2 (*B. subtilis* BaBc-1 + *T. asperellum* + *Bacillus* sp. BaOu-1) recorded significantly higher fungal population followed by T_5 (control) with a population of 4.67 x 10^4 cfu g^{-1}. No actinomycetes could be detected in any of the treatments. The population of BaBc-1, BaOu-1 and *T. asperellum* was maximum (28.67 x 10^6 cfu g^{-1}, 17 x 10^6 cfu g^{-1} and 7

x 10^4 cfu g^{-1} respectively) in T_2. The treatment T_4 recorded maximum population of *B. subtilis* and *G. butleri* (26.67 x 10^6 cfu g^{-1} and 1.67 x 10^4 cfu g^{-1} respectively).

Based on the weight reduction, enzyme activity and microbial population on 21 DAI, best two consortia (T_1- consortium I and T_2- consortium II) were selected for pilot scale experiment to evaluate their efficacy in KAU smart biobin.

Table 20. Effect of microbial consortia on weight reduction of biosolid waste

Treatments	Weight reduction (Per cent)
T_1 (*G. butleri* + *S. roseofulvus* AcOu-1 + *B. subtilis*)	8.75
T_2 (*B. subtilis* BaBc-1 + *T. asperellum* + *Bacillus* sp. BaOu-1)	10.00
T_3 (*B. subtilis* BaBc-1 + *G. butleri* + *Bacillus* sp. BaOu-1)	8.75
T_4 (*B. subtilis* + *G. butleri* + *B. subtilis* BaBc-1)	10.00
T_5 (Control)	6.25
CD (0.05)	NS

NS: Non-significant

Table 21. Enzyme activity of consortial formulations during degradation of biosolid waste

Treatments	*Enzyme activity (U ml^{-1})		
	Cellulase	Amylase	Protease
T_1 (*G. butleri* + *Streptomyces roseofulvus* AcOu-1 + *B. subtilis*)	0.62^c	4.88^a	57.91^c
T_2 (*B. subtilis* BaBc-1 + *T. asperellum* + *Bacillus* sp. BaOu-1)	0.96^b	5.33^a	107.50^a
T_3 (*B. subtilis* BaBc-1 + *G. butleri* + *Bacillus* sp. BaOu-1)	0.17^d	3.00^b	41.56^d
T_4 (*B. subtilis* + *G. butleri* + *B. subtilis* BaBc-1)	1.53^a	2.44^c	91.26^b
T_5 (Control)	0.07^d	1.77^d	36.98^d
CD (0.05)	0.277	0.466	7.676

*Mean of three replications

The values followed by same letter in each column do not differ significantly according to DMRT

Table 22. Microbial population in biosolid waste inoculated with consortia

Treatments	*Microbial population (cfu ml^{-1}) 21 DAI							
	Bacteria (x 10^6)				Fungi (x 10^4)			Actinomycetes (x 10^3)
	Total bacteria	BaBc-1	BaOu-1	*B. subtilis*	Total fungi	*G. butleri*	*T. asperellum*	AcOu-1
T_1 (*G. butleri* + AcOu-1+ *B. subtilis*)	29.67 (1.46)[c]	Not detected	Not detected	9.00	2.00 (0.30)[c]	0.67	Not detected	0
T_2 (BaBc-1 + *T. asperellum* + BaOu-1)	49.33 (1.69)[b]	28.67	17.00	Not detected	9.00 (0.95)[a]	Not detected	7.00	Not detected
T_3 (BaBc-1 + *G. butleri* + BaOu-1)	18.33 (1.24)[c]	7.67	5.67	Not detected	1.67 (0.20)[c]	0.67	Not detected	Not detected
T_4 (*B. subtilis*+ *G. butleri* + BaBc-1)	90.00 (1.95)[a]	18.00	Not detected	26.67	1.67 (0.20)[c]	1.67	Not detected	Not detected
T_5 (Control)	23.33 (1.34)[c]	Not detected	Not detected	Not detected	4.67 (0.65)[b]	Not detected	Not detected	Not detected
CD (0.05)	0.241				0.248			

DAI : Days After Inoculation

*Mean of three replications. The values followed by same letter in each column do not differ significantly according to DMRT

Log transformed values are given in parentheses

4.12. Evaluation of consortial formulations in degradation of biosolid waste (Pilot scale)

Pilot scale experiment was conducted to evaluate the efficiency of the selected microbial consortia in KAU smart biobin. Quality of compost was assessed based on physical, chemical and biological parameters of compost.

4.12.1. Effect of microbial consortia on composting process

Physical parameters such as volume reduction, variation in temperature, duration of composting process and yield of compost were recorded.

4.12.1.1. Volume reduction of biosolid waste

Reduction in volume of the biosolid waste was recorded at specific intervals. 12 kg of biosolid waste was added in biobin and first set of observations on volume reduction were recorded at 15 days after inoculation. Afterwards, 20 kg waste was added and observations on volume reduction were recorded at 15 and 30 days after inoculation. Data on per cent volume reduction during specific intervals are presented in Table 23 and Fig. 8.

During the first interval, volume reduction of T_2 (*B. subtilis* + *G. butleri* + *B. subtilis* BaBc-1) (74.21 per cent) was found to be significantly higher, which was on par with T_1 (65.62 per cent). T_4 (uninoculated control) recorded the least reduction in volume.

During the second interval, T_1 (*B. subtilis* BaBc-1 + *T. asperellum* + *Bacillus* sp. BaOu-1) recorded maximum volume reduction of 68.12 per cent, and this was on par with T_2 (volume reduction 64.61 per cent). The treatments T_3 and T_4 recorded lower volume reduction of 58.94 and 55.9 per cent respectively. A similar trend was observed 30 days after inoculation also, whereas T_1 (*B. subtilis* BaBc-1 + *T. asperellum* + *Bacillus* sp. BaOu-1) recorded the maximum volume reduction of 74.81 per cent, which was statistically on par with T_2 (73.56 per cent).

Table 23. Effect of selected consortial formulations on volume reduction during composting in biobin

Treatments	Volume reduction of biosolid waste (%)		
	First interval	Second interval	
	(15 DAI)	(15 DAI)	(30 DAI)
T_1 (*B. subtilis* BaBc-1 + *T. asperellum*+ *Bacillus* sp. BaOu-1)	65.62[a]	68.12[a]	74.81[a]
T_2 (*B. subtilis* + *G. butleri* + *B. subtilis* BaBc-1)	74.21[a]	64.61[a]	73.56[a]
T_3 (Cow dung slurry)	54.80[b]	58.94[b]	64.58[b]
T_4 (Control)	37.97[c]	55.69[b]	61.13[b]
CD (0.05)	10.430	5.593	5.491

*Mean of five replications

The values followed by same letter in each column do not differ significantly according to DMRT

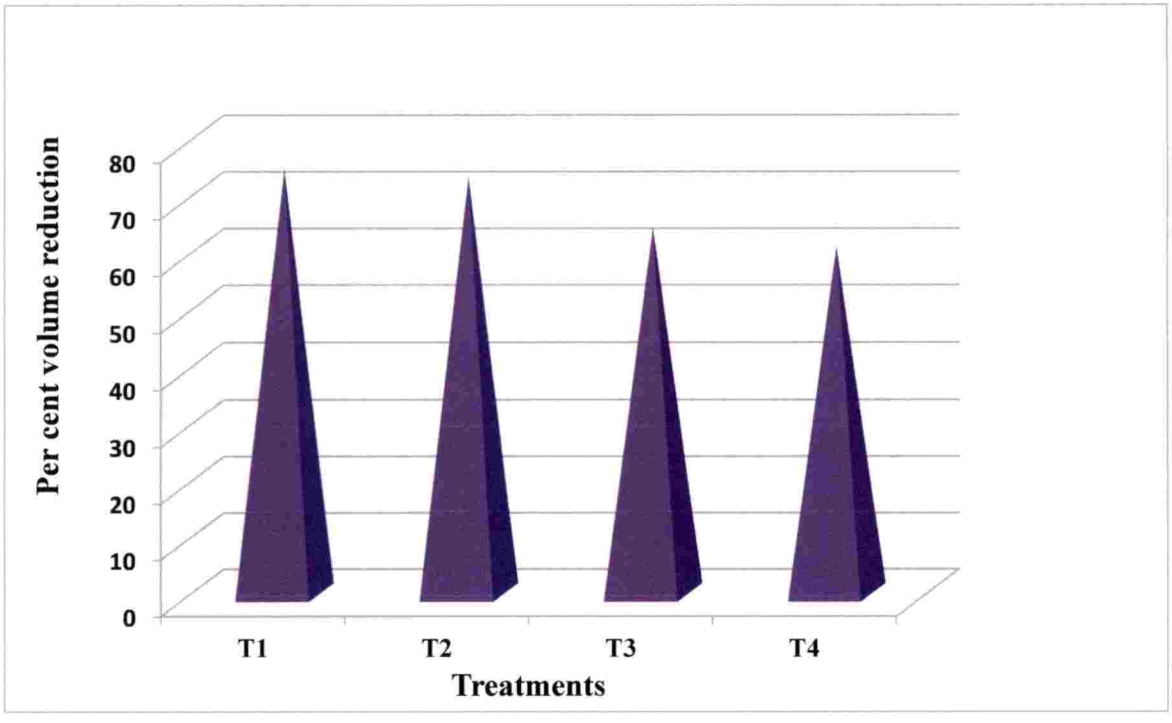


Fig. 8. Volume reduction of biosolid waste in KAU smart biobin 30 DAI

T_1 : *B. subtilis* BaBc-1 +*T. asperellum* + *Bacillus* sp. BaOu-1

T_2 : *B. subtilis* + *G. butleri* + *B. subtilis* BaBc-1

T_3 : Cow dung slurry

T_4 : Uninoculated control

4.12.1.2. Temperature variation during composting

Temperature during composting was recorded daily. Initial temperature range was 31.94 to 38.94^{0}C (Table 24, Appendix III and Fig. 9). Irrespective of the treatments; temperature showed a gradual increase to a peak value at three to four days after inoculation and later declined to a normal range. The variation in temperature during first interval (after the addition of 12 kg biosolid waste) and second interval (after the addition of 20 kg biosolid waste) followed the same trend. In all treatments, highest maximum temperature was recorded during second interval. After 24 days, the temperature was stable (28.6 to 31.1^{0}C).

The treatment T_2 (*B. subtilis* + *G. butleri* + *B. subtilis* BaBc-1) recorded maximum temperature of 48.32^0 C on third day of composting. The final temperature was recorded after 30 days, and T_4 (uninoculated control) recorded maximum final temperature of 31.1^{0}C.

Table 24. Effect of consortial formulations on temperature variation during composting

Treatments	No. of days taken to reach maximum temperature	Temperature (^{0}C)		
		Initial (Day 1)	Maximum	Final (Day 40)
T_1 (*B. subtilis* BaBc-1 + *T. asperellum* + *Bacillus* sp. BaOu-1)	3	38.62	44.72	28.6
T_2 (*B. subtilis* + *G. butleri* + *B. subtilis* BaBc-1)	3	38.94	48.32	29.2
T_3 (Cow dung slurry)	4	31.94	44.32	30.2
T_4 (Control)	4	38.48	42.24	31.1

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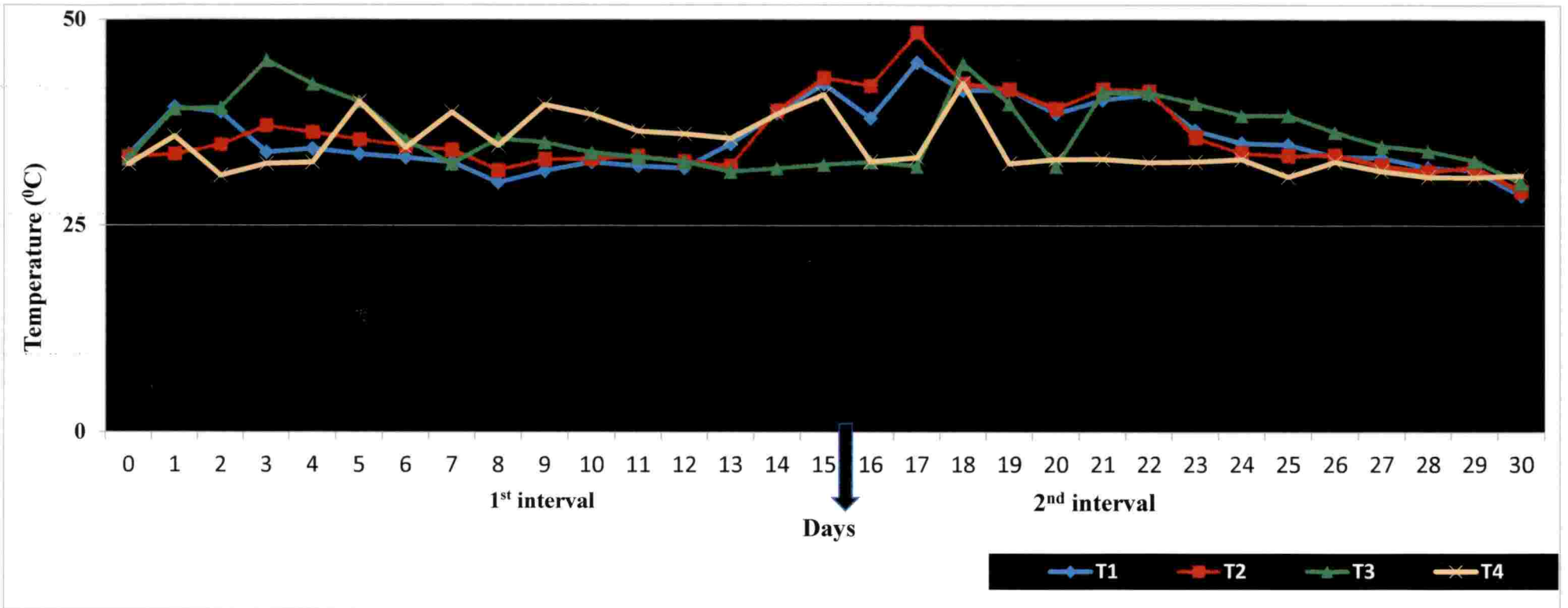


Fig. 9. Daily variation in temperature during composting in KAU smart biobin

T_1 : *B. subtilis* BaBc-1+ *T. asperellum*+ *Bacillus* sp. BaOu-1

T_2 : *B. subtilis*+ *G. butleri*+ *B. subtilis* BaBc-1

T_3 : Cow dung slurry

T_4 : Uninoculated control

4.12.1.3. Composting period

Observations on number of days taken for initiation of composting were recorded in each treatment. The treatment T_1 (*B. subtilis* BaBc-1 + *T. asperellum* + *Bacillus* sp. BaOu-1) recorded minimum number of days (17 days) followed by T_2 (*B. subtilis* + *G. butleri* + *B. subtilis* BaBc-1) (23 days). Compost formation was initiated in uninoculated control (T_4) after 40 days of inoculation (Table 25 and Fig. 10).

Table 25. Effect of consortial formulations on composting period

Treatments	Number of days for initiation of compost formation
T_1(*B. subtilis* BaBc-1 + *T. asperellum* + *Bacillus* sp. BaOu-1)	17
T_2(*B. subtilis* + *G. butleri* + *B. subtilis* BaBc-1)	23
T_3 (Cow dung slurry)	30
T_4 (Control)	40

4.12.1.4. Yield of compost

The data on yield of compost and residue material left after composting are given in Table 26 and Fig 11. The final yield of compost collected after 40 days, from different treatments was calculated by deducting the residue weight from total compost obtained. The final yield of compost after deducting the residue material varied from 0.88 to 5.35 kg (Plate 25). Considering the yield from 32 kg of biosolid waste, significantly higher yield of compost was recorded in T_1 (*B. subtilis* BaBc-1 + *T. asperellum* + *Bacillus* sp. BaOu-1) which was on par with T_2 (*B. subtilis* + *G.*

127a

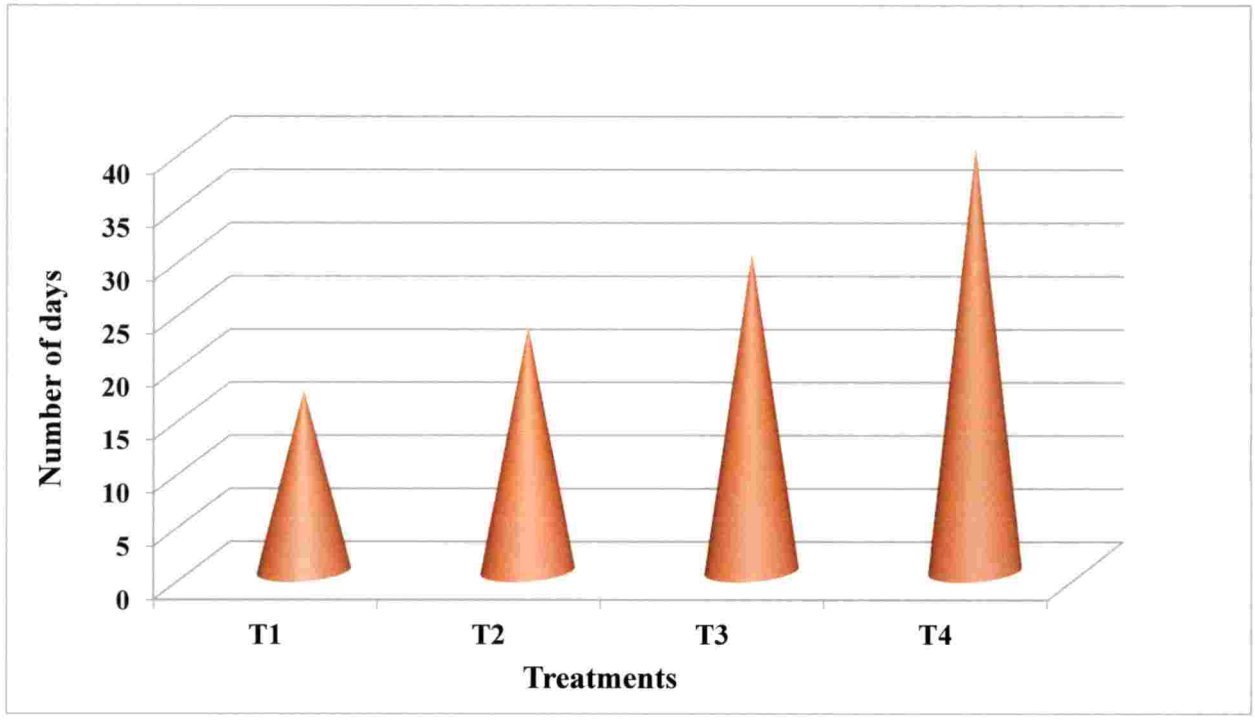


Fig. 10. Number of days taken for initiation of compost formation in KAU biobin

T_1 : *B. subtilis* BaBc-1+*T. asperellum* + *Bacillus* sp. BaOu-1

T_2 : *B. subtilis* + *G. butleri* + *B. subtilis* BaBc-1

T_3 : Cow dung slurry

T_4 : Uninoculated control

T_1 **Control**

T_2 **Control**

T_3 **Control**

T_1- *B. subtilis* BaBc-1 + *T. asperellum* + *Bacillus* sp. BaOu-1
T_2- *B. subtilis* + *G. butleri* + *B. subtilis* BaBc-1
T_3- Cow dung slurry
T_4- Uninoculated

Plate 25: Compost from KAU smart biobin

butleri + *B. subtilis* BaBc-1) and the average yield was 5.35 kg and 4.58 kg respectively. The uninoculated control recorded significantly lower quantity of compost (0.88 kg) and the residue weight was significantly higher in T_4. The per cent recovery of compost was significantly higher in T_1 (*B. subtilis* BaBc-1 + *T. asperellum* + *Bacillus* sp. BaOu-1) and T_2 (*B. subtilis* + *G. butleri* + *B. subtilis* BaBc-1).

Table 26. Yield of compost as influenced by consortial formulations (40 days after inoculation)

Treatments	Yield of compost (kg)	Residue weight (kg)	Compost recovery (Per cent)
T_1 (*B. subtilis* BaBc-1 + *T. asperellum* + *Bacillus* sp. BaOu-1)	5.35 (0.713) [a]	2.42 (0.33)[b]	16.73 (1.21)[a]
T_2 (*B. subtilis* + *G. butleri* + *B. subtilis* BaBc-1)	4.58 (0.646) [a]	3.41 (0.52)[b]	14.30 (1.15)[a]
T_3 (Cowdung slurry)	2.30 (0.344) [b]	6.60 (0.81)[a]	6.76 (0.81)[b]
T_4 (Control)	0.88 (-0.090) [b]	7.46 (0.86)[a]	2.76 (0.40)[b]
CD (0.05)	0.203	0.211	0.201

*Mean of five replications

The values followed by same letter in each column do not differ significantly according to DMRT

Log transformed values are given in parentheses.

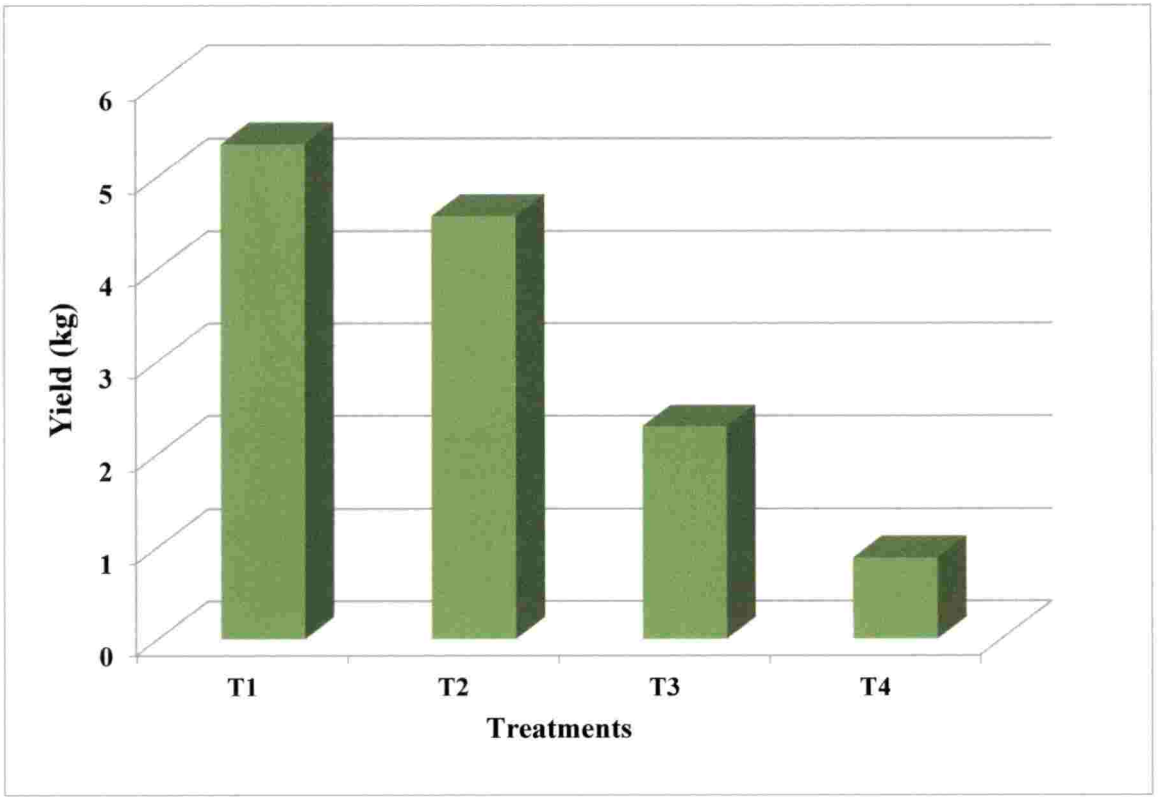


Fig. 11. Compost yield from KAU biobin

T_1 : *B. subtilis* BaBc-1 +*T. asperellum*+ *Bacillus* sp. BaOu-1

T_2 : *B. subtilis*+ *G. butleri* + *B. subtilis* BaBc-1

T_3 : Cow dung slurry

T_4 : Uninoculated control

4.12.2. Colour and odour of compost

Colour and odour of the mature compost samples from different treatments was assessed after 40 days of inoculation and it was dark brown without any foul odour.

4.12.2.1. Moisture content of compost

Moisture content of compost collected from all the four treatments was determined after 40 days of inoculation. The results showed that moisture content of T_3 (cow dung slurry) was significantly high (37.52 per cent) compared to all other treatments. The treatments T_1 (*B. subtilis* BaBc-1 + *T. asperellum* + *Bacillus* sp. BaOu-1), T_2 (*B. subtilis* + *G. butleri* + *B. subtilis* BaBc-1) and T_4 (uninoculated control) were showed comparable values. The data on moisture content of compost is given in Table 27.

Table 27. Effect of consortial formulations on moisture content of compost

Treatments	Moisture (%)
T_1 (*B. subtilis* BaBc-1 + *T. asperellum* + *Bacillus* sp. BaOu-1)	15.08 (3.807) [b]
T_2 (*B. subtilis* + *G. butleri* + *B. subtilis* BaBc-1)	15.10 (3.779) [b]
T_3 (Cow dung slurry)	37.52 (6.122) [a]
T_4 (Control)	24.50 (4.897) [b]
CD (0.05)	1.24
FCO standard	<25

*Mean of five replications

The values followed by same letter in each column do not differ significantly according to DMRT

$\sqrt{x+0.5}$ transformed values are given in parentheses.

4.12.2.2. Bulk density of compost

Bulk density of compost from different treatments did not exhibit any significant difference. The bulk density of compost samples ranged from 1.47 to 1.8 g cm^{-3} with a highest value recorded in control treatment (Table 28).

Table 28. Effect of consortial formulations on bulk density of compost

Treatments	Bulk density (g cm^{-3})
T_1(*B. subtilis* BaBc-1 + *T. asperellum* + *Bacillus* sp. BaOu-1)	1.47
T_2(*B. subtilis* + *G. butleri* + *B. subtilis* BaBc-1)	1.58
T_3 (Cow dung slurry)	1.79
T_4 (Control)	1.80
CD (0.05)	NS
FCO standard	<1

4.12.3. Chemical parameters of compost

Chemical parameters of compost collected from all the four treatments were estimated after 40 days of inoculation. Analysis of chemical parameters included pH, electrical conductivity (EC), organic carbon (OC), C:N ratio, nitrogen (N), phosphorous (P), potassium (K), copper (Cu), zinc (Zn) and heavy metal contents in compost. The details of chemical parameters presented in Table 29.

4.12.3.1. pH and electrical conductivity of compost

pH of all the compost samples were found to be in the alkaline range. The treatment T_2 (*B. subtilis* + *G. butleri* + *B. subtilis* BaBc-1) recorded maximum pH of 10.28 and this was on par with T_1 (pH 9.23).

Significantly high EC of 16.25 dS m^{-1} was recorded in T_2 (*B. subtilis* + *G. butleri* + *B. subtilis* BaBc-1). All other treatments were statistically on par with each other.

4.12.3.2. Organic carbon and C:N ratio in compost

Analysis on organic carbon in compost from different treatments did not exhibit any significant difference. It ranged from 14.53 (in T_2) to 17.18 per cent (in T_4).

The C:N ratio in compost samples ranged from 8.22 to 11.44:1. C:N ratio was significantly higher (C:N ratio 11.44:1) in T_1, and this was statistically on par with T_2 (C:N ratio 10.96:1). The compost samples from T_3 and T_4 recorded lower C:N ratio of 8.68:1 and 8.22:1 respectively.

4.12.3.3. Nitrogen, potassium and phosphorus contents in compost

N content in compost samples ranged from 1.29 to 1.68 per cent and there was no significant difference among these samples. Maximum N content (1.68 per cent) was observed in uninoculated control and minimum was in T_2 (1.29 per cent).

Analysis on P content in compost revealed that, there was no significant difference in P content. Maximum P content (1.21 per cent) was recorded in uninoculated control and the minimum (0.84 per cent) was in T_2.

P content in different compost samples ranged from 3.49 to 5.84 per cent and there was no significant difference among samples. However, maximum P content (5.84 per cent) was recorded in T_2 and the minimum P content (3.49 per cent) was in T_4.

4.12.3.4. Micronutrient contents in compost

Significantly higher Cu content of 22.65 ppm was recorded in T_3 followed by T_1 (19.71 ppm). This was on par with T_2 (18.65 ppm) and T_4 (19.70 ppm). Analysis of compost samples revealed that, the Zn content was not significantly different. Highest Zn content of 227.28 ppm was recorded in T_1 and lowest Zn content of 122.89 ppm in T_4.

4.12.3.5. Heavy metal contents in compost

Results of heavy metal analysis indicated the absence of cadmium (Cd) and arsenic (As) in compost collected different treatments. There was no significant difference among samples. However, traces of lead (Pb), chromium (Cr) and nickel (Ni) were observed in all the four treatments, which were less than maximum permissible limits (maximum permissible limit for Pb- 100 mg/kg, Cr and Ni-50 mg/kg).

4.12.4. Biological parameters of compost

Total microbial population, phytotoxicity and presence of human pathogens in compost were assessed after 40 days of inoculation.

Table 29. Effect of consortial formulations on chemical parameters of compost

Treatments	pH	EC ($dS\ m^{-1}$)	OC (%)	C:N	N (%)	P (%)	K (%)	Cu (ppm)	Zn (ppm)	Pb (ppm)	Cd (ppm)	Cr (ppm)	Ni (ppm)	As (ppm)
T_1	9.23^{ab}	8.94^{b}	15.84	11.44^{a}	1.41	0.90	4.45	19.71^{b}	227.78	5.82	NDL	37.66	13.19	NDL
T_2	10.28^{a}	16.25^{a}	14.53	10.96^{a}	1.29	0.84	5.84	18.65^{b}	168.53	6.48	NDL	44.45	13.26	NDL
T_3	9.00^{b}	9.37^{b}	15.87	8.68^{b}	1.59	0.94	4.34	22.65^{a}	226.75	5.24	NDL	31.63	13.28	NDL
T_4	8.70^{b}	8.43^{b}	17.18	8.22^{b}	1.68	1.21	3.49	19.70^{b}	122.89	4.91	NDL	28.35	15.12	NDL
CD (0.05)	1.087	4.913	NS	1.406	NS	NS	NS	2.459	NS	NS	-	NS	NS	-
FCO standard	6.5-7.5	<4	>14	<20:1	>0.8	>0.4	>0.4	<300	<1000	<100	<50	<50	<50	<50

NDL- Non detectable level

*Mean of five replications

In the case of pH, EC, C:N ratio and Cu each column values followed by same letter do not differ significantly according to DMRT

Treatments found to be non-significant in OC, N, P, K and Zn, Pb, Cr and Ni

4.12.4.1. Microbial population

Bacterial population predominated in all compost samples from different treatments (Table 30). This was followed by fungal and actinomycetes population. The treatment T_1 (*B. subtilis* BaBc-1 + *T. asperellum* + *Bacillus* sp. BaOu-1) recorded significantly higher bacterial population (110.33 x 10^6 cfu g^{-1}) which was statistically on par with T_2 (*B. subtilis* + *G. butleri* + *B. subtilis* BaBc-1) with a population of 85.67 x 10^6 cfu g^{-1}. Minimum bacterial population (13.67 x cfu g^{-1}) was recorded in T_4.

A similar trend was observed in fungal population also, T_1 (*B. subtilis* BaBc-1 + *T. asperellum* + *Bacillus* sp. BaOu-1) and T_2 (*B. subtilis* + *G. butleri* + *B. subtilis* BaBc-1) recorded significantly higher fungal population of 21 x 10^4 cfu g^{-1} and 14.67 x 10^4 cfu g^{-1} respectively. Minimum fungal population was recorded in T_3 (4.67 x 10^4cfu g^{-1}). Actinomycetes population was significantly higher in T_1 (33.67 x 10^5 cfu g^{-1}) and this was on par with T_2 (28.67 x 10^5 cfu g^{-1}). In T_3, where cow dung slurry was used as the inocula, actinomycete population could not be detected. The microbial population in cow dung was determined and compared with that of treatments. Bacterial population was predominant (33.5 x 10^6 cfu g^{-1}), followed by fungi (9.00 x 10^4 cfu g^{-1}) and actinomycetes (2.15 x 10^4 cfu g^{-1} (Table 31).

Table 30. Effect of consortial formulations on microbial population in compost

Treatments	*Microbial population (cfu g^{-1}) 40 DAI		
	Bacteria ($\times 10^6$)	Fungi ($\times 10^4$)	Actinomycetes ($\times 10^3$)
T_1 (*B. subtilis* BaBc-1+*T. asperellum* + *Bacillus* sp. BaOu-1)	110.33 (2.04)[a]	21.00 (1.32)[a]	33.67 (1.52)[a]
T_2 (*B. subtilis*+ *G. butleri* + *B. subtilis* BaBc-1)	85.67 (1.93)[a]	14.67 (1.17)[a]	28.67 (1.46)[a]
T_3 (Cow dung slurry)	23.67 (1.37)[b]	4.67 (0.65)[c]	0.00 (0.00)[b]
T_4 (Control)	13.67 (1.11)[c]	8.00 (0.89)[b]	0.00 (0.00)[b]
CD (0.05)	0.201	0.187	0.215

*Mean of three replications

The values followed by same letter in each column do not differ significantly according to DMRT

Log transformed values are given in parentheses

Table 31. Microbial population in cow dung

Microorganism	*Microbial population (cfu g^{-1})
Bacteria ($\times 10^6$)	33.5
Fungi ($\times 10^4$)	9.00
Actinomycetes ($\times 10^4$)	2.15

*Mean of three replications

4.12.4.2. Phytotoxicity

The effect of compost extract on germination of tomato seeds was studied, in order to detect the phytotoxicity of compost.

Per cent germination of tomato seeds on 5th day was significantly high (100 per cent germination) in T_1 and T_4 (100 per cent germination) followed by T_3 (96.67 per cent germination) and T_2 (83.33 per cent germination) (Table 32).

4.12.4.3. Presence of human pathogens

To detect the presence of human pathogens in compost samples, serial dilution plating was done for *E.coli, Shigella* sp. and *Salmonella* sp. The results revealed that, all the five compost samples were free from human pathogens.

Table 32. Effect of consortial formulations on germination of tomato seeds

Treatments	Germination (%) (5th day)
T_1 (*B. subtilis* BaBc-1 + *T. asperellum* + *Bacillus* sp. BaOu-1)	100.00 (10)^a
T_2 (*B. subtilis* + *G. butleri* + *B. subtilis* BaBc-1)	83.33 (9.3)^b
T_3 (Cow dung slurry)	96.67 (9.8)^b
T_4 (Control)	100.00 (10)^a
Distilled water (Control)	61.66 (7.8)^c
CD (0.05)	0.33

*Mean of five replications
The values followed by same letter in each column do not differ significantly according to DMRT
$\sqrt{x+0.5}$ transformed values are given in parentheses.

Considering the volume reduction, duration of composting process, yield of compost, microbial population and phytotoxicity of compost, T_1 (consortium I) was found to be the best treatment in KAU smart biobin and this consortial formulation was selected for large scale experiment.

4.13. Evaluation of consortial formulation in degradation of biosolid waste (Large scale)

Consortium I (*B. subtilis* BaBc-1 + *T. asperellum* + *Bacillus* sp. BaOu-1), selected as the best consortial formulation in the pilot scale experiment, was evaluated for its efficiency to enhance the process of composting of biosolid waste, using Thumburmuzhi composting units. A positive control using cow dung and a negative control without inoculum served as positive and negative controls respectively. The results on volume reduction, temperature variation, yield and quality of compost are given below.

4.13.1. Effect of consortial formulation on composting process

Physical parameters of composting such as volume reduction, variation in temperature and yield of compost were recorded.

4.13.1.1 Volume reduction of biosolid waste

Volume reduction of biosolid waste in different treatments was recorded at different time intervals (15, 30, 45 and 80 days after inoculation) (Plate 26). The results showed that, there was no significant difference in volume reduction. Volume reduction at 15 days after inoculation ranged from 44.9 to 48.19 per cent, and the maximum volume reduction (volume reduction 48.19 per cent) was recorded in T_1. After 30, 45 and 80 days of inoculation, the volume reduction of biosolid waste was maximum (volume reduction 56.90, 64.63 and 73.02 per cent respectively) in T_1 and

minimum (volume reduction 54.60, 59.86 and 66.11 per cent) in T_2. Data on per cent volume reduction during different time intervals are presented in Table 33.

Table 33. Effect of selected consortial formulation on volume reduction during composting

Treatments	Volume reduction of biosolid waste (%)			
	15 DAI	**30 DAI**	**45 DAI**	**80 DAI**
T_1 (*B. subtilis* BaBc-1 + *T. asperellum* + *Bacillus* sp. BaOu-1)	48.19	56.90	64.63	73.02
T_2 (Cow dung)	44.9	54.60	59.86	66.11
T_3 (Control)	44.9	56.08	62.50	69.07
CD (0.05)	NS	NS	NS	NS

4.13.1.2. Temperature variation during composting

Temperature during composting was recorded daily for 30 days and then once in a week. Initial temperature ranged from 39.7 to 45.98^{0}C (Table 34, Appendix IV and Fig. 12). Irrespective of the treatments; temperature gradually increased to a maximum value and later declined to a stable range. Initial temperature was maximum in T_1 (45.98^{0}C). The highest maximum temperature (64^{0}C) was recorded in T_1, on third day after inoculation. The highest final temperature was recorded in uninoculated control and lowest final temperature was in T_1 (*B. subtilis* BaBc-1+ *T. asperellum* + *Bacillus* sp. BaOu-1).

Initial volume

30 DAI

45 DAI

80 DAI

T_1- *B. subtilis* BaBc-1 + *T. asperellum* + *Bacillus* sp. BaOu-1

Plate 26: Volume reduction in Thumburmuzhi composting unit (T_1)

Table 34. Effect of microbial consortia on composting temperature

Treatments	No. of days taken to reach maximum temperature	Temperature (^{0}C)		
		Initial (1st day)	Maximum	Final (80th day)
T_1 (*B. subtilis* BaBc-1 + *T. asperellum* + *Bacillus* sp. BaOu-1)	3	45.98	64.00	32.62
T_2 (Cow dung)	4	39.70	51.78	33.90
T_3 (Control)	4	40.40	53.74	40.52

4.13.1.3. Yield of compost

After 80 days of inoculation, the compost collected from each treatment was sieved and the total compost yield, residue material left after composting and recovery per cent was recorded. The yield of compost was significantly higher (104.17 kg) in T_2 followed by T_1 (26.30 kg) and the lower compost yield (19.21 kg) was observed in T_3 (Table 35 and Plate 27). A similar trend was observed in compost recovery per cent also, significantly higher compost recovery (13.03 per cent) was in T_2 followed by T_1 (3.75 per cent) and the minimum recovery per cent (2.74 per cent) was observed in T_3. The weight of residue materials did not differ significantly among treatments.

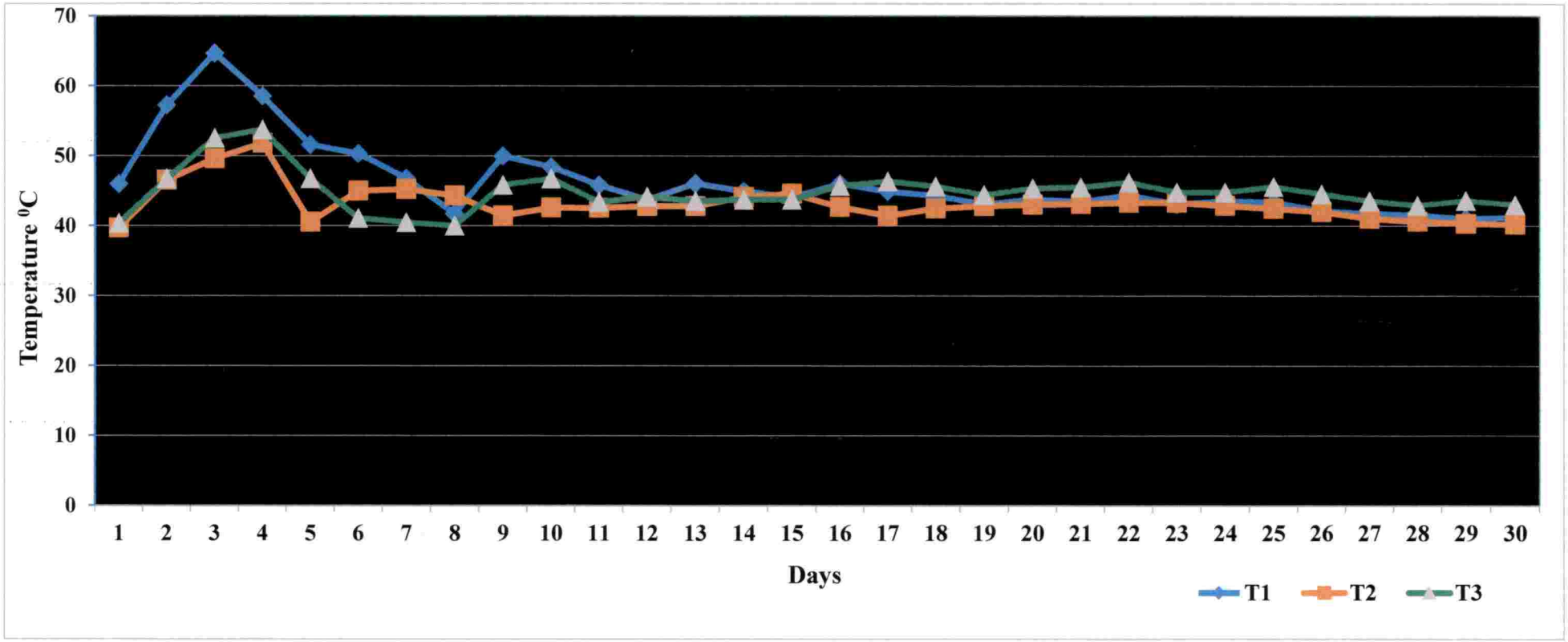


Fig. 12. Daily variation in temperature during composting in Thumburmuzhi composting unit

T_1 : *B. subtilis* BaBc-1+ *T. asperellum*+ *Bacillus* sp. BaOu-1

T_2 : Cow dung

T_3 : Uninoculated control

Table 35. Effect of consortial formulation on yield of compost

Treatments	Yield (kg)	Residue (kg)	Compost recovery 80 DAI (%)
T_1 (*B. subtilis* BaBc-1 + *T. asperellum* + *Bacillus* sp. BaOu-1)	26.30 (1.39)[b]	60.00	3.75 (0.54)[b]
T_2 (Cow dung)	104.17 (2.01)[a]	128.00	13.03 (1.11)[a]
T_3 (Control)	19.21 (1.24)[c]	122.00	2.74 (0.39)[c]
CD (0.05)	0.229	NS	0.229

*Mean of three replications

The values followed by same letter in each column do not differ significantly according to DMRT

Log transformed values are given in parentheses

4.13.2. Colour and odour of compost

Colour of the compost was assessed after 80 days of inoculation and all the compost samples were found to be in dark brown colour, without any foul odour.

4.13.2.1. Moisture content of compost

Moisture content in compost samples was analyzed after 80 days of inoculation. The range of moisture content was 21.7 to 25 per cent (Table 36) and hence, it was within the specifications (<25 per cent) given in Fertilizer Control

T_1

T_2

T_3

T_1- *B. subtilis* BaBc-1 + *T. asperellum* + *Bacillus* sp. BaOu-1
T_2- Cow dung
T_3- Uninoculated

Plate 27: Compost from Thumburmuzhi composting unit

Order (FCO, 1985). There was no significant difference in moisture content among the treatments. However, T_2 recorded maximum moisture content of 24.3 per cent and T_1 recorded the minimum (21.7 per cent).

Table 36. Effect of consortial formulation on moisture content of compost

Treatments	Moisture (%)
T_1 (*B. subtilis* BaBc-1 + *T. asperellum* + *Bacillus* sp. BaOu-1)	21.7
T_2 (Cow dung)	25
T_3 (Uninoculated control)	24.3
CD (0.05)	NS
FCO standard	<25

4.13.2.2. Bulk density of compost

Bulk density of compost collected from all the three treatments was assessed after 80 days of inoculation. The results showed that, significantly higher (0.92 g cm^{-3}) bulk density was recorded in T_2 and the lower (0.67 g cm^{-3}) bulk density was recorded in T_1 (Table 37). In all the samples, the bulk density was within the prescribed limit of less than one (FCO, 1985).

Table 37. Effect of consortial formulation on bulk density of compost

Treatments	Bulk density (g cm^{-3})
T_1 (*B. subtilis* BaBc-1 + *T. asperellum* + *Bacillus* sp. BaOu-1)	0.67[c]
T_2 (Cow dung)	0.92[a]
T_3 (Uninoculated control)	0.87[b]
CD (0.05)	0.045
FCO standard	<1

*Mean of three replications

The values followed by same letter in each column do not differ significantly according to DMRT

4.13.3. Chemical parameters of compost

Analysis of chemical parameters of compost included pH, EC, OC, C:N ratio, N, P_2O_5, K, Cu, Zn and heavy metal contents of compost. The details of chemical parameters presented in Table 38.

4.13.3.1. pH and electrical conductivity of compost

pH of all the compost samples were in alkaline range (pH 8.07 to 8.74). The treatment T_2 recorded significantly high pH of 8.74 and this was statistically on par with T_3 (pH 8.61).

Analysis on EC in compost samples revealed that, T_3 recorded significantly high (4.69 dS m^{-1}) EC and the lower (2.59 dS m^{-1}) was in T_1.

4.13.3.2. Organic carbon and C:N ratio in compost

The organic carbon content in compost from different treatments ranged from 30.13 to 18.99 per cent. Significantly high organic carbon content of 30.13 per cent was recorded in T_3 and the lower (18.99 per cent) was in T_1.

The C:N ratio in compost was significantly higher (24.73) in T_3 and the lower C:N ratio (11.37) was recorded in T_1.

4.13.3.3. Nitrogen, potassium and phosphorus contents in compost

The nitrogen content in compost samples ranged from 1.38 to 2.01 per cent. Significantly high nitrogen content of 2.01 per cent was recorded in T_2 and the lower (1.38 per cent) was in T_3.

The phosphorous content in different compost samples ranged from 0.16 to 0.23 per cent and there was no significant difference among samples. The treatment T_3 recorded maximum phosphorus content (0.23 per cent) and T_2 recorded the minimum (0.16 per cent).

The potassium content of compost samples ranged from 0.16 to .79 per cent. Significantly higher potassium content (0.79 per cent) was recorded in T_2 and lower (0.16 per cent) was in T_3.

4.13.3.4. Micronutrient contents in compost

Analysis of copper content in compost revealed that, T_3 recorded maximum Cu content (24.7 ppm), which was statistically on par with T_1 (23.21 ppm). The Zn content in compost varied from 72.22 to 161.27 ppm. Compost collected from T_3 recorded significantly higher (161.27 ppm) Zn content, T_1 and T_2 recorded the minimum (72.22 and 64.87 ppm respectively).

4.13.3.5. Heavy metal contents in compost

Analysis of the heavy metal content in compost samples revealed that, traces of Pb, Cr and Ni were detected in all the compost samples, but within the maximum permissible limits (maximum permissible limit for Pb- 100 mg/ kg, Cr and Ni-50 mg/ kg). The Pb content of compost was maximum (12.98 ppm) in T_1 followed by T_2 and T_3 (7.16 and 8.3 ppm respectively). Content of Cr was maximum (47.62 ppm) in T_2, which was on par with T_3 (46.76 ppm). The minimum Cr content was recorded in T_1 (37.65 ppm). The nickel content of compost was significantly higher in T_2 (12.26 ppm), followed by T3 (9.24 ppm) and minimum in T_1 (6.54 ppm).

4.13.4. Biological parameters of compost

In vitro evaluation of biological parameters of compost such as microbial population, phytotoxicity in seed germination and presence of human pathogens in compost samples was assessed.

4.13.4.1. Microbial population

The total population of bacteria, fungi and actinomycetes was recorded in each compost sample. Bacterial population dominated in all the compost samples and it ranged from 7.33 to 45 x 10^6 cfu g^{-1}. Significantly higher population (45 x 10^6 cfu g^{-1}) was recorded in T_1 and lower population (7.33 x 10^6 cfu g^{-1}) in T_3 (Table 39). Maximum fungal population of 29 x 10^4 cfu g^{-1} was recorded in T_1 followed by T_2 (11.33 x 10^4 cfu g^{-1}) and the lowest fungal population observed in T_3 (1 x 10^4 cfu g^{-1}). None of the treatments recorded presence of actinomycetes.

Table 38. Effect of microbial consortium on chemical parameters

Treatments	pH	EC ($dS\ m^{-1}$)	OC (%)	C : N	N (%)	P (%)	K (%)	Cu (ppm)	Zn (ppm)	Pb (ppm)	Cr (ppm)	Ni (ppm)
T_1 (*B. subtilis* BaBc-1+ *T. asperellum*+ *Bacillus* sp. BaOu-1)	8.07^b	2.59^c	18.99^c	11.37^c	1.38^c	0.19	0.48^b	23.21^a	72.22^b	12.98^a	37.65^b	6.54^c
T_2 (Cow dung)	8.74^a	3.87^b	23.86^c	14.98^b	2.01^a	0.16	0.79^a	19.03^c	64.87^b	7.16^b	47.62^a	12.26^a
T_3 (Uninoculated control)	8.61^a	4.69^a	30.13^a	24.73^a	1.81^b	0.23	0.16^c	24.7^a	161.27^a	8.30^b	46.76^a	9.24^b
CD (0.05)	0.14	0.21	3.27	2.31	0.16	NS	0.08	3.16	11.37	1.73	7.06	1.55
FCO standard	6.5-7.5	<4	>14	<20:1	>0.8	>0.4	>0.4	<300	<1000	<100	<50	<50

*Mean of five replications

The values followed by same letter in each column do not differ significantly according to DMRT

Table 39. Effect of consortial formulation on microbial population in compost

Treatments	*Microbial population (cfu g^{-1}) 80 DAI	
	Bacteria (x 10^6)	**Fungi (x 10^4)**
T_1 (*B. subtilis* BaBc-1 + *T. asperellum* + *Bacillus* sp. BaOu-1)	45.67 (1.66)[a]	29.00 (1.47)[a]
T_2 (Cow dung)	26.00 (1.41)[b]	11.33 (1.05)[b]
T_3 (Control)	7.33 (0.85)[c]	1.00 (0.20)[c]
CD (0.05)	0.213	0.479

*Mean of three replications

The values followed by same letter in each column do not differ significantly according to DMRT

Log transformed values are given in parentheses

4.13.4.2. Phytotoxicity

The effect of compost extract on germination of tomato seeds was studied, in order to detect the phytotoxicity of compost. All the samples resulted in 100 per cent of germination on fifth day (Table 40 and Plate 28).

Table 40. Effect of consortial formulation on per cent germination of tomato seeds

Treatments	Germination (%) (5th day)
T_1 (*B. subtilis* BaBc-1 + *T. asperellum*+ *Bacillus* sp. BaOu-1)	100
T_2 (Cow dung)	100
T_3 (Uninoculated Control)	100
Distilled water (Control)	100
CD (0.05)	NS

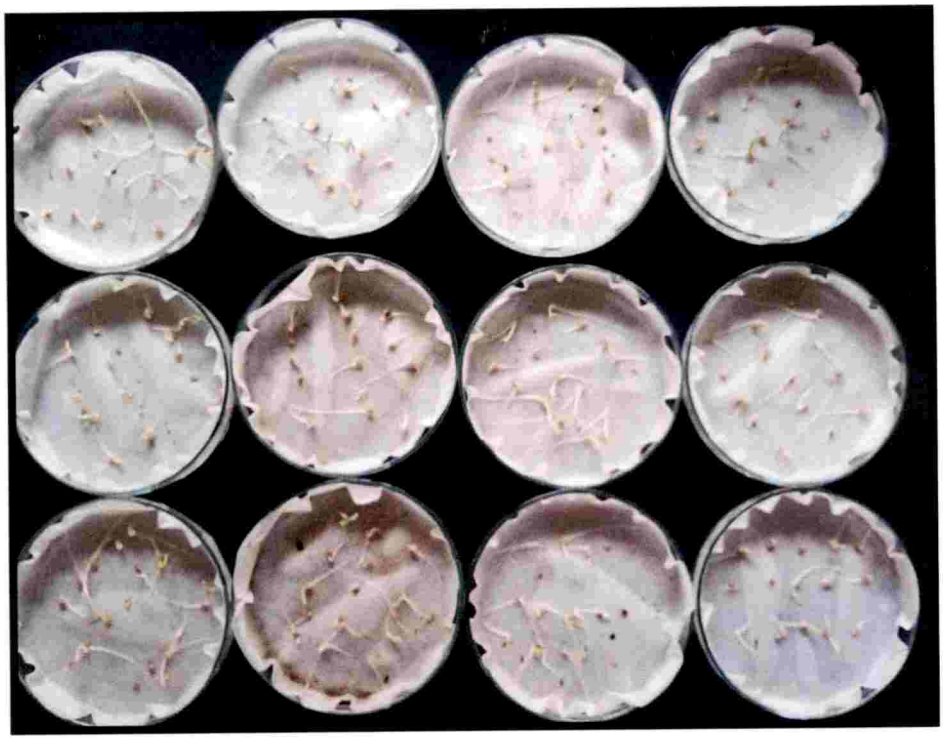
*Mean of five replications

The values followed by same letter in each column do not differ significantly according to DMRT

$\sqrt{x+0.5}$ transformed values are given in parentheses.

4.14.4.3. Presence of human pathogens

Compost samples collected from different treatments were subjected to serial dilution and plating technique to detect the presence of human pathogens. Presence of *E. coli*, *Salmonella* sp. and *Shigella* sp. could not be detected in any of the compost samples (Plate 29).



T_1 **T_2** **T_3** **T_4**

T_1- *B. subtilis* BaBc-1 + *T. asperellum* + *Bacillus* sp. BaOu-1

T_2- Cow dung

T_3- Uninoculated

T_4- Distilled water

Plate 28: Effect of compost from Thumburmuzhi composting units on germination of Tomato seeds

Control

Absence of *E. coli*

Control

Absence of *Salmonella* & *Shigella*

Plate 29: Human pathogens in compost from Thumburmuzhi composting unit

4.15. Economics of composting in Thumburmuzhi composting unit

Benefit cost analysis was carried out for composting in Thumburmuzhi composting unit (Table 41 and Appendix IV). It was found that among the different treatments, BC ratio was maximum in T_2 (BC ratio 1.00) and lower BC ratio (0.19) was noted in T_3.

Table 41. Benefit cost ratio of composting in Thumburmuzhi composting unit

Treatments	Total cost (Rs)	Total returns (Rs)	BC ratio
T_1 (BaBc-1 + *T. asperellum* + BaOu-1)	3280	841.6	0.26
T_2 (cow dung)	3350	3333.44	1.00
T_3 (control)	3200	614.72	0.19

*Cost of compost → Rs. 8.00 per kg

Discussion

5. DISCUSSION

Annual organic waste production in the world estimated to be about 38 billion metric tons (Seth, 2010) and it is becoming a major threat to the environment. Considering the case of India, the range of per capita national municipal solid waste generation is between 200 and 600 g and for Kerala it is comes to 178 g (Varma *et al.*, 2004; CPCB, 2010 and Zeinhom *et al.*, 2010). Composting of organic waste materials is one of the best alternatives for effective waste disposal. However, natural composting needs long period ranging from four to six months for the composting process (Misra *et al.*, 2003). Conventionally, cow dung is used as the source of microbes for enhancing degradation process in composting. In bio-composting, inoculated microorganisms enhance the rate of degradation and reduce the time for composting process. Earlier reports indicate that mixed cultures of microorganisms (consortia) are more efficient than individual cultures, in reducing the composting period and preventing foul smell during composting (Pan *et al.*, 2012; Game *et al.*, 2017). It has also been reported earlier that indigenous microorganisms increased the efficiency of composting process than introduced flora (Hanim *et al.*, 2012; Abu-Bakar and Ibrahim, 2013). In this context, the present study was carried out to explore the possibility of enhancing degradation of biosolid waste using microbial inoculants in aerobic composting.

Isolation of indigenous microorganisms with potential degradation activity was attempted from different compost samples including coir pith compost, kitchen waste compost and Oushadhi ayurvedic compost. Earlier reports indicate that effective hydrolytic microorganisms are available in compost. Pan *et al.* (2011) isolated different strains of *Bacillus subtilis* and *Pseudomonas* sp. from various compost samples. Rajeswari (2017) isolated and prepared consortia containing *Bacillus* sp., *Mucor* sp., and *Fusarium* sp., from fish waste compost. Organic waste materials contain bacteria, fungi and actinomycetes. These microbes are able to produce specific enzymes which plays major role in degradation of cellulose and

lignocelluloses content of waste materials. Among these microorganisms *Trichoderma*, *Aspergillus*, *Penicillium*, *Humicola* like cellulolytic fungi and *Bacillus* sp., *Pseudomonas* sp., *Clostridium* sp., *Streptomyces* sp. like bacteria are most discussed types (Wood *et al.*, 1988 and Gautam *et al.*, 2010).

In the present study, the compost samples from different sources exhibited variability with respect to microbial population. In general, bacterial population was found to be more, compared to fungal and actinomycetes population. The variability of bacterial, fungal and actinomycetes population in compost samples depended upon the nature and physicochemical properties of the raw material used (Balasundaram, 2009). The compost sample from KAU biobin recorded highest bacterial population (215.67×10^6 cfu g^{-1}), whereas the population of fungi was maximum in kitchen waste compost (42.67×10^4 cfu g^{-1}). Actinomycetes could not be detected in any of the samples except Oushadhi ayurvedic waste compost (129×10^3 cfu g^{-1}). Rebollido *et al.* (2008) also found that, population of bacteria was maximum in compost samples, followed by actinomycetes and fungi. They reported that among the microbial isolates, genera of *Bacillus* and *Streptomyces* were more predominant. Balasundaram (2009) also assessed microbial population in different compost samples and reported highest population of bacteria in ayurvedic herbal waste compost (20.2×10^6 cfu g^{-1}), actinomycetes in tea waste compost (12.8×10^5 cfu g^{-1}) and fungi in ayurvedic herbal waste compost (91.7×10^3 cfu g^{-1}).

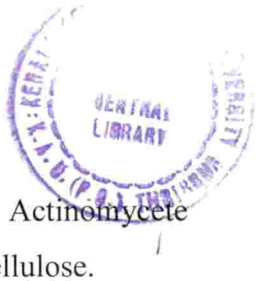
Fourteen microbial isolates including eight bacteria, four fungi and two actinomycetes were obtained from different compost samples. Pure cultures of these isolates were preserved as slants at 4^0C and also as glycerol stock at -80^0C for further use.

Biosolid waste comprises complex organic compounds like cellulose, starch, proteins, lignin, lipids, chitin and pectin (de Bertoldi, 1983). In order to develop microbial inoculum for rapid decomposition of biodegradable waste, it is essential to

have microbial isolates efficient in degrading all the components. Hence all the fourteen microbial isolates were screened for their ability to utilize specific biomolecules present in biosolid waste, by culturing them on selective media. Apart from these isolates, two bacterial isolates (*Bacillus subtilis* and *B. niabensis*) and two fungal isolates (*Gongronella butleri* and *Trichoderma asperellum*) available in the repository of the Department of Agricultural Microbiology, College of Horticulture, Vellanikkara were also used as reference cultures for screening. These were already used in waste management, both at house hold level and for municipal solid waste (MSW) management.

Carboxy methyl cellulose agar, lignin sulphonate, starch agar, calcium caseinate agar, pectic agar, colloidal chitin medium and spirit blue agar medium were used as selective media, for screening of cellulose, lignin, starch, protein, pectin, chitin and lipid decomposing microorganisms respectively. The ability of the microbe to utilize the specific molecule was indicated by the formation of a clear zone around the colony. Ability of microorganisms to degrade the carbon sources was expressed as degradation index (the ratio of diameter of clear zone and colony diameter) (Dida *et al.*, 2018) or zone ratio (Ahmad *et al.*, 2013) or hydrolytic value (Gupta *et al.*, 2012).

In the present study, majority of the selected isolates were able to utilize cellulose, starch, protein and lipid, as indicated by the clear zone around the colonies. Cellulose was more efficiently degraded by fungi, than bacteria. The two reference cultures of fungi, *G. butleri* and *T. asperellum* recorded maximum clear zone on CMC agar. These isolates have already been identified as efficient degraders of cellulose and lignin and hence the former isolate, along with *Bacillus subtilis* is being used in the preparation of composting inoculum (Girija *et al.*, 2016). Lynd *et al.* (2002) observed that, fungi as the good cellulose degrader. Devi and Kumar (2012) had also reported that, among ten cellulase producing fungal isolates, *Aspergillus* sp.



and *Trichoderma* sp. recorded maximum diameter of clear zone. Actinomycete isolates used in the present study were found to be poor in degrading cellulose.

The cellulose degradation index was highest in fungal isolates and the maximum degradation index (1.7) was noted in *G. butleri* followed by *T. asperellum* (1.6). However, many of the earlier workers have reported that bacterial isolates efficiently degraded cellulose. The clear zone produced by mesophilic cellulose degrading bacteria obtained from flower stalk and vegetable waste ranged from 2.55 to 6.40 cm (Lu *et al.*, 2005). Hatami *et al.* (2008) isolated cellulolytic aerobic bacterial isolates from farming and forest soil. The clear zone to colony ratio of the isolates was in the range of 1.38 to 2.33 and 0.15 to 1.37 for isolates from farming soil and forest soil respectively. Gupta *et al.* (2012) recorded higher degradation index values (4.3 to 9) in cellulose degrading bacterial isolates. Ahmad *et al.* (2013) observed 15 cellulose degrading bacterial isolates with clear zone to colony diameter ratio in the range of 2.5 and above.

With respect to lignin, though all the isolates tested were found to produce growth, no clear zone was visible around the colonies. Lignin is known to be a complex organic molecule and hence very slowly decomposed by microorganisms in soil. Due to the complexity of the lignin substrate, the number of microbes in soil, which are capable of efficiently degrading lignin is also reported to be a few (Brown and Chang, 2014; Datta, 2017).

In the case of starch, bacteria were found to be more efficient than fungal isolates tested. The maximum clear zone on starch agar was recorded by the bacterial isolate BaCp-1. Starch degradation index ranged from 1.1 to 1.6. The highest starch degrading index (1.6) was observed in *B. cereus* BaCp-1. Mishra and Behera (2008) recorded maximum clear zone formation by *Bacillus* sp. on starch agar plate. Singh *et al.* (2014) observed highest starch degradation by *Bacillus* sp. Dida *et al.* (2018) found that, starch degrading index of rhizobacteria ranged from 1.23 to 2.15.

The bacterial isolate BaOu-1 produced maximum clear zone on calcium caseinate agar medium with a degradation index of 1.6, indicating its ability to degrade protein. None of the fungal and actinomycete isolates degraded proteins. Narendra *et al.* (2012) noted maximum zone formation around the colony of *Bacillus* sp. during the isolation and characterization of protease producing bacterial isolates. Alnahd (2012) could isolate proteolytic bacteria from marine environment and large areas of clear zone were exhibited by *Bacillus* strain.

None of the isolates obtained from various compost samples in the study degraded pectin and chitin. Only two bacterial isolates (BaBc-1 and *B. subtilis*) were able to degrade lipid. *B. subtilis* BaBc-1 (10 mm) recorded a higher lipid degradation index of 1.3. Bonola and Mangmoori (2013) conducted qualitative assay for screening of lipid degrading isolates and the high zone of clearance was observed in *Bacillus tequilensis.*

All ten microbes screened on lignin sulphonate medium, colloidal chitin medium and pectin agar medium exhibited growth but did not form clear zones. This reflected the lack of ability of isolates to utilize the test substrate in the selective medium. Likewise, *G. butleri* and *T. asperellum* also showed growth and did not develop any clear zone on most of the selective media. This may be due to the growth of fungus from the carbon source available in the inoculum disc (Abdel-Raheem and Shearer, 2002).

Screening of microbial isolates on selective media is only a semi-quantitative test to assess the degradation ability of microorganisms. Quantifying the activity of enzyme or production of enzyme by the microorganisms may be a better tool to assessing the degradation efficiency. Hence, selected eight isolates were tested for enzyme production.

Enzyme assay for cellulase, β-1,3 glucanase, β-glucosidase, laccase, amylase, protease, pectinase and lipase was carried out to quantify the amount of enzyme produced by these selected isolates. It is noted that, isolates which did not exhibit clear zone on selective media also expressed enzyme activity in quantitative assy. Interestingly, all the eight isolates were showed significant difference in enzyme activity except in case of lipase activity.

Cellulase is a group of enzymes including exoglucanase, endoglucanase and β-glucosidase which are known as cellulase complex. This cellulase complex involved in the degradation of cellulose in to glucose (Shuangqi *et al.*, 2011). All these three enzymes contribute to cellulase activity. Therefore, present study was conducted to determine the activity of these three enzymes. Significantly higher cellulase activity was recorded by *G. butleri* and this is in accordance with the result of qualitative assay for cellulose degradation. There are many reports that, fungal cultures are able to show high cellulase activity. Akinola *et al.* (2012) recorded cellulase production of 12 *Trichoderma* strains and the highest production was noted in *T. reesei*.

In the present study, the bacterial isolates *B. subtilis* (KAU isolate), *B. subtilis* BaBc-1 and *Bacillus* sp. BaOu-1 showed maximum β-1,3 glucanase activity. Narasimhan *et al.* (2013) recorded maximum β-1,3 glucanase activity in *B. subtilis* when the study was conducted at 30^0 C and pH 7. Dewi *et al.* (2016) also reported the production of β- glucanase by *B. subtilis* strain SAHA 32.6 and the specific activity was 32.6 U mg^{-1}.

The complex polymer cellulose is degraded by several microorganisms, each producing one or two cellulose degrading enzymes. In case of β-glucosidase activity, *G. butleri* (KAU isolate), *T. asperellum* (KAU isolate), *B. subtilis* BaBc-1 and *B. subtilis* (KAU isolate) recorded more activity. Santos *et al.* (2016) studied the β-glucosidase activity of *G. butleri* and he found that, activity reached extremely high (21.5 U ml^{-1}). β-glucosidase activity of *T. asperellum* was reported by Bech *et al*,

(2015) and Marx *et al*, (2013). According to Akinola *et al.* (2012) β-glucosidase production of *T. viride* was varied at different temperature and maximum production was found at 35^0C (4.7422 x 10^{-7} U ml^{-1}). In the present study β-glucosidase activity of *B. subtilis* BaBc-1 and *B. subtilis* (KAU isolate) were 0.28 U ml^{-1} and 0.27 U ml^{-1} respectively. Similar results have been recorded by Bagudo *et al.* (2014) and the β-glucosidase activity of *B. subtilis* was 0.23 U ml^{-1} when glucose used as the carbon source.

Laccase is one of the ligninolytic enzymes which are involved in the degradation of lignin. In the present study, significantly higher laccase activity was noticed in *Bacillus* sp. BaOu-1. Muthukumarasamy *et al.* (2015) examined *Bacillus subtilis* MTCC 2414 to optimize the production of laccase enzyme at different condition such as using different agrowaste substrates, temperature and pH. Guo *et al.* (2017) reported that, laccase producing novel strain of *Bacillus* sp. was isolated from forest soil and the maximum laccase activity by *Bacillus* sp. was in LB (Luria-Bertani) medium (3.9 U ml^{-1}). The present study in which laccase activity was maximum in *Bacillus* sp, BaOu-1(6.33 U ml^{-1}), is in agreement with this report.

In the present study, *S. roseofulvus* AcOu-1 showed amylase activity of 0.25 U ml^{-1}. Islam *et al.* (2014) also found *Streptomyces* sp. as good source of amylase. They isolated a total of 24 indigenous *Streptomyces* sp. from different part of Bangladesh and the highest activity was recorded as 2.67 U ml^{-1}. Soil samples collected from mangrove areas of Muthupetu were subjected for isolation of *Streptomyces*. Out of 23 isolates, eight of them indicated positive results for amylase activity, both in qualitative and quantitative studies (Rengasamy and Thangaprakasam, 2018). Likewise, Ragunathan and Padhmadas (2013) also suggested that, *Streptomyces* sp. can be very good source for amylase production.

The protease activity in the present investigation indicated that, *G. butleri* (KAU isolate) and *T. asperellum* (KAU isolate) were good candidates for protease

production to breakdown the organic waste substrates. Whu *et al.* (2017) reported that, *T. asperellum* GDFS1009 could secret protease enzyme. Gajera and Vakharia (2012) found 1.41 U ml^{-1} of protease production by *T. harzianum* isolates 1051 and TVC after 72 hours of growth. The results presented by Simkovic *et al.* (2008) suggested that *T. viride* grown in the presence of proteins could secrete proteases from the starting of the exponential growth stage, whereas in the absence of inducer, protease secretion was observed in the stationary phase. Kredics *et al.* (2005) also observed the protease activity exhibited by *T. viride*.

The isolate *B. subtilis* BaBc-1 exhibited significantly higher pectinase activity (19.85 U ml^{-1}) in this study. Several reports showed the potentiality of *Bacillus* sp. in the production of pectinase and its hydrolytic action. Tripathi *et al.* (2014) recorded maximum specific activity of pectinase (4.5 U mg^{-1}) from *Bacillus subtilis*. The isolation of test bacteria was carried out from soil and mixed fruit waste sample. Furthermore, Mathur *et al.* (2014) also purified pectinase from *Bacillus subtilis*. Sridevi *et al.*, (2015) could isolate pectinolytic *Bacillus* strain from fruit waste dumping yards of mango industry. All these studies revealed that, *Bacillus subtilis* could serve in the production of pectinase enzyme, to degrade pectic substances in the biosolid waste.

In the present study, all the tested isolates exhibited lipase activity. Microbial lipase has recently gained more attention because of its availability, yield and catalytic nature. Studies showed the evidence for lipase activity exhibited by microorganisms. Bonala and Mangamoori (2013) isolated *Bacillus tequilensis* Nrrl B-41771 from oil mill waste and this isolate showed highest lipase activity of 40 U ml^{-1} at a temperature of 35^0C and pH of 7. Present study recorded maximum lipase activity of 3.57 U ml^{-1} and this difference may be due to the variation in temperature and incubation period.

The six microbial isolates, which exhibited maximum enzyme production were selected for further studies.

Cultural, morphological and biochemical characterization of the ten isolates under the present study revealed that, the bacterial isolates were Gram positive. They were also characterized by the presence of endospores. The three bacterial isolates (BaBc-1, BaCp-1 and BaOu-1) showed resemblance with *Bacillus* and the actinomycete colony exhibited characters similar to *Streptomyces*. The results of cultural, morphological and biochemical studies of the present study is supported by results of Sahu (2016) and Rajeswari (2017).

In order to confirm the results of cultural, morphological and biochemical characterization, the three bacterial isolates and actinomycete were further identified by 16S rRNA gene sequencing. 16S rRNA gene sequencing is a powerful tool that has been used to trace phylogenetic relationships among bacteria, for species definition and identification of bacteria (Rossello-Mora and Amann, 2001; Clarridge, 2004; Raoult *et al.*, 2004). Sequence analysis by BLASTn revealed the accessions in NCBI database, showing homology with the query sequences. Accordingly, the isolates BaBc-1, BaCp-1, BaOu-1 and AcOu-1 identified as *Bacillus subtilis*, *B. cereus*, *Bacillus* sp. and *Streptomyces roseofulvus* respectively. These relationships were further illustrated by creating a phylogenetic tree and the results of phylogenetic analysis were similar to that of 16S rRNA homology analysis. All these isolates have been earlier reported to be potential agents for decomposition. There are several studies on the efficacy of *Bacillus* sp. in composting process. Girija *et al.* (2011) isolated *Bacillus* sp. from cow dung and the findings supported the suitability of these bacteria in decomposition of different agricultural waste. Ryckebore *et al.* (2003) noticed that *Bacillus* was most predominant bacteria during decomposition of vegetable, fruit and garden wastes. The actinomycete *Streptomyces* sp. is well known to the decomposition of lignin (Mc Carthy and Broda, 1984).

Several fungi like *Trichoderma harzianum*, *Pleurotus ostreatus*, *Polyporus ostriformis* and *Phanerochaete chrysosporium* are known to play important role in degradation of organic matter (Singh *et al.*, 2012). Zayed and Motaal (2005) found that, *Trichoderma* sp. as good lignocellulose degrader during composting of lignocellusic substrates. Rapid decomposition of organic municipal solid waste was achieved by the application of *T. viride* (Gautam *et al.*, 2012).

For formulating a successful microbial consortium containing two or more than two microorganisms, it is important to have compatibility among them. Compatibility of selected bacterial isolates, actinomycete and the reference fungal cultures were tested by cross streak and dual culture method. Two bacterial cultures were tested by cross streak method and on bacterium with a fungus was carried out by dual culture method. Any inhibition of any one of the cultures by the other was observed. The results indicated that all the tested isolates were compatible with each other except *B. subtilis* BaBc-1 vs *S. roseofulvus* AcOu-1, *Bacillus* sp. BaOu-1 vs *B. subtilis* (KAU isolate) and *T. asperellum* (KAU isolate) vs *G. butleri* (KAU isolate). Similar study was conducted by James and Mathew (2017), who evaluated the mutual compatibility between bacterial isolates by cross streak method and found no lysis at the juncture. Compatible actinomycetes were also found in the same study. They tried dual culture method to evaluate the mutual compatibility of fungal isolates and noticed many incompatible isolates and five highly compatible isolates.

The fungal isolates *G. butleri* and *T. asperellum* were found to be mutually incompatible, as indicated by an inhibition per cent of more than 50. *T. asperellum* inhibited *G. butleri*. *Trichoderma* spp. have long been recognized as biocontrol agents and it parasitizes other fungi through antibiotic production (Ha *et al.*, 2010; Anita *et al.*, 2012). Mohammed *et al.* (2011), reported compatible, partially compatible and incompatible reactions among fungal isolates, during evaluation for oil palm waste degradation. A study was conducted by Balasundaram (2009) for

developing microbial consortium to degrade different kinds of organic waste such as ayurvedic herbal waste, coir pith and saw dust. Compatibility was observed between *Aspergillus* sp., *Bacillus* sp. and *Streptomyces* sp. Mary (2015) reported the compatibility of thirty six combinations of nine fungi for the preparation of microbial consortium for composting. The main benefit of using compatible isolates in composting is to overcome the slow degradation activity and accelerate the decomposition process by their mutual action.

Based on the enzyme activity and compatibility, suitable combinations of six potential isolates were selected, and four potential microbial consortia were formulated to evaluate the *in vitro* efficiency of selected isolates. Inclusion of at least one microorganism capable of degradation of each of the components *viz.* cellulose, starch, protein, pectin and lipid, in each consortium, was ensured. Consortium I (*B. subtilis*, *G. butleri* and *S. roseofulvus* AcOu-1), consortium II (*B. subtilis* BaBc-1 and *T. asperellum*, *Bacillus* sp. BaOu-1), consortium III (*B. subtilis* BaBc-1, *G. butleri* and *Bacillus* sp. BaOu-1) and consortium IV (*B. subtilis*, *G. butleri* and *B. subtilis* BaBc-1) were T_1, T_2, T_3 and T_4 respectively. All these microbial consortia were evaluated for their efficacy to degrade biosolid waste under *in vitro* conditions for three weeks, with an uninoculated control (T_5). Rapid degradation (as indicated by the formation of slurry) was observed in all the inoculated treatments on third day of incubation. Maximum degradation was noticed in T_2 and T_4 on 21st day after inoculation and this reflected the suitability of these microbial consortia for biosolid waste degradation in flask culture. Furthermore, these isolates have proven their degradation efficiency in qualitative and quantitative assays. In uninoculated control, degradation was initiated on 6 days after inoculation and was incomplete even after 21 days of incubation. Steam sterilization of vegetable biomass might have destroyed all microflora and this may have delayed decomposition process in uninoculated control.

Sahu (2016) conducted a study to evaluate the potentiality of 11 fungal isolates and 6 bacterial strains for degradation of kitchen waste. The visual rate of decomposition revealed that, only four fungal isolates (three isolates of *T. viride* and one isolate of *T. harzianum*) and one bacterial strain were efficient in decomposition of kitchen waste. Similar study was conducted by Sarkar *et al.* (2011) where they carried out lab trial for degradation of kitchen waste using different consortia and the observation on visual rate of degradation (for 15 days) indicated that, inoculated treatments showed more degradation efficiency which were further selected for large scale experiment.

Assay for three major enzymes involved in organic matter degradation (cellulose, amylase and protease) was carried out after 3 weeks of incubation. All the treatments recorded activity for cellualse, amylase and protease. Cellulase activity was found to be maximum in T_4 (*B. subtilis* + *G. butleri* + *B. subtilis* BaBc-1). This may be due to the presence of isolate *G. butleri* in the consortium, which is a good producer of cellulase enzyme. Similarly, the isolates *B. subtilis* BaBc-1 and *B. subtilis* (KAU isolate) in T_4 are good producers of β-1,3 glucanase and β-glucosidase. There are several reports on cellulase activity of *G. butleri* and *B. subtilis*. Rawat and Tewari, (2012) reported that due to the high level secretion of extracellular cellulolytic enzymes by *Bacillus subtilis* it is considered as a major microorganism for cellulase production. Cellulase activity of *Bacillus subtilis* was also reported by Deka *et al.* (2013); Siu-Rodas *et al.* (2018) and Sreena and Sebastian, (2018).

The highest amylase activity in T_2 (*B. subtilis* BaBc-1 + *T. asperellum* + *Bacillus* sp. BaOu-1) and T_1 (*G. butleri* + *S. roseofulvus* AcOu-1 + *B. subtilis*) might be to the presence of *Bacillus* sp. BaOu-1 and AcOu-1 in their respective consortium. Islam *et al.* (2014) observed that *Streptomyces* sp. is a good source of amylase. Likewise, activity of *T. asperellum* could have increased the activity of protease in T_2

(*B. subtilis* BaBc-1 + *T. asperellum* + *Bacillus* sp. BaOu-1). Simkovic *et al.* (2008) and Kredics *et al.* (2005) observed the protease activity of *T. viride*.

Population of microorganisms in the decomposed biosolid waste indicates the efficiency of decomposition process. Microbial population of biosolid waste inoculated with consortia was assessed after 21 days of incubation. The presence of microbes in degraded biosolid waste can be considered as a major parameter for dynamics of composting (Tiquia *et al.*, 2001). In the present investigation, the treatment T_4 (*B. subtilis* + *G. butleri* + *B. subtilis* BaBc-1) recorded significantly higher bacterial population whereas T_2 (*B. subtilis* BaBc-1 + *T. asperellum*+ *Bacillus* sp. BaOu1) recorded significantly higher fungal population. Actinomycete population could not be detected in any of the treatments, including T_1 which included the actinomycete AcOu-1. Earlier *in vitro* experiments proved that this isolate produced maximum quantity of amylase, it may have not been able to survive in the vegetable waste contained in flasks, indicating that it may not be a candidate isolate for further experiments.

Based on visual observations, per cent weight reduction, enzyme activity and microbial population on 21 DAI in flask culture, the best two consortia (T_2- consortium II and T_4- consortium IV) were selected for pilot scale experiment. In T_2 and T_4, the vegetable waste showed complete degradation on 21st day of incubation and maximum weight reduction. Amylase and protease activity were maximum in T_2, whereas T_4 recorded maximum cellulase activity. Fungal population was maximum in T_2 and bacterial population in T_4. Therefore these treatments were selected for the pilot scale experiment under *in vivo* conditions. Game *et al.* (2017) also evaluated microbial consortia containing *Bacillus* sp., *Aspergillus terreus* and *Streptomyces* sp. on rural and urban waste materials under *in vivo* composting experiments.

The fact that microbes are able to degrade a particular component in the nutrient media under *in vitro* condition does not necessarily confirm their potential for the

same under *in vivo*. Therefore, their efficiency was evaluated using KAU Smart Biobin. This is a device developed in the Department of Agricultural Microbiology, Kerala Agricultural University, Thrissur, to promote decentralized, at source management of household kitchen waste (Girija *et. al.*, 2016). It promotes aerobic degradation of kitchen waste, with the help of microbial consortia, impregnated with coir pith. This has been popularized by Thrissur Corporation in 2016 and 2017 and about 1500 units have been distributed.

The best two treatments in the previous experiment were selected as T_1 and T_2. There was also a positive control with cow dung slurry (T_3) and a negative control with no source of microbes (T_4). Volume reduction of biosolid waste after 30 days was maximum in T_1 (*B. subtilis* BaBc-1 + *T. asperellum* + *Bacillus* sp. BaOu-1) and T_2 (*B. subtilis* + *G. butleri* + *B. subtilis* BaBc-1), which were statistically on par. Minimum reduction in volume was found in uninoculated treatment. Many researchers have opined that, self-heating activity during aerobic bio-degradation could reduce the volume of waste by 40 to 50 per cent of the actual volume, which results in good quality compost (Finstein and Morris, 1975; Pare *et al.*, 1999). Similar results were also obtained in a study conducted by Chanurungroung *et al.* (1998). Khan (2016) noticed high volume reduction in effective microorganisms (EM) treated agricultural waste and municipal waste, with the per cent volume reduction of 42 and 38 respectively. The uninoculated treatments showed significantly low volume reduction of 27 per cent in agricultural waste and 23 per cent in municipal waste.

Temperature being a significant factor in composting process, daily temperature was monitored in the current investigation. All the treatments recorded a gradual increase in temperature, reached maximum (42.24 - 48.32^{0}C), and thereafter, there was a gradual decline. The treatment T_2 recorded the maximum temperature after three days. The temperature stabilized after 24 days. Under optimal conditions, composting generally proceeds through three phases mesophilic, thermophilic, or high-temperature phase, and finally cooling and maturation phase (Amir *et al.*, 2008;

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Zeng *et al.*, 2009). Tiquia (2005) observed that temperature increased rapidly from 31 to 65^{0}C in compost piles after 2-4 days and later became stable to an ambient range. Initial increase of temperature might be due to fast degradation and mineralization of simpler organic compounds (Rajeswari, 2017). Adhikari *et al.* (2009) suggested that temperature above 45^{0}C (thermophilic temperatures) should be attained within a few days of composting process. Prolonged high temperature (55-60^{0}C) about five to six weeks was lasting the degradation process and delaying the maturation stage of composting (Zucconi and de Bertoldi, 1987). Balasundaram (2009) reported a temperature range of 30 to 35^{0}C, during final maturation stage of composting. The observed final temperature in the present study gave assurance that final product is safe for use in agricultural uses.

Nolan *et al.* (2011) reported that temperature reached a maximum of 60^{0}C on second day of composting, followed by 1 to 2 weeks of thermophilic stage and finally the temperature turned down during the cooling stage. According to Gowda (1996) the efficient decomposition proceeds at high temperature of 40-70^{0}C range. Composting releases exothermic energy which can generate more heat to create a favorable environment for thermophilic organisms (Gaur, 1997).

Rapid degradation of biosolid waste in a period of 17 days was observed in T_1 (*B. subtilis* BaBc-1 + *T. asperellum* + *Bacillus* sp. BaOu-1). However, compost formation was initiated in uninoculated control (T_4) only after 40 days of inoculation. Rapid decomposition of biosolid waste with the help of microbial consortia has earlier been reported in several studies. Vegetable waste, flower waste, fruits waste and municipal solid waste could be completely decomposed within 30 days, using microbial constoria (Kalaivani *et al.*, 2011). The study of Kausar *et al.* (2014) using *Trichoderma* and *Aspergillus* inoculated rice straw, showed early bioconversion within two to three weeks as indicated by physical and chemical parameters. According to Khan (2016) uninoculated municipal solid waste took 65 days for

maturation of compost whereas EM treated municipal waste was took nearly half the maturation time of uninoculated treatment. In the same study, composting period of agricultural waste could be reduced from 82 to 51 days, with the help of EM.

Sarkar *et al.* (2011) conducted lab trials for the degradation of kitchen waste using best three consortia. Fifty per cent degradation was achieved within 21 days, at the same time control recorded only 36 per cent degradation. Game *et al.* (2017) inoculated rural waste with microbial consortium, it took only 92 days for complete degradation and this took 22.68 per cent less time period than that of control treatment. Similar trend was noticed during the composting of urban waste. Test consortium treated waste reduced the decomposition period to 71 days, since the per cent decrease in time period over commercial consortium and uninoculated control was about 18.39 per cent and 10.12 per cent respectively. Gaur (1982) also found that, inoculation of mesophilic fungi could reduce the duration of composting by one month. Decrease of composting period because of application of cellulolytic microorganisms in the composting process has been reported by Raut *et al.* (2008) and Iqbal *et al.* (2010). All these studies revealed that, it is possible to enhance the degradation rate as well as degradation period with help of efficient microbial consortium.

With respect to yield of compost from 32 kg of biosolid waste, highest yield and recovery of compost were recorded in T_1 and T_2. Undecomposed materials were also significantly lower in inoculated treatments. This might be due to the efficient conversion of biosolid waste in to compost by inoculated microorganisms.

The moisture content in finished compost from cow dung treatment possessed high moisture content compared to all other treatments. According to Anthonis (1994) the best range of moisture content in mature compost was about 15-25 per cent. In the present study, treatment with microbial consortium and uninoculated control recorded optimum range of water content. Indumathi (2017) recorded a

moisture content of 33 per cent in *Bacillus* sp. treated vegetable waste compost, at the same time moisture content of control treatment was 41 per cent.

Bulk density in all the compost samples were within the recommended range of FCO standards (<1 g cm^{-3}). Other physical properties of compost such as colour and odour of compost did not show much variation among compost samples. It was dark brown without any foul odour.

Several chemical parameters have been used to estimate the quality of compost including pH, EC, OC, C:N ratio, N, P, K, Cu, Zn and heavy metal contents. In the present investigation, pH of all the compost samples were found to be in the alkaline range (8.7 to 10.28) particularly the two treatments which were inoculated with microbial consortia. As per the Fertilizer Control Order (FCO, 1985), the pH of the final product must be preferably between 6.5 and 7.5. The pH observed in the experiment was much higher than the specified values. However, this could be beneficial with respect to Kerala soils, which are acidic in nature. Compost obtained with a high pH could act as ameliorant, to correct the soil acidity. Many studies revealed that microbial activity in compost can results in alkaline pH. Zucconi and de Bertoldi (1987) opined that increased pH of compost is due to the release of ammonia by breakdown of proteins. McKinley and Vestal (1985) also concluded that, decomposition of organic acids with the involvement of microorganisms can generate ammonia by volatilization and further rise in pH of compost.

In the present study, high EC was found in all the compost samples (8.43 to 16.25 dS m^{-1}). It was higher than optimum range (<4 dS m^{-1}). The biosolid waste collected from the vegetable market was used for the present study. The source of biosolid waste may be a reason for increased EC. According to Rajeswari (2017) high EC in compost may be due to the release of mineral salts at the time of organic matter decomposition.

All the compost samples were found to have organic carbon in the optimum range (FCO standard for OC >14 per cent). The C:N ratio in compost samples ranged from 8.22 to 11.44 :1. The carbon to nitrogen (C:N) ratio is one of the important factors affecting the composting process as well as the properties of the end product, C:N ratio between 25:1 and 30:1 is usually considered as the optimum ratio for composting (Zhu, 2007; Chang and Hsu, 2008). Pan *et al.* (2012) composted common organic waste with microbial consortia containing *Bacillus subtilis* and *Pseudomonas* sp. and the C: N ratio of finished compost was observed in the range of 25 – 30:1. Similar findings were reported by Brito *et al.* (2008) and Chandna *et al.* (2013). A wide C:N ratio of compost may affect the standing crop, as soil microflora will utilize the N available and result in N deficiency symptoms on the crop (Jakobsen, 1994; Baker, 1997).

Nutrient content of compost is an important factor to consider, hence it is essential to evaluate the N, P, K and micronutrients (Cu and Zn) status in compost. No significant difference was recorded in the primary nutrient (N, P and K) content and all the samples recorded optimum range. Cu content differed significantly among compost samples, but was well below the FCO specified limit of 300 ppm in all the samples. The maximum Cu content was observed in T_3, in which cow dung was used as the source of microbes. This might be due to the presence of copper in cow dung, as reported by Ntui *et al.* (2014). Zn content was also within the FCO prescribed limit of 1000 ppm. Heavy metals Cd and Ar was absent in all the treatments. Traces of Pb, Ni and Cr were observed in all four treatments, which were less than maximum permissible limits (maximum permissible limit for Pb- 100 mg/ kg, Cr and Ni-50 mg/ kg).

Among the various groups of microorganisms, bacteria was predominant in all compost samples. The treatments T_1 and T_2 recorded maximum population of bacteria, fungi and actinomycetes. According to Umsakul (2010) during composting

of water hyacinth, higher bacterial population was recorded, as compared to that of fungi and actinomycetes. Actinomycetes population was observed only in treatments inoculated with microbial consortia. None of the consortia contained actinomcetes and therefore, these could have been introduced by coir pith, which was used as a carrier material in the inoculum. Presence of actinomycetes in coir pith has been reported earlier by Paramanandham and Ross (2016). Higher population of microbes in compost inoculated with consortia clearly indicates that these microbes are efficient in organic matter degradation. This also indicates the possibility of reusing the compost as inoculum for subsequent composting process. This will also enable the self-sustenance of such aerobic composting units, since there is no need to purchase the inoculum regularly from an external agency.

Earlier reports also indicated the ability of *Bacillus* sp. to survive in hot compost (Blanc *et al.* 1997). Umsakul (2010) concluded that presence of EM in compost could produce sugars, proteins and peptides in available forms which will further increase the number of microorganisms. Survival capacity of *Bacillus* sp. under varied conditions was reported by many researchers and they also mentioned that, it can continue to live in compost pile because of their adaptability to mesophilic temperature.

Phytotoxicity indicates the presence of organic chemicals in mature compost which can hinder the germination of seeds and growth of plants. In the present experiment, all the compost samples were non-phytotoxic, as indicated by germination of more than 80 per cent in tomato seeds. Higher germination per cent was observed in T_1 and T_4. According to the report of Zucconi *et al.* (1985) and Emino and Warman (2004), below 50 per cent germination index indicates high phytotoxicity; 50 to 80 per cent, moderate level of phytotoxicity and more than 80 per cent, the absence of phytotoxicity. Sahu (2016) studied the effect of kitchen waste compost prepared by different decomposing microbial isolates on seed germination.

The results indicated non-pytotoxicity, with highest per cent seed germination in *T. harzianum* (98.33 %) followed by *T. viride* (98 %) treated compost.

Presence of human pathogen in compost also considered as an important biological parameter. Standards of the US-EPA suggested that, class A composts should not go beyond maximum limits for *Salmonella* sp. and thermotolerant coliforms (less than 3 MPN/4 g and 1000 MPN g^{-1}). Results of the present study showed that all five treatments were free of human pathogens (*E. coli, Shigella* sp. and *Salmonella* sp.). It has been reported earlier, the increased temperature during composting, suppressed the growth of pathogens in compost (Ryckeboer *et al.*, 2003).

The results of pilot scale experiment indicated that with regard to volume reduction, duration of composting process, yield of compost, microbial population and phytotoxicity of compost, T_1 (consortium I) performed as the best consortium. Hence, this consortial formulation was selected for large scale experiment in Thumburmuzhi composting units.

Thumburmuzhi model aerobic composting unit was developed by Dr. Francis Xavier, at Kerala Veterinary University, Thrissur for composting of dead cattle using cow dung as inoculum. Later cow dung was replaced by microbial inoculants based on studies conducted at the Department of Agricultural Microbiology, KAU, Vellanikkara. This technology is now being practiced for municipal solid waste recycling in large scale in various municipalities and corporations of Kerala, for municipal solid waste management.

In the present investigation, volume reduction of biosolid waste, recorded at different time intervals (15, 30, 45 and 80 days), was found to be 63 to 76 per cent. However, there was no significant difference among treatments, in volume reduction. Indumathi (2017) observed clear decrease in volume ($3/4^{th}$ of the original volume) of vegetable waste on application with inoculum.

The variation in temperature followed the similar trend as that of pilot scale experiment. The temperature gradually increased, reached the maximum on 3rd or 4th day and then declined to a stable range. The maximum temperature was observed in T_1 (64^0C) on 3rd day. This might be due to the increased microbial activity during composting. During the last week of composting, temperature declined to 32.62 to 40.52^0C. The final temperature was higher (40.52^0C) in T_3 as compared to other treatments, which indicated that the decomposition process was still going on in control. This is in agreement with the report of Tiquia (2005) that the temperature increased rapidly from an initial value of 31^0C to 55^0C in two days during composting. The maximum temperature recorded was 69^0C and after a period of rise in temperature, it stabilized to an ambient range. Similar observations were made by Game *et al.* (2017), where the maximum temperature (60.2 to 63.4^0C) was attained in the compost pits within seven days and hereafter it decreased gradually. According to Taiwo and Oso (2004) highest temperature during first week of composting was 70^0C. Similar trend has also been reported by Gazi *et al.* (2007); Goyal *et al.* (2005); and Himanen and Hanninen (2011).

Yield of compost obtained from Thumburmuzhi composting units varied significantly, among treatments. Maximum yield and compost recovery were observed in T_2 (104.17 kg and 13.03 per cent). This yield increase in T_2 could be attributed to the addition of 100 kg cow dung along with biosolid waste, as a source of microbes, in this treatment. These results are in agreement with the report of Narayanan (2015), who recorded maximum compost yield from cow dung added treatment followed by inoculated with EM and *B. subtilis*.

Analysis of moisture content in compost revealed that, all the treatments contained moisture below the maximum level specified in FCO (<25 %). The bulk density of all compost samples were below 1 gcm^{-3} which indicated better porosity of compost samples. All other physical properties of compost such as colour and odour

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did not show much difference. All the compost samples were dark brown and free of foul odour.

pH of the compost samples were in the alkaline range (8.07 to 8.74), as in case of pilot experiment. T_2 recorded significantly higher pH of 8.74. Rajeswari (2017) also reported that, the range of pH recorded in the matured compost was 6.3 to 8.4.

EC was within the recommended limit of FCO standard (<4 dS m^{-1}), for the treatments T_1 and T_2, whereas in uninoculated treatment, EC was slightly higher (4.69 dS m^{-1}). Similar findings were obtained in the study of Bakari *et al.* (2016) and the EC of EM inoculated compost was in the range of 3.59 to 4.48 dS m^{-1}. In uninoculated control, a much higher EC of 6.21 dS m^{-1} was recorded.

Microorganisms obtain their energy from organic carbon and hence its content in compost is important. The organic carbon content in the present study ranged from 18.99 to 30.13 per cent. Inoculated treatment (T_1) recorded minimum organic carbon content and maximum was recorded in uninoculated treatment (T_3). Degradation of waste substrates by microorganisms causes a reduction in organic carbon during composting (Mondini *et al.*, 2003). The decrease in organic carbon content during composting might be a direct indication of organic substrate decomposition. Studies conducted by Gupta *et al.* (2004); and Naik (2007) showed that, the inoculation of different microbial consortia resulted in rapid decomposition with decreased organic carbon content. Findings of the present study are comparable with the results obtained by Narayanan (2015), where the organic carbon content ranged from 14.39 to 23.11 per cent. *B. subtilis* inoculation resulted in an organic carbon content of 18.18 per cent.

The C:N ratio of compost samples ranged from 11.37 to 24.73:1. The minimum C:N ratio of compost was recorded in treatment with microbial inoculum.

This is indicative of the efficiency of the microorganisms present in the consortium. In uninoculated control C:N ratio (24.73:1) was slightly higher than recommended limit of FCO standard (<20:1). This could be indicative of a slow degradation process due to lack of efficient microorganisms in this treatment. Natural microbial flora could be present in vegetable waste, but these may not be efficient in degrading organic substrates. Hardy *et al.* (1993) suggested that, the C:N ratio in the range of 25 to 30:1 indicated the end of composting process. Balasundaram (2009) also reported that, the C:N ratio of matured compost from ayurvedic herbal waste, weed waste and coir pith were 10.31:1, 9.78:1 and 18.63:1 respectively.

Nitrogen content was found to be sufficient in all the compost samples (1.38 to 2.01 per cent) and significantly higher nitrogen content was noted in T_2 (cow dung added treatment). The compost might be useful to promote plant growth, as it contains sufficient level of nitrogen (Keeling *et al.*, 1995; Namasivayam and Bharani, 2012). Narayanan (2015) also observed significantly higher nitrogen content (2.13 %) in cow dung added treatment.

Phosphorous content ranged from 0.16 to 0.23 per cent, which was less than the FCO specified level (>0.4 per cent). The available P content in Kerala soils is high and hence, the low content of P in compost could not pose any problem (Krishnakumar, 1991; Sureshkumar, 1999). Narayanan (2015) reported a phosphorous content of 0.18 to 0.33 per cent in compost.

Potassium content in compost samples varied from 0.16 to 0.79 per cent. The lowest value was recorded in T_3 (uninoculated control). For T_1 and T_2, K content was sufficient in the finished product (FCO specification >0.4 per cent). Khater (2012) revealed that, cattle manure and sugar cane plants residues (50:50) compost showed a potassium level of 0.62 per cent. According to Khan (2016) nitrogen, phosphorous and potassium content of municipal waste compost treated with EM were 1.87, 0.56 and 0.51 per cent respectively.

Cu and Zn status of the compost samples were below the limits specified in the FCO (<300 and <1000 ppm respectively). Among the samples, significantly higher Cu (24.7 ppm) content was recorded in T_3 (uninoculated control), followed by T_1 (23.21 ppm). Zn content was also significantly high in T_3. Narayanan (2015) also reported that Cu content in compost was 25.3 ppm in *B. subtilis* inoculated treatment.

Heavy metal content in compost samples (Pb, Cr and Ni) was within the maximum permissible limits (Pb <100 ppm, Cr and Ni <50 ppm) specified by the FCO. Absence of heavy metal contents (Cr, Cd, Ni, Pb, Hg) in compost was earlier reported by Ignatowic and Brenko (2011). Mary (2015) also carried out analysis of heavy metals in compost and detected traces of Cr and Ni in ayurvedic compost and Pb in all the samples which were within the acceptable limit.

Total microbial population in the compost samples after 80 days of inoculation showed that T_1 recorded highest bacterial and fungal population (45.67 x 10^6 cfu g^{-1} and 29 x 10^4 cfu g^{-1} respectively). Actinomycetes were absent in all the treatments. This may be due to the dominance of bacteria and fungi, which could have affected the growth of actinomycetes. There is also a possibility that actinomycetes population increased during mesophilic stage, but decreased during thermophilic stage, at a temperature of 64^0C. Chandna *et al.* (2013) also reported that bacterial population showed differences between mesophilic, thermophilic and maturing stages of compost. By using metagenomic tools for assessing bacterial diversity, they were able to detect actinomycetes (6.1 per cent). This indicated that unculturable bacteria including actinobacteria were present in compost and detection of these may require metagenomic analysis.

Higher microbial population in inoculated treatment is a clear indication of the survival and proliferation of microbes in the consortial formulation. The ambient temperature in the inoculated treatment during final stage of composting might have also contributed to the population of mesophilic microorganisms. These findings are

in agreement with the reports by Game *et al.* (2017), who observed highest microbial population in test consortium inoculated treatment during composting of urban waste. The population of bacteria was found to be higher compared to the population of fungi and actinomycetes. Ryckeboer *et al.* (2003) suggested that generally bacterial population were higher than fungal population due the difference in growth rate. Dominance of bacteria might be due to their smaller size and more surface area, which allows quick absorption of soluble substrates by bacteria.

Compost from all the treatments recorded 100 per cent germination of tomato seeds, indicating the absence of phytotoxicity. The per cent germination was 100 in all the treatments, whereas in the compost collected from biobin in the earlier experiment, the corresponding value ranged from 83.33 to 100. This could be attributed to the longer period (80 days) given for composting process in Thumburmuzhi units, when compared to that in biobin (30 days).

Compost samples obtained were evaluated for the presence of human pathogens. Presence of *E. coli*, *Salmonella* sp. and *Shigella* sp. could not be detected in any of the treatments. These results are in accordance with the study conducted in KAU biobin. The high temperature in Thumburmuzhi units could be the reason for the absence of human pathogens. Weil *et al.* (2013) reported that the population of human pathogens (*Listeria, E. coli* and *Salmonella*) introduced in the substrate, was reduced to non-detectable levels during the second phase of composting. All the human pathogens disappeared beyond a temperature of 54.4^0C. Hassen *et al.* (2001) found that, population of *E. coli* was decreased as a result of high temperature (60-65°C) and population of *Salmonella* and *Shigella* disappeared completely from compost due to a temperature of 55°C.

The large scale experiment conducted in Thumburmuzhi composting units revealed that, consortium I inoculated treatment, T_1 (*B. subtilis* BaBc-1+ *T.asperellum*+ *Bacillus* sp. BaOu-1) developed maximum temperature (64^0C) during

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composting period, recorded higher volume reduction of biosolid waste and maximum microbial population in compost.

The economics of composting in Thumburmuzhi composting unit was estimated, BC ratio was favorable for T_2. The cost of cow dung was comparatively less and the yield of compost was also high in T_2, this is the major reason for maximum BC ratio. However, the availability of cow dung is an important factor to consider. The use of microbial consortium could be recommended in urban and peri-urban areas, where the availability of cow dung is a problem. Moreover, the major objective in bio-solid waste management is to reduce the volume of the waste and recycle it into usable organic manure, through an eco-friendly method.

Hence, after considering all the results of the experiment the following conclusion can be made from the present study. Efficient microflora was isolated from different compost samples and subjected to qualitative and quantitative enzyme assay. The selected isolates were used for the formulation of microbial consortia, and then tested for their ability to degrade biosolid waste *in vitro*, pilot scale and large scale in Thumburmuzhi model composting units. The good quality compost with desirable physical, chemical and biological properties was obtained within a short period of composting.

The highly efficient microbial isolates obtained in this study could be used for the management of agricultural and municipal solid wastes in large scale. Apart from the usage of these isolates in waste management, possibilities of antagonistic activities against plant pathogens could also be studied in future, as many of the isolates were earlier reported to possess antagonistic properties. Future studies could be conducted to explore the possibilities of imparting thermal and drought tolerance to plants. As the composting process involved a thermophillic phase with the highest temperature peak reaching 64^0C, the isolates might also possess thermal tolerance that could efficiently be used for imparting abiotic stress tolerance. The degradation

capacities found to be high in theses isolates could indicate that these are only a part of the wide spectrum of activities they could perform. These isolates could also be screened and further exploited for various plant growth promoting activities and antagonistic properties against soil borne plant pathogens, so that we could derive multiple benefits from these microorganisms.

Summary

6. SUMMARY

Large quantities of organic waste are generated from municipalities, households, flats and various industries. Management of this biosolid waste has become a serious environmental and economic issue. Composting is considered as the suitable treatment method for conversion of organic waste into simpler compounds. Efficient microorganisms that degrade components of biosolid waste can accelerate the process of composting.

The study entitled 'Microbial inoculants for enhancing degradation of biosolid waste in aerobic composting' was conducted in the Department of Agricultural Microbiology, College of Horticulture, Vellanikkara, during 2016- 2018. The major objective of the study was exploring the possibility of enhancing degradation of biosolid waste using microbial inoculants in aerobic composting. The main findings of the study are summarized below.

- For the isolation of microorganisms capable of enhancing degradation of biosolid waste, samples were collected from four different sources *viz.,* KAU smart biobin compost, coir pith compost, Oushadhi ayurvedic and kitchen waste compost.
- Maximum bacterial population was recorded in compost from KAU biobin, fungal population was highest in kitchen waste compost and Oushadhi waste compost recorded maximum actinomycetes population.
- A total of fourteen isolates including bacteria, actinomycetes and fungi were obtained from the compost samples, for further experiments.
- Two bacterial isolates (*Bacillus subtilis* and *B. niabensis*) and two fungal isolates (*Gongronella butleri* and *Trichoderma asperellum*) available in the repository of the Department of Agricultural Microbiology, College of Horticulture, Vellanikkara were also used as reference cultures for screening.

- Screening of efficient microorganisms for degradation of major components in biosolid waste (cellulose, lignin, starch, protein, pectin, chitin and lipid) was performed on selective media.
- Based on the ability to degrade the chemical components in the selective medium, four isolates (BaBc-1, BaCp-1, BaOu-1 and AcOu-1) and four reference cultures (*Bacillus subtilis*, *Bacillus niabensis*, *Gongronella butleri* and *Trichoderma asperellum*) were selected for quantitative assay.
- Enzyme assay was carried out for selected microbial isolates to determining the activity of cellulase, laccase, amylase, protease, pectinase and lipase. The isolate *G. butleri* exhibited highest cellulase activity. BaBc-1, *B. subtilis* and BaOu-1 recorded significantly higher β- 1, 3 glucanase activity. β- glucosidase activity was highest in *G. butleri*, *T. asperellum*, BaBc-1 and *B. subtilis*. Significantly higher laccase, amylase and pectinase activity was observed in BaOu-1, BaBc-1 and AcOu-1 respectively. Maximum protease activity was recorded in fungal isolates *G. butleri* and *T. asperellum*.
- Cultural, morphological, biochemical and molecular characterization was carried out for the selected isolates. Accordingly, the isolates BaBc-1, BaCp-1, BaOu-1 and AcOu-1 were identified as *Bacillus subtilis*, *B. cereus*, *Bacillus* sp. and *Streptomyces roseofulvus* respectively.
- The isolates showing maximum enzyme production were tested for compatibility using cross streak and dual culture method. Based on the results of enzyme analysis and compatibility test four different microbial consortia were developed (consortia I, II, III and IV). These microbial consortia were evaluated for degradation of vegetable waste in conical flasks along with an uninoculated treatment.
- Visual observation, enzyme activity and microbial population 21 DAI in flask culture proved that, consortium II (*B. subtilis* BaBc-1 + *T. asperellum* + *Bacillus*

sp. BaOu-1) and consortium IV (*B. subtilis* + *G. butleri* + *B. subtilis* BaBc-1) were the promising ones.

- Pilot scale experiment was carried out in KAU smart biobin with best two treatments from *in vitro* degradation, consortium II as T_1, consortium IV as T_2. There was a positive control with cow dung slurry (T_3) and a negative control with no source of microbes (T_4).
- T_1 (consortium I) found to be effective on volume reduction, duration of composting, yield, microbial population and phytotoxicity of compost.
- Consortium I was selected for large scale composting study in Thumburmuzhi composting unit, along with cow dung added (T_2) and uninoculated treatment (T_3).
- Consortium I inoculated treatment, T_1 (*B. subtilis* BaBc-1 + *T. asperellum* + *Bacillus* sp. BaOu-1) recorded maximum temperature during composting period (64^0C), highest volume reduction of biosolid waste and maximum microbial population in compost. Hence, T_1 (consortium I) was selected as best treatment in Thumburmuzhi composting unit.
- Microbial consortium formulated with *B. subtilis* BaBc-1, *T. asperellum* and *Bacillus* sp. BaOu-1 enhanced the degradation of biosolid waste in KAU smart biobin and Thumburmuzhi composting unit.
- Good quality compost with desirable physical, chemical and biological properties was obtained within a short period of composting.

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Appendices

APPENDIX- I

MEDIA USED AND COMPOSITION

a) Carboxy methyl cellulose medium (CMC)

Sodium nitrate	: 2.0 g
Dipotassium phosphate	: 1.0 g
Magnesium sulphate	: 0.5 g
Potassium chloride	: 0.5 g
Carboxy methyl cellulose	: 2.0 g
Peptone	: 0.02 g
Agar	: 17.0 g
Distilled water	: 1000 ml

b) Calcium Caseinate Agar

Peptic digest of animal tissue	: 4.000
Meat extract	: 2.000
Casein enzymic hydrolysate	: 2.000
Calcium caseinate	: 3.500
Calcium chloride $2H_2O$	: 0.200
Tri-potassium citrate H_20	: 0.350
Disodium hydrogen phosphate	: 0.105
Potassium dihydrogen phosphate	: 0.035
Sodium chloride	: 5.000
Agar	: 13.000
Final pH (at 25°C)	: 7.0±0.2

c) Colloidal chitin agar medium

Na_2HPO_4	: 6 g
KH_2PO_4	: 3 g
NH_4Cl	: 1 g
NaCl	: 0.5 g
Yeast extract	: 0.05 g

Agar	: 15 g
Colloidal chitin	: 10 g
Distilled water	: 1000 ml

d) Lignin sulphonate medium

Malt extract	: 15.0 g
Tannic acid	: 5.0 g
Agar	: 20.0 g
Lignin sulphonate	: 1.0 g
Distilled water	: 1000 ml

e) Nutrient agar

Beef extract	: 3.00 g
Peptone	: 5.00 g
NaCl	: 5.00 g
Agar	: 29.00 g
Distilled water	: 1000 ml
pH	: 6.8-7.2

f) Kenknight and Munaier's media

Dextrose	: 1 g
K_2HPO_4	: 0.1 g
NH_4SO_4	: 0.1 g
KCl	: 0.1 g
$MgSO_4$	: 0.1 g
Agar	: 20 g
Distilled water	: 1000 ml
pH	: 7

g) Potato dextrose agar

Potato	: 200 g
Dextrose	: 20.00 g
Agar	: 20.00 g

Distilled water	: 1000 ml
pH	: 5.1

h) Starch agar

Peptone	: 0.5 g
KCl	: 0.1 g
$MgSO4 .7H_2O$	: 0.5 g
$(NH4)_2SO_4$	: 0.1 g
NaH_2PO_4	: 0.1 g
Starch	: 2.00 g
Agar	: 8.00 g
Distilled water	: 1000 ml

APPENDIX- II

Temperature variation during composting in KAU smart biobin

No. of days	Temperature (^{0}C)			
	T_1	T_2	T_3	T_4
1	38.62	38.94	31.94	38.48
2	39.34	33.62	39.08	35.8
3	38.74	34.76	39.26	31.04
4	33.86	37.1	45	32.5
5	34.3	36.28	42.14	32.64
6	33.64	35.38	40	40
7	33.22	34.7	35.36	34.4
8	32.66	34.18	32.48	38.72
9	30.24	31.72	35.5	34.7
10	31.64	33.04	35.08	39.64
11	32.66	33.1	33.84	38.44
12	32.2	33.44	33.3	36.42
13	31.98	32.82	32.72	36.1
14	34.86	32.22	31.52	35.54
15	38.62	38.94	31.94	38.48
16	42.18	42.92	32.4	40.86
17	44.72	48.32	32.18	33.22
18	40.78	41.9	32.66	32.74
19	41.36	42.2	44.62	42.24
20	41.36	41.5	39.76	32.5
21	38.52	39.18	32.16	33.02
22	40.22	41.54	41.1	33.04
23	40.84	41.28	41.06	32.64
24	36.52	35.62	39.82	32.72
25	34.96	33.74	38.32	33.02
26	34.78	33.4	38.32	30.9
27	33.34	33.52	36.32	32.7
28	33.18	32.22	34.62	31.56
29	32	31.48	34.02	30.88
30	31.7	32.12	32.86	30.84

APPENDIX- III

Temperature variation during composting in Thumburmuzhi composting unit

No. of days	Temperature (^{0}C)		
	T_1	T_2	T_3
1	45.98	39.7	40.4
2	57.26	46.52	46.76
3	64.66	49.6	52.52
4	58.54	51.78	53.74
5	51.58	40.52	46.76
6	50.28	45	41.1
7	46.8	45.2	40.5
8	41.72	44.32	40
9	49.96	41.44	45.84
10	48.42	42.58	46.76
11	45.82	42.56	43.4
12	43.7	42.82	44.14
13	46.06	42.8	43.54
14	44.94	44.12	43.74
15	44.08	44.58	43.74
16	46.02	42.72	45.72
17	44.92	41.44	46.4
18	44.34	42.48	45.66
19	43.1	42.84	44.44
20	43.76	43.02	45.34
21	43.48	43.16	45.54
22	44.4	43.32	46.24
23	43.12	43.32	44.8
24	43.54	42.9	44.8
25	43.42	42.48	45.6
26	42.12	42	44.56
27	41.78	41.04	43.56
28	41.52	40.64	42.92
29	41	40.3	43.6
30	41.28	40.2	43.04

APPENDIX- IV

Benefit cost ratio of composting in Thumburmuzhi composting unit

	T_1	T_2	T_3
Fixed cost			
Fixed cost of one Thumburmuzhi composting unit	10000	10000	10000
Life period	10	10	10
Depreciation (10 %)	1000	1000	1000
Repair and maintenance (2 %)	200	200	200
Total	1200	1200	1200
Variable cost			
Cost of inoculum	80	150	-
Labour cost	2000	2000	2000
Total	2080	2150	2000
Total cost	3280	3350	3200
Returns	841.6	3333.44	614.72
BC ratio	0.26	1.00	0.192

MICROBIAL INOCULANTS FOR ENHANCING DEGRADATION OF BIOSOLID WASTE IN AEROBIC COMPOSTING

By

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ABSTRACT OF THE THESIS

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ABSTRACT

Solid waste management is a major challenge throughout the world, especially in urban areas, due to the rapid growth of population along with urbanization. Earlier, centralized management of biosolid waste was being practiced. However, due to problems in transportation and segregation, management at source is being promoted. Aerobic composting has been practiced from time immemorial for recycling of biosolid waste, using various processes and containers. Recently, more importance is being given to bio- composting, considering the efficiency of microorganisms in enhancing degradation of organic substrates by their multiple mode of action. Hence, this study was taken up to explore microorganisms which can enhance the process of aerobic composting of biosolid waste.

Isolation of potential decomposing microorganisms was attempted from different compost samples including coir pith compost, kitchen waste compost and Oushadhi ayurvedic compost. A total of 14 isolates were obtained from different compost samples. All the isolates were assigned names depending upon the type of microorganism and the source from which they were isolated. Based on the ability to degrade the chemical components in selective medium, four isolates (BaBc-1, BaCp-1, BaOu-1 and AcOu-1) and four reference cultures (*Bacillus subtilis*, *Bacillus niabensis*, *Gongronella butleri* and *Trichoderma asperellum*) were selected for quantitative assay. Enzyme assay was carried out for selected isolates and the isolate *G. butleri* exhibited highest cellulase activity. BaBc-1, *B. subtilis* and BaOu-1 recorded significantly higher β- 1, 3 glucanase activity. Glucosidase activity was found to be significantly high in *G. butleri*, *T. asperellum*, BaBc-1 and *B. subtilis*. Significantly higher laccase, amylase and pectinase activity was recorded in BaOu-1, BaBc-1 and AcOu-1 respectively. Maximum protease activity was recorded in fungal isolates *G. butleri* and *T. asperellum*. Potential isolates were further subjected to cultural, morphological, biochemical and molecular characterization. The isolate BaBc-1 showed

maximum homology to *Bacillus subtilis,* BaCp-1 to *Bacillus cereus*, BaOu-1 to *Bacillus* sp. and the actinomycete isolate AcOu-1 to *Streptomyces roseofulvus*.

The compatible combinations of selected isolates with high enzyme activity were selected for formulation of microbial consortia and the consortia were evaluated for degrading vegetable waste under *in vitro* condition. All the inoculated treatments showed faster degradation compared to uninoculated control. Based on visual observations, per cent weight reduction, enzyme activity and microbial population on 21 DAI in flask culture, consortium II (*B. subtilis* BaBc-1+ *T.asperellum*+ *Bacillus* sp. BaOu-1) and consortium IV (*B. subtilis*+ *G. butleri* +*B. subtilis* BaBc-1) were selected for pilot scale experiment.

The efficiency of selected consortia was evaluated in KAU smart biobin along with cow dung slurry and uninoculated treatment. In T_1 (*B. subtilis* BaBc-1+ *T.asperellum*+ *Bacillus* sp. BaOu-1) compost formation was initiated within 17 days after inoculation. Based on the volume reduction, duration of composting process, yield of compost, microbial population and phytotoxicity of compost, consortium I (*B. subtilis* BaBc-1+ *T.asperellum*+ *Bacillus* sp. BaOu-1) was selected as best performing consortium in KAU smart biobin. Hence, this consortial formulation was selected for large scale experiment in Thumburmuzhi composting units. Cow dung was used as inoculum in positive control and uninoculated treatment served as negative control. The treatment T_1 (*B. subtilis* BaBc-1+ *T.asperellum*+ *Bacillus* sp. BaOu-1) recorded maximum temperature (64^0C) during composting period, faster volume reduction and maximum microbial population in compost. Based on these results, T_1 was found to be the best treatment in Thumburmuzhi composting unit.

The study revealed that, consortial formulation of *B. subtilis* BaBc-1, *T. asperellum* and *Bacillus* sp. BaOu-1 could be exploited for enhancing degradation of biosolid waste in aerobic composting. This can be used in future for the management of agricultural and municipal solid waste. The plant growth

promoting (PGP) activities of these isolates could be an added advantage in improving the growth and yield of plants.