FRUIT WASTE UTILISATION FOR PECTINASE PRODUCTION THROUGH SOLID STATE FERMENTATION

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled **"Fruit waste utilisation for pectinase production through solid state fermentation"** is a bonafide record of research work done by me during the course of research and that it has not been previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled **"Fruit waste utilisation for pectinase production through solid state fermentation"** is a record of research work done independently by **Mr. Venkatesh . M** under my guidance and supervision and that it has not formed the basis for the award of any degree, diploma, fellowship or associateship to him.

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TO MY LOVING MOTHER

and

INDIAN ARMY

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LIST OF ABBREVIATIONS

Sl.No	Abbreviation	Expansion
1.	BCR	Bromo Cresol Red
2.	BPB	Bromo Phenol Blue
3	CC	Calcium Chloride
4	DW	Distilled Water
5	cfu	colony forming unit
6	cm	centimeters
7.	CPBMB	Center for Plant Biotechnology and Molecular Biology
8.	DMRT	Dunken Multiple Range Test
9	IMTECH	Institute of Microbial Technology
10.	KAU	Kerala Agricultural University
11.	MR	Methyl Red
12.	PDA	Potato Dextrose Agar
13.	PE	Pectin Esterase
14.	smf	submerged fermentation
15.	ssf	solid state fermentation

Introduction

1. INTRODUCTION

Enzymes are proteinaceous biocatalysts known for their selectivity and specificity and are active under mild temperature and pressure conditions, which enable their effective use in food processing to modify, alter and enhance the quality of food. Recently extraction of enzymes from biowastes using the technology fermentation gained much importance as the enzymes have great demand by different industries (Textile, detergent, wool processing, beverage and food processing industry) and biowastes serve as ideal medium for their production. Extraction of valuable by products from wastes will be a viable proposition for their effective disposal as well.

Among the biowastes, fruit and vegetable wastes attracted the attention of scientists and large volume of data has been generated on various fruit and vegetable wastes defining their content of valuable constituents and mode of extraction of by products from them. An important biochemical component present in fruit and vegetable wastes is reported to be pectin (Sudhakar and Maini, 1999., Yu and Delvalle, 1999. and Madhav, 2001)

Many microorganisms are capable of producing enzyme in the presence of an inducer in the medium in which they are cultured. Pectinase, being an inducible enzyme, presence of pectin in fruit wastes can induce pectinase in the presence of suitable microorganisms. Pectinase is widely employed by fruit processing industries especially for maceration of fruit pulp and depectinisation of juices, which results in higher extractability of juices from pulp and its clarification. Pectinase have been also successfully used for clarification of wine.

Commercial enzymes are obtained from plants, animals and microbial sources. However microbial sources are generally preferred for production of most of the commercial enzymes due to the several advantages such as purity, economy, consistency, ease in process modification and availability of cheaper substrates as well as technology (Mcneil and Harvey, 1990). Enzymes from microbial sources are estimated to account for 80 per cent of the total enzyme production (Rose, 1980).

In contrast to the extensive use of microbial pectinase in processing foods in the world, the status of its use in India is still in its infancy mainly because of high cost involved in the enzyme aided processes. If some economically efficient technologies for its production in terms of source, strain, condition, e.c. could be standardised it will be of immense use to the food processing industries, as the enzymes could be made available at relatively cheaper rate.

The microorganisms produce enzymes both in solid state fermentation (ssf) of substrates or submerged state fermentation (smf). But ssf technique offers many well recognised advantages such as lower capital and recurring expenses, higher concentration of product at less energy input, substantial saving in down streaming processing and negligible effluent output (Singh, 2000). It also approximates the growth of microorganisms that occur in the nature on moist solids and is well proved to be economical even at lower scale of production. These advantages of ssf could be exploited for pectinase production from fruit wastes, which are available in plenty.

The driving force behind this study is that zero waste approach, concern of clean environment and quality product. The enzyme production through fermentation largely depends upon the factors such as media composition and conditions provided (temperature, pH, duration of fermentation, microorganisms etc.) for fermentation. Hence to achieve maximum enzyme production through microbial fermentation, each and every factor is to be made available at an optimum level. This necessitates standardisation of condition for enzyme production.

The present study was undertaken with the following objectives:

1. To isolate microorganisms capable of producing pectinase in pectin medium.
2. To analyse the efficiency of isolated microorganisms to produce pectinase in ssf of different fruit wastes.
3. To standardise the conditions for pectinase production in ssf of fruit wastes.

Review of literature

2. REVIEW OF LITERATURE

Fruit wastes are highly perishable and their disposal is a problem to the processing industries and pollution monitoring agencies. There are many ways by which, they could be profitably exploited for the conversion into value added products (Anand and Maini, 1997). In that way utilising the fruit wastes as source of product diversification fetches additional income to the processor. There are amble literatures available which highlight, the different uses of wastes. The wastes can be effectively utilised as raw material for animal feed preparation, antibiotic preparation, biogas production, composting, organic acid production, pectin extraction and enzyme production.

2.1 FRUIT WASTE UTILISATION

Shankaranand and Lonsane (1994) reported that coffee husk could be employed as an inexpensive substrate for production of citric acid in ssf. Stuzenberger (1994) reported extracellular enzyme like cellulase, xylanase and beta glucosidase could be produced by fermentation of baggasse. Xavier and Lonsane (1994) utilised sugarcane press mud as novel and inexpensive substrate for production of lactic acid. Garg and Hang (1995) reported microbial production of citric acid and lactic acid from carrot processing waste. Cabbage waste was utilised by Krishna and Chandrasekaran (1995) for the production of different enzymes like protease and cellulase.

Pineapple waste was identified as novel substrate for citric acid production (Tran and Mitchell, 1995). Jha *et al.* (1996) observed that soyhull could be utilised for single cell protein production in ssf. Joshi and Sandhu (1997) reported that ethanol and animal feed could be produced from apple pomace. Fruit waste like mango peel and guava pomace was used as substrate for the production of acetic acid and citric acid through microbial fermentation (Garg *et al.*, 1998 and Girdharilal *et*

al., 1998). Production of single cell protein from food industry waste using ssf technology was reported by Yun *et al.* (1998). Production of xylanolytic enzyme from agricultural waste material using *Aspergillus niger* was reported by Ferraria *et al.* (1999). Roukas (1999) reported possibility of producing citric acid from carob pod by ssf. Sethi (2000) reported that horticultural waste could be effectively utilised as source of fat, pectin, organic acid and animal feed.

2.1.1 Pectin from fruit waste

Almost all fruit wastes contain varying quantities of pectin (Santhosh, 1982; Srivastava and Kumar, 1994). Reports on extraction of quality pectin from nutmeg rind (Pruthi and Krishnamurthy, 1985), mango peel (Ranganna, 1986; Garg *et al.*, 1994; Sudhakar and Maini, 1999 and Yu and Delvalle, 1999), apple pomace (Anand and Maini, 1997), jackfruit rind (Srivastava and Kumar, 1994 and Anand and Maini, 1997), pineapple rind (Joshi and Joshi, 1990), citrus peel (Anand and Maini, 1997) and passion fruit rind (Srivastava and Kumar, 1994) highlight the pectin content in fruit waste.

The work conducted in KAU by Madhav (2001) revealed that jack fruit rind, mango peel, passion fruit rind and nutmeg rind are good sources of pectin and quality jelly can be produced from them.

2.2 ENZYMES IN FOOD PROCESSING INDUSTRY

In fruits, vegetables, cereals and oilseeds one or more enzymes catalyse many desirable or undesirable changes. These enzymes affect the ultimate quality of the food or beverages in which they are present. From the dawn of history, man has used enzyme system for food preservation, fermentation and for bread making.

Many of the most useful, but least understood, uses of enzymes are in the food industries, where they are used together with endogenous enzymes, to produce or process foodstuffs, which are only rarely refined (Cui *et al.*, 1988).

Great advances in enzyme chemistry over the past few decades have enabled food technologist to select specific enzyme to achieve a desirable end product. Food technologist is playing an important role in expanding food industry by improving the flavour, texture, organoleptic and nutritional quality of all type of processed products.

Enzymes can be defined as the biocatalyst, which break down the macromolecule to simpler forms, without undergoing any change by themselves (Pelwzer *et al.*, 1996).

Enzymes employed in food industry can be broadly categorised into two main groups hydrolase, which includes α amylase, β amylase, glucanase, pectinase, cellulase, invertase, rennet, protease and non-hydrolase like glucose isomerase, glucose oxidase etc. (Srivastava and Kumar, 1994). The application of enzymes in food processing industry is unavoidable, unparallel and innumerable. The profound usage is well observed tangibly in fruit juice processing, beverage industry, confectionery industry and diary industry (Norttingham University, 1997)

The above said enzymes are commercially employed in the food industries. Application of enzyme in food processing industries can be categorized as follows

1. Improvement of functionality (Jona and Fonta, 1997)
2. Bioconversion (Pandey *et al.*, 2000)
3. Clarification of beverages (Singh, 2000)
4. Better extraction of juices (Landbo and Meyer, 2001)
5. Flavour development (Teren *et al.*, 2001)

Singh (2000) summarised the advantages of using enzymes in food processing industries as follows

1. Ingredient substitution: Enzymes substitute cheap raw material in lieu of costlier one.
2. Efficient processing: It enables to overcome processing hurdles, especially in the case of clarification of juice and modified cheese production.

3. It increases industries capacity, by making operations easier and reduces cost of production.
4. It generates minimal amount of hazardous by-products, unlike chemical processing.
5. It aids in development of unique product, which is other way not possible, like aspartame production.

The pectin in fruit wastes offers great potential for exploiting them for the production of pectinase enzymes. The microorganisms that are capable of utilizing pectin for its growth can induce pectinase in the medium (Maheswari, 2001). The enzyme pectinase find immense applications in food processing industries.

2.2.1 Uses of pectinase in food processing industry

The pretreatment of beet pulp with a combination of pectinase and cellulase increased the saccharification efficiency and is used for converting pulp to ethanol (Kayabachi *et al.*, 1994). Jona and Fonta (1997) compared the efficiency of juice extraction from grape cv Barbera and kiwifruit cv Hayward using two protocols viz., chemical method and enzymatic method. The protocol using pectinase (polygalacturase) was found superior compared to others. In apple juice processing, maceration of fruit and clarification of juice is achieved by a mixture of pectinolytic enzyme and hemicellulase (Srivastava and Sanjeevkumar, 1994).

Enzymatic release of antioxidants and phenolic substance from grape pomace was very high when treated with pectinase (Meyer *et al.*, 1998). Patil and Pai (1998) studied the effect of various methods of clarification of pomegranate juice. They opined that treatment with pectinase enzyme at the rate of 0.5 per cent yielded excellent quality juice with high clarity, high yield, good retention of vitamin C and better acceptability.

The extraction efficiency of natural colorants from annatto, carrot, and spinach were improved when treated with pectinase enzyme (Aparnathi and Borhkatriya, 1999). In citrus fruits naringin, a glycolated flavonone responsible for bitterness was converted to less bitter compound by enzyme known as naringinase.

Another bitter principle called limonin, which associated with naval orange, was made less bitter by enzymatic degradation using limonate dehydrogenase (Norttingham University, 1997)

Singh (2000) reported that pectinase are used by food processing industries mainly for fruit juice extraction and clarification of fruit juices. In the preparation of lime cordial, clarity of juice is very important and it could be achieved by treating the juice with pectin esterase and polygalactourase. Teren *et al.* (2001) extracted glucovanillin from vanilla green pods and simultaneously transformed to vanillin by treatment with a combination of pectinase and cellulase. The high amount of pulp consistency of banana fruit is a limiting factor, for extraction of juice from it. This can be overcome by addition of pectinase at the rate of 0.5 per cent, which yielded better recovery (85%) of juice (Biocon, 2002).

2.3 FERMENTATION

Fermentation is the technology employed from the time immemorial. Technically the term is defined as "the anaerobic oxidation of compounds by enzymatic action of microorganism, neither gaseous nor respiratory oxygen chain is involved in the energy yielding process. An organic compound is the electron acceptor" (Pelwzer *et al.*, 1996)

Generally two methods of fermentation are employed (Fadel, 2001)

1. Submerged state fermentation and
2. Semi solid/ solid state fermentation

Among the methods, solid state fermentation method finds its importance in the enzyme, organic acid and antibiotic production.

2.3.1 solid state fermentation (ssf)

At present ssf processes are used commercially for production of different type of fermented products. Apart from their use in food industries, the process is

successfully used for large scale production of fungal metabolites and for conversion of plant, animal and domestic waste into useful products (Aidoo *et al.*, 1982)

The advantages reported for ssf can be listed as follows

1. The medium is relatively simple
2. The space required by the fermentation equipment is small
3. Neither seed tank nor performed inoculums is necessary
4. The condition in which microorganism grows are like condition in the natural habitat.
5. Aeration is easily obtained.
6. The desired product may be readily extracted from the vessel.
7. The substrate is dry, so there is reduced possibility of contamination.
8. The ssf required low energy in comparison with other types.

(Aidoo *et al.*, 1982)

Tangerdy (1985) stated that the advantage of solid state fermentation lies in its simplicity and its closeness to the natural way of life for many microorganisms. Since large amount of water is not required to the biological material, fermentar volume remains small. This type of fermentation is especially suitable for growing mixed culture of microorganisms, where symbiosis stimulate better growth and productivity.

In ssf, culture medium is impregnated in a carrier substrate such as baggasse, wheat bran, potato pulp and the organism is allowed to grow in this medium. This method allows greater surface area for growth of organism, production of desirable substance and its easy recovery (Tauro *et al.*, 1997).

2.4 SOLID STATE FERMENTATION FOR ENZYME PRODUCTION

Boccas *et al.* (1994) reported the production of pectinase from coffee pulp by solid state fermentation using *Aspergillus niger*. Hornecka *et al.* (1994) attempted to produce cellulase using mutants of *Aspergillus niger* in the medium containing

wheat bran and beet pulp at 4:1 ratio, in the solid state fermentation. Zychlinska *et al.* (1994) reported xylanase production at the tune of 20 XU ml^{-1} in the medium containing wheat bran (75%), beet pulp (20%) and apple pomace (5%) when *Chaetomium globosum* was used. The organism *Aspergillus niger* produced 45 XU ml^{-1} of xylanase in same medium.

Asheh and Dejack (1995) recorded maximum phytase activity in ssf of canola meal using *Aspergillus niger*. Coronel *et al.* (1995) studied the glucoamylase production using different strains of *Aspergillus niger* in spent brewer grain (SBG), rice bran (RB), powdered sweet potato (SP) and coconut water (CW). The optimum substrate identified was SBG: RB: SP (4:1:0.05) mixed at1:1 ratio with CW at 35^0 C. Jain (1995) reported maximum xylanase production along with acetyl esterase in solid state fermentation than in submerged fermentation, in the sugarcane baggasse and wheat straw medium when *Melanocarpus albomyces* IIS-68 was inoculated to the medium. Jha *et al.* (1995) reported the production of cellulase from soy hull using *Phanerochaete chrysoporium* in solid state fermentation. Krishna and Chandrasekaran (1995) economically utilized the cabbage for the production of different enzymes viz., α amylase, protease and cellulase in solid state fermentation using native microflora. Production of xylanolytic enzyme from agricultural waste material using *Aspergillus niger* was reported by Ferreria *et al.* (1999). Gessesse and Mamo (1999) reported a xylanase yield of about 720 Ugm^{-1} in the wheat bran substrate added with Na_2NO_3 of 10 per cent, when subjected to ssf using *Bacillus* sp. AR 009.

Use of spoiled casein for protease production using *Aspergillus niger* in solid state fermentation was reported by Mulamani and Patil (1999). Selvakumar *et al.* (1999) produced insulinase from rice bran, coconut oilcake, and cornflour using *Staphylococcus* sp. and *Kluyvermoces marxiamus* in ssf. Gomez *et al.* (2000) opined that, invertase production in the solid state medium containing sucrose at 100 gm l^{-1} using *Aspergillus niger* was higher than the submerged state fermentation in the

same medium. The better productivity in solid state was due to a better mould growth. Jecu (2000) reported that, agricultural waste is an excellent carbon source for enzyme production. He reported endoglucanase activity 14.80 IU in solid state fermentation, of the substrate containing wheat straw: wheat bran (9:1) inoculated with *Aspergillus niger.*

Regalado *et al.* (2000) worked on production of β mannanase in copra waste and spent coffee. They revealed that *Aspergillus niger* produced higher β mannanase activity on the copra waste medium, in solid state fermentation.

Fadel (2001) tried the xylanase production in sorghum flour medium. He reported highest xylanase activity (438 U gm^{-1}) in medium containing sorghum flour at moisture rate of 1:1.2, pH 4.5 at 33^0 C, using *Trichoderma harzianum* F 416, in solid state fermentation. Alkaline protease of 49950 U gm^{-1} was obtained in the medium of pigeon pea residue in pH 8.5 at 45^0 C using *Aspergillus niger* in solid state fermentation (Verma *et al.*, 2001).

2.4.1 solid state fermentation for pectinase production

Pectinase is a group of enzymes consisting Poly Galacturonase (PG), Pectin Methyl Esterase (PME) and Pectin lyase (PL) (Rose, 1980).

Production of pectinase is commonly from the microbial sources like bacteria and fungi. Both are commercially exploited in large scale for pectinase production (Rose, 1980; Mcneil and Harvey, 1990).

Production of pectinase by ssf of wheat bran using native microflora was reported by Ghildyal *et al.* (1981). Babitskaya *et al.* (1993) reported the production of pectin lyase by *Erwinia carotovora* subsp *atroseptica* on medium containing potato tuber and flax straw in ssf. Nair *et al.* (1995) reported the production of pectinase by *Aspergillus niger* NCIM 548, in corn flour medium supplemented with glucose (1.3 U) in 36 hours after fermentation in ssf.

Dahm (1996) showed the production of pectin lyase by *Cylindrocarpus destructans* in *in vivo* condition on the cellulose medium. Kollar (1998) produced

extracellular pectinase in the medium containing galacturonic acid supplemented with one per cent pectin, using fungus *Venturia inequalis* by ssf.

Extracellular production of pectinolytic enzymes (Polygalacturonase and Pectin methyl esterase) by *Scelortium rolfisii* on chick peas was reported by Chattopadhay *et al.*, 1999. Production of pectinolytic enzyme (Pectin lyase and Polygalacturonase) by *Cantharellus cibarius* on ssf of paddy straw was reported by Dahm *et al.* (1999). Laurant *et al.* (2001) reported pectin lyase production by *Erwinia carotovora* subsp *carotovora*, in the vegetable medium using ssf technology.

2.4.2.1 Bacterial source

Zucker *et al.* (1972) and Chatterjee *et al.* (1979) showed the production of inducible and extra cellular endopolygalacturonase by *Pseudomonas fluorescens* and *Erwinia*. Bacteria like *Bacillus, Pseudomonas* and *Micrococcus* isolated from retting flax, jute, sisal, and coir and *Erwinia* from coffee fruit are reported to possess the ability to degrade pectin by producing pectinolytic enzymes (Chesson, 1980). Fogarty and Kelly (1983) listed many microorganisms that are capable of degrading pectin. Millan *et al.* (1992), Heikinheimo *et al.* (1995), Liao (1996) and Weber *et al.* (1996) found that many species of *Erwinia, Xanthomonas* and *Pseudomonas* are capable of producing pectinolytic enzymes.

The determination of total counts and total pectinolytic counts on the whole coffee fruits and on pulped fruits indicated that pectinolytic bacteria are the important fraction of the microbial population (Roussos *et al.*, 1995). The microbial flora of coffee pulp consisted of mostly enterobacteriaceae particularly the genus *Erwinia*, which produce pectinolytic enzymes during fermentation.

2.4.2.2 Fungal source

Many fungal species are capable of degrading pectin by producing different pectinolytic enzymes. The fungus, *Alternaria sesami* produced pectinolytic enzymes viz., polygalacturonase (Rajpurohit and Prasad, 1982). Shindia (1995) reported that temperature variation during garbage composting led to corresponding changes in the distribution of pectin degrading fungi in the compost and the most common

pectinolytic fungi were *Aspergillus niger, A. flavus, Penicillum* sp, *Fusarium moniliforme, Alternaria alternata, Cladosporium cladosporioids* and *Trichoderma reesei.*

2.5 FACTORS CONTROLLING ENZYME PRODUCTION UNDER SOLID STATE FERMENTATION

According to Pandey (1992), the major factors that affect the microbial synthesis of enzyme in solid state fermentation include selection of suitable substrate, particle size of the substrate, water content and water activity of the substrate, relative humidity, type and size of inoculum, control of temperature of the fermenting matter, removal of metabolic heat, period of cultivation, maintenance of uniformity in the environment of solid state fermentation system and the gaseous atmosphere.

Polygalacturonase secretion by *Fusarium oxysporum* was found maximum in the fermentation medium of paddy straw at pH 4 to 5 (Mehta and Mehta, 1985). The control of moisture level within a relatively narrow range is essential for optimising solid state fermentation. A properly moistured substrate could have surface film of water to facilitate dissolution and mass transfer of nutrients and oxygen, but interparticle channel would be kept free to permit oxygen diffusion and heat dissipation (Tengerdy, 1985). Hours *et al.* (1988) observed maximum pectinolytic activity from apple pomace at pH 4.0 and temperature 45^0 C.

Maximum production of polygalacturonase occurred on four days after inoculation and the optimum incubation period for the production of pectinolytic enzymes varied from strains to strains and species to species.

Murad (1989) reported maximum endo polygalactouranase activity under ssf of agrowastes by *Myrothecium verrucaria* four days after incubation of medium. *Alternaria macrospora* showed endo polygalactouranase activity within 10 to 14 days of incubation and *Rhizoctonia solani* produced polygalactouranase within four days.

Prusky *et al.* (1989) reported that Polygalactouranase are more stable and active at room temperature (25 to 37 ^{0} C). The biggest hurdle in ssf process is reported to be heat build up. This was found to cause evaporative heat loss, stoppage of vegetative growth and induction of protective, but non productive growth of organism. Murad and Foda (1992) reported the progressive increase in production of polygalacturonase from dairy waste by yeast as the pH value of the medium reduced.

Dudeja *et al.* (1993) reported that production of pectinase, cellulase and xylanase by cowpea *Rhizobia* group of microorganism was low when temperature of growth medium, was elevated. Newman (1993) reported that the activity of enzyme is also depend upon temperature of fermenting media. The enzyme phytase is extremely heat stable with temperature optimum of 75 ^{0}C and pH optimum of 6.0 to 7.0. Donaghy and Mckay (1994) observed maximum production of pectin from citrus peel at 37^{0} C, when polygalactouranase was used.

In solid state fermentation using *Aspergillus niger* Pectinolytic enzymes are stable when compared to smf. Maximum endo polygalacturonase activity in ssf was reported at 60^{0} C (Acuna arguelles *et al.*, 1995). The efficiency of microorganism is an important factor controlling enzyme production under ssf. Kanotra and Mathur (1995) proved that fungus *Trichoderma rescei* GM 9414, a mutant, produces twenty five times more xylanase in culture of one per cent xylan than in the culture of one per cent cellulase. This mutant strain mixed with *Pleurotus sajurcaju* reported maximum degradation of paddy straw in solid state fermentation. Yao *et al.* (1996) reported that the polygalactouranase produced from apple pomace was heat liable and active at pH 5.5. Production of polygalactouranase by *Penicillium* sp was higher (1.91 U/mg protein) in solid culture when compared to submerged (1.18 U mg^{-1} protein) culture (Gupta *et al.*, 1997). Production of enzyme was observed to be 120 hours after incubation at pH 5.5 and temperature 45^{0} C.

Fattah *et al.* (1998) investigated three strains of *Aspergillus niger*, *Aspergillus japonicum* MSM 101, *Aspergillus aculeatus* MSM 102 and *Aspergillus awamori* MSM 103 for xylolytic activity. All the cultures show xylolytic activity on solid

medium but *Aspergillus japonicum* MSM 101 was the best xylanase producer. Ikasari and Mitchell (1998) conducted an experiment to study the effect of temperature and gas changes during solid state fermentation of rice bran. They reported that decrease in oxygen concentration from 21 to 0.5 percent did not alter protease production by *Rhizopus oligoporous*, but retarded amyloglucosidase production. The increase in temperature from 37^0 C to 50^0 C decreased the activity of both the enzymes.

Reddy *et al.* (1998) reported that, β amylase (1.79 U) and pullulanase (2.18) could be produced by solid state fermentation, in the medium containing one part wheat bran to 0.5 to 2.5 parts of yeast extract medium, using *Clostridium thermosulfurogenes* SV2. Ridder *et al.* (1998) reported maximum xylanase activity (716 Ugm^{-1} of bran), in a media containing 55 per cent moisture, when inoculated at depth of 1.5 cm after 5 days of fermentation.

Suresh and Chandrasekaran (1998) succeeded in production of chitinase from prawn processing waste. They concluded that maximum chitinase yield was 248 unit gm^{-1} in a medium containing 5:1 ratio of (w/v) of prawn waste and sea water, supplemented with one per cent NaCl and 2.5 per cent potassium di-hydrogen phosphate, using *Beaveria bassiaria* BTMF 310 in solid state fermentation.

Chelvi and Purushothamam (1999) compared two strains of *Aspergillus niger* for the production of glucoamylase. The results revealed that the strain *Aspergillus niger* F.ST.SW.1 was better in medium of pearl millet residue under solid state fermentation. Reddy *et al.* (1999) reported production of pullalanase by *Clostridium thermosulfurogenes* SV2 in solid state fermentation. The enzyme production was high in a medium containing 16.5 per cent potato starch, 2.5 per cent corn steep, 0.015 per cent ferrous sulphate and 14 per cent pearl millet flour.

Jecu (2000) attempted the endo glucanase production from agricultural waste and he observed that production was higher in a waste medium mixed with wheat straw and wheat bran (9:1), at moisture content of 74 per cent and pH range of 4.5 to 5.5. Mandiwala and Khire (2000) employed agricultural residues, wheat bran,

mustard cake, cowpea meal, coconut cake, cotton cake and black bean flour for thermostable phytase production. Maximum phytase activity ($108 Ugm^{-1}$) was obtained with cowpea meal, in the medium containing 100 gm residue with 10 mg KH_2PO_4, using *Aspergillus niger* in solid state fermentation.

Valino *et al.* (2000) attempted the production of exo β 1,4 glucanase from sugarcane baggasse, using *Aspergillus fumigatus* 155. He reported the optimum stage for enzyme production was 48 h after inoculation at pH of 5.6 to 5.7.

Materials and Methods

3. MATERIALS AND METHODS

A study was conducted in the Department of Processing Technology, College of Horticulture, Vellanikkara during 2001-2003 period to analyse the possibilities of producing pectinase through solid state fermentation (ssf) process of different fruit wastes. The study consisted of five parts as follows

1. Isolation and pure culturing of microorganisms from different fruit and vegetable wastes.
2. Preliminary screening of isolated microorganism for their efficiency to grow in pectin medium.
3. Second stage screening of microorganism for their efficiency to produce pectinase.
4. Assessment of the efficiency of screened microorganisms to produce pectinase in solid state fermentation (ssf) of fruit wastes.
5. Standardisation of media and conditions for pectinase production in ssf.

3.1 ISOLATION AND PURE CULTURING OF MICROORGANISMS FROM DIFFERENT FRUIT AND VEGETABLE WASTES

3.1.1 Isolation

To isolate microorganisms, decaying fruit and vegetable wastes were collected from different locations as given below

Material	Source of collection
Pineapple peel and core	Compost / waste pit of the Department of Processing Technology
Jack fruit rind	Pits in Instructional farm, Vellanikkara and waste pit of men's hostel, College of Horticulture

Vegetable wastes (Ashgourd, pumpkin, watermelon), banana peel, stalk, mango peel and stone	Waste pit, Department of Olericulture and Department of Processing Technology, College of Horticulture.

The collected samples were subjected to serial dilution technique (Parkinson *et al.*, 1971) and inoculated in Potato Dextrose Agar (PDA) medium for isolating different microorganisms. The PDA was prepared as described by Kennedy *et al.* (1994). For isolating fungi, inoculation was done at 10^{-2} and 10^{-3} dilution and for isolating bacteria, inoculation was done at 10^{-5} and 10^{-6} dilution. The cultures were maintained at room temperature (Plate 1).

3.1.2 Pure culturing of microorganisms

When the microorganisms exhibited enough vegetative growth to distinguish among themselves, they were repeatedly sub cultured on PDA medium itself till pure cultures were obtained. Different fungi were isolated by hyphal tip method and the bacteria through streak plate method as suggested by Kennedy *et al.* (1994). The pure cultures were maintained on PDA slants. Apart from these pure culture of *Aspergillus foetidus* 115 obtained from Institute of Microbial Technology, Chandigarh was maintained in Czapek medium for standard pectinolytic culture. The description of the microorganism as well as the composition of Czapek medium is as follows (IMTECH, 2003)

Czapek medium

Czapek concentrate- 10ml

KH_2PO_4 – 1*g*

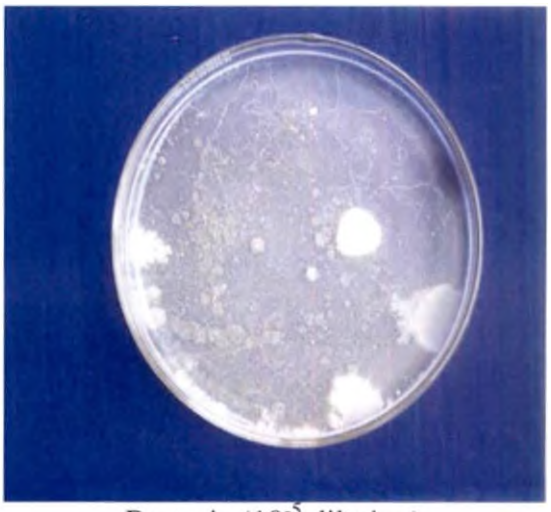
Yeast extract – 5*g*

Sucrose – 30*g*

Agar – 15*g*

Distilled water – 1000ml

Fungi (10^{-3} dilution)



Bacteria (10^{-5} dilution)

Plate 1. **Microbial isolates on PDA Medium**

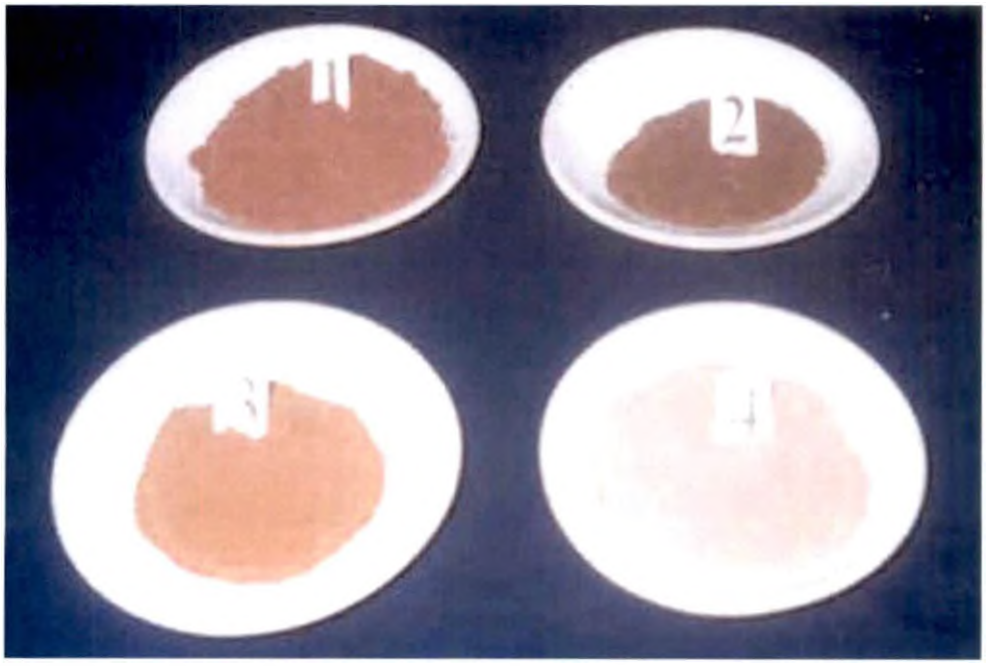


Plate 2. **Powdered wastes for preparing media**

1. Grape
2. Banana
3. Cashew apple
4. Pineapple

Composition of czapek concentrate

$NaNO_3$ – 30*g*

KCl – 5g

$MgSO_4.7H_2O$ – 5g

$FeSO_4.7H_2O$ – 0.1g

Distilled water – 100ml

Condition

Aerobic

Temperature: 30°C

Incubation: 7 days

Subculture: 30 days

The isolates obtained from fruit and vegetable waste were subjected to preliminary screening for their efficiency to grow in pectin medium.

3.2 PRELIMINARY SCREENING OF MICROORGANISMS FOR THEIR EFFICIENCY TO GROW ON PECTIN MEDIUM

Isolates of bacteria and fungi were cultured in petridishes with solid medium containing pectin as the sole carbon and energy source, to assess their efficiency to use pectin and exhibit growth.

Composition of pectin medium

Pectin – 2g

Urea – 0.05g

Ammonium sulphate – 0.15g

Agar – 20 *g*

Distilled water – 1000 ml

pH of the medium – 5.5

3.2.1 Screening of fungal isolates on solid pectin medium

The growth parameter of the fungal isolates *viz.*, hyphal growth was recorded daily from third day after inoculation, upto seven days. The fungi, which exhibited good growth performance, were also cultured in liquid medium to assess their capacity to grow utilizing pectin.

3.2.2 Screening of fungi in liquid culture

The different fungi isolated through preliminary screening based on the growth performance on pectin medium were subjected to another screening in liquid culture. In this, the organisms obtained from Department of Plant Pathology (*Trichoderma viridae* and *Trichoderma harzianum*) were also included in this experiment. The liquid pectin medium was prepared by excluding agar from the media prescribed for pectin solid medium. The fungi were inoculated in 100 ml liquid culture, in 250 ml conical flask, and were incubated at 35° C in rotary shaker at 100 rpm. After five days of incubation, the medium was filtered through Whatman No. 1 filter paper and dried in hot air oven at 60° C until the fungal mass recorded constant weight consecutively for three days. The turbidity of the broth was observed. Intensity of turbidity was recorded using plus mark.

Those isolates which, exhibited good growth performance both in solid and liquid medium were selected and subjected to second screening in dye containing media and media containing different quantities of pectin.

3.2.3 Assessment of bacterial cultures

The pure bacterial cultures maintained on the PDA slants as well as those obtained from CPBMB, Vellanikkara (*Pseudomonas fluorescens* and *Azotobactor* sp.) were streaked on PDA and incubated at room temperature for two days. On second day, two colonies were picked up with the help of an inoculation loop and suspended in 10 ml sterile water. The suspension was mixed thoroughly by vortexing and 100 µl from this was plated on the solid pectin medium by spread plate technique (Kennedy *et al.*, 1994). The number of bacterial colonies formed was noted after 24 hours of incubation for three days.

The data recorded were subjected to DMRT analysis and the microorganisms were ranked for their growth performance. The fungal and bacterial cultures in solid medium were continuously monitored to observe the hydrolysed zone produced if any, through hydrolysis of pectin (Boccas *et al.*, 1994)

3.3 SECOND STAGE SCREENING OF MICROORGANISMS TO PRODUCE PECTINASE

3.3.1 Screening in dye containing media

Hydrolysis of pectin leads to formation of acids and therefore a drop in pH. The pectin medium (pH 5.5) was modified by incorporating different dyes, so that change in the pH would be indicated by the colour change of the media.

Medium	Composition	Colour change
MR medium	Pectin medium + methyl red dye	Red to yellow at pH 4.2 – 6.3
BCR medium	Pectin medium + bromo cresol green	Yellow to blue at pH 3.6 – 5.2
BPB medium	Pectin medium + bromo phenol blue	Yellow to violet at pH 2.8 – 4.6

The isolates used in second stage screening were also cultured. Growth parameters recorded were same as that given in the experiment 3.2.1. Number of days taken to bring about colour change in the medium due to pectin degradation was also noted.

3.3.2 Screening in media containing different quantities of pectin

The microorganisms screened out through preliminary screening and the fungus *Aspergillus foetidus* 115, from IMTECH were inoculated in media containing different quantities of pectin and other nutrients as follows.

Medium	Pectin (g)	Urea (g)	Ammonium sulphate (g)	Agar (g)	Distilled water (ml)
A	4	0.05	0.15	20	1000
B	3	0.05	0.15	20	1000
C	4	0.1	0.15	20	1000
D	3	0.05	-	20	1000
E	2	-	0.15	20	1000

The growth parameter of the microorganisms *viz.*, hyphal length in the case of fungi and number of colonies in the case of bacteria were recorded. The cultures were closely observed for the production of hydrolysed zone due to pectolytic activities of the organisms.

Number of medium - 5

Replications - 3

Design – CRD

3.4 PERFORMANCE OF SELECTED MICROORGANISMS IN SSF OF FRUIT WASTES

Bulk quantity of fruit wastes were collected from the processing unit attached with the Department of Processing Technology, College of Horticulture, Vellanikkara. The fruit wastes collected include mango (peel), jackfruit (rind), cashew apple (pomace), grape (pomace) and banana (peel).

All the samples were initially subjected to sun drying in the open air for three days to bring down the moisture content in the range of 20 to 30 per cent. Then they were dried in the hot air oven at 60^0 C, till the moisture content was around 8 to10 per cent. The dried samples were made into small pieces and ground in mills. The powder was sieved to fine mesh and used for preparing media (Plate 2).

The various isolates selected through the experiment 3.1 and 3.2, were inoculated on wastes of grape, banana, cashew apple and pineapple. Each medium was prepared with 5g of fruit waste powder, 0.05g of urea, 0.25g ammonium sulphate and 2g of agar and autoclaved at 121° C. The observations recorded were as given in experiment 3.2.1.

Number of microorganisms – 5 (3 fungi and 2 bacteria)

Number of media – 4

Replications – 2

Design - CRD

3.4.1 Test for pectinase production in the culture medium

3.4.1.1 Liquid culture

The isolates selected for their efficiency to grow on the different fruit wastes in experiment 3.4.2 (wastes of grape, banana, cashew apple and pineapple) were again inoculated on the liquid media containing 5g fruit waste along with 0.25g of ammonium sulphate and 0.05g of urea taken in 250ml conical flask. The cultures were incubated at room temperature and kept on rotary shaker rotating at 100 rpm. The extract was filtered after five days of inoculation, centrifuged at 10000 rpm. The supernatant was taken and designated as crude enzyme extract.

The presence of pectinase in the extract was tested by noting its efficiency to clarify banana pulp (Maheswari, 2001). The extract was added to banana pulp at the rate of 0.5 per cent of pulp weight and kept at 45° C for three hours (Biocon, 2002). The volume of clarified juice obtained was measured.

3.4.1.2 Solid culture

The isolates selected for their efficiency to grow on fruit wastes were again inoculated on the media used for primary screening (3.4.1) contained in 250 ml conical flask. Five days after inoculation different media were extracted using 100 ml distilled water and tested for the presence of pectinase by adding it to banana pulp.

3.4.2 Identification of effective pectinase producing microorganism.

The microorganisms selected for having the potency to produce pectinase were identified using standard keys like mycelial character, spore character, colour of hyphae.

3.5 STANDARDISATION OF MEDIA FOR PECTINASE PRODUCTION

The effect of composition of medium on growth of microorganisms was studied in this experiment. For this 5g of dried waste material was mixed with 2g of agar dissolved in 100ml distilled water with varying quantity of urea and ammonium sulphate as follows and inoculated with the microbial cultures.

Medium	Composition of the medium
G_1	5*g* grape waste + 0.05*g* urea + 0.25*g* ammonium sulphate
G_2	5*g* grape waste + 0.075*g* urea + 0.25*g* ammonium sulphate
G_3	5*g* grape waste + 0.05*g* urea + 0.3*g* ammonium sulphate
G_4	5*g* grape waste + 0.075*g* urea + 0.3*g* ammonium sulphate
B_1	5*g* banana waste + 0.05*g* urea + 0.25*g* ammonium sulphate
B_2	5*g* banana waste + 0.075*g* urea + 0.25*g* ammonium sulphate
B_3	5*g* banana waste + 0.05*g* urea + 0.3*g* ammonium sulphate
B_4	5*g* banana waste + 0.075*g* urea + 0.3*g* ammonium sulphate
C_1	5*g* cashew apple waste + 0.05*g* urea + 0.25*g* ammonium sulphate
C_2	5*g* cashew apple waste + 0.075*g* urea + 0.25*g* ammonium sulphate
C_3	5*g* cashew apple waste + 0.05*g* urea + 0.3*g* ammonium sulphate
C_4	5*g* cashew apple waste + 0.075*g* urea + 0.3*g* ammonium sulphate
P_1	5*g* pineapple waste + 0.05*g* urea + 0.25*g* ammonium sulphate
P_2	5*g* pineapple waste + 0.075*g* urea + 0.25*g* ammonium sulphate
P_3	5*g* pineapple waste + 0.05*g* urea + 0.3*g* ammonium sulphate
P_4	5*g* pineapple waste + 0.075*g* urea + 0.3*g* ammonium sulphate

Each medium was inoculated with the selected isolates along with the standard culture *Aspergillus foetidus* 115. Observations recorded were same as that of experiment 3.2.1.

3.6 STANDARDISATION OF CONDITIONS FOR PECTINASE PRODUCTION

The temperature, duration of fermentation and extractant for crude enzyme were standardized in this experiment.

The selected isolates were further grown on the best medium identified for their growth with respect to each waste (Plate 3a). Apart from the wastes included in the experiment 3.5 two more wastes *viz.*, jackfruit rind and mango peel were also subjected in ssf. The media composition used was same as that identified for other wastes. The different media prepared were taken in big trays of size 10 x 7 x 4 cm. The trays were white in colour, transparent having airtight lid of same colour. To each medium water was added to keep it in moist condition. Thoroughly mixed medium was autoclaved at 121^0C for 20 minutes, to kill the native microorganisms present in the waste samples. The autoclaved samples were cooled to ambient temperature and each medium was inoculated with spore suspension of each organism at the rate of 2×10^7 g^{-1} of dry material.

3.6.1 Temperature

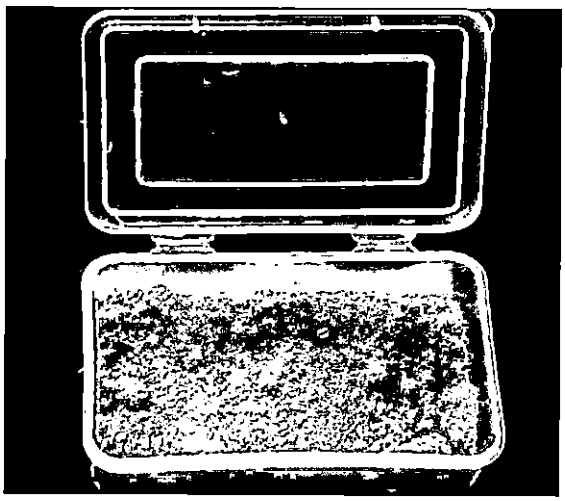
The media were then incubated at three different temperatures *viz.*, 25°, 30° and 40° C. Moisture content was adjusted to approximately 50 per cent and maintained at the same by adding sterile water.

3.6.2 Duration of fermentation

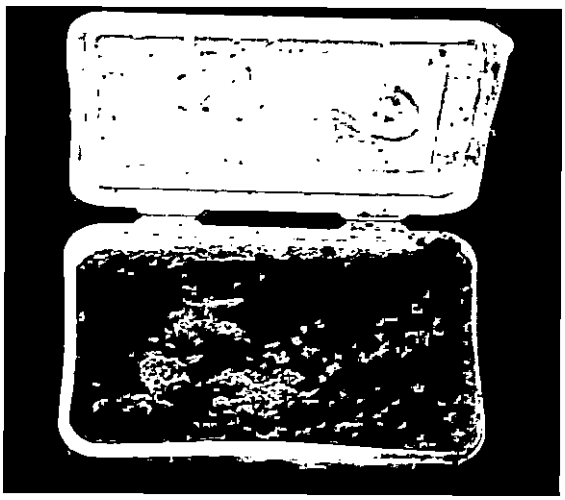
To find the optimum time for fermentation the incubated media were allowed to ferment for different duration *viz.*, five and eight days (Plate 3b).

3.6.3 Extractant

Enzymes from the fermented wastes were extracted with different solvent *viz.*, two per cent calcium chloride and distilled water on fifth and eighth day of



a. Before inoculation



b. Eight days after inoculation

Plate 3. **Medium with cashew apple waste**

fermentation. In each case extraction was done twice and the extracts were pooled and centrifuged at 10000 rpm and the supernatants were used as crude enzyme extract. It was kept for analysing the enzyme activity.

3.7 Analysis of pectin esterase activity

Pectin esterase activity was analysed by measuring the amount of methanol released or increase in free carboxyl group by titration against NaOH (Talboys and Busch, 1970). Twenty milliliters of one per cent pectin dissolved in 0.15 M NaCl (pH 7.0) and four ml of crude enzyme extract were taken in a beaker and incubated for one hour. After incubation, the solution was titrated against 0.02 N NaOH to reach the pH 7.0 using phenolphthalein as indicator. The heated crude enzyme extract was used as control. Pectin esterase activity was calculated by using the following formula.

$$\text{Pectin esterase activity} = V_S - V_b \times (\text{Normality of NaOH}) \times 100 / t$$

Where,

v_s = volume of NaOH used to titrate sample (ml)

v_b = volume of NaOH used to titrate blank (ml)

t = Reaction time (hours)

The data were analysed to draw interpretation.

3.7.1 Test for pectinase production

The extracts from different wastes subjected to ssf under varying conditions were ranked based on their PE activity. The extractants which recorded higher values for PE activity were selected and added to banana pulp for testing the efficiency to yield clarified juice.

STATISTICAL ANALYSIS

The data were subjected to DMRT analyses using MSTAT - C package developed by Harvard University.

Results

4. RESULTS

The data and observations of the present study "Fruit waste utilization for pectinase production in solid state fermentation (ssf)" were analysed and the results are presented in this chapter.

4.1. ISOLATION AND PURE CULTURING OF MICROORGANISMS

4.1.1. Isolation

The inoculation of decaying fruit and vegetable materials on PDA medium after serial dilution resulted in growth of enormous microorganisms on them. At this stage, it was not possible to make records of the different microorganisms that grew, but it served as the basic culture from which different organisms could be separated through pure culturing.

4.1.2. Pure culturing

Following pure culturing technique, 19 different types of fungi and nine bacteria were isolated from wastes of pineapple, jackfruit, ashgourd, watermelon, banana and mango by pure culturing technique. The isolates from each waste were given a code related to the name of waste and numbered separately as given in the Table 1.

4.2. PRELIMINARY SCREENING OF MICROORGANISMS

4.2.1 Performance of fungi in solid pectin medium

The hyphal growth recorded for different fungi in pectin medium for seven days from the date of inoculation is presented in Table 2. All organisms showed tendency to grow in pectin medium but the magnitude of growth differed. The organisms PF_3, JF_3, BP_2, BSF_1, MF_2 and MF_3 exhibited better hyphal elongation viz., above 2.25cm on third day after inoculation. Also when observed continuously

Table 1.**Pure cultures of fungi and bacteria isolated**

Sl. No	Fruit waste	Number of microorganism	
		Fungi	Bacteria
1	Pineapple peel and core	3 (PF_1, PF_2 and PF_3)	1 (PB_1)
2	Jackfruit rind	4 (JF_1, JF_2, JF_3 and JF_4)	2 (JB_1 and JB_2)
3	Ashgourd and water melon	4 (AF_1, AF_2, WF_1 and WF_2)	2 (AB_1 and AB_2)
4	Banana peel and stalk	4 (BF_1, BF_2, BF_3 and BSF_1)	1 (BB_1)
5	Mango peel	4 (MF_1, MF_2, MF_3 and MF_4)	3 (MB_1, MB_2 and MB_3)

P – Pineapple
J – Jackfruit
A – Ashgourd
W – Water melon
B – Banana peel
BS- Banana stalk
M – Mango

F – Fungus
B – Bacteria

Table 2. **Growth of different fungal isolates in solid pectin medium**

Sl. No	Fungal isolate	Hyphal length (cm) Days after inoculation				
		3	4	5	6	7
1	PF_1	2.06 ^{D}	2.56 ^{D}	2.93 ^{E}	3.36 ^{D}	3.93 ^{D}
2	PF_2	1.16 ^{F}	1.80 ^{EF}	1.73 ^{H}	1.90 ^{G}	2.56 ^{FGH}
3	PF_3	2.63 ^{AB}	3.36 ^{AB}	4.03 ^{BC}	4.76 ^{B}	5.53 ^{B}
4	JF_1	1.13 ^{F}	1.53 ^{EF}	1.93 ^{GH}	2.26 ^{EFG}	2.50 ^{GH}
5	JF_2	1.16 ^{F}	1.53 ^{EF}	1.90 ^{H}	2.2 ^{EFG}	2.53 ^{FGH}
6	JF_3	2.46 ^{BC}	3.13 ^{BC}	3.86 ^{CD}	4.46 ^{BC}	5.10 ^{C}
7	JF_4	1.23 ^{F}	1.70 ^{EF}	2.33 ^{F}	2.53 ^{E}	2.76 ^{EFG}
8	AF_1	1.20 ^{F}	1.56 ^{EF}	1.83 ^{H}	2.13 ^{FG}	2.33 ^{H}
9	AF_2	1.10 ^{F}	1.50 ^{EF}	1.86 ^{H}	2.06 ^{G}	2.40 ^{H}
10	WF_1	1.13 ^{F}	1.43 ^{F}	1.73 ^{H}	2.06 ^{G}	2.40 ^{H}
11	WF_2	1.10 ^{F}	1.43 ^{F}	1.86 ^{H}	2.20 ^{EFG}	2.53 ^{FGH}
12	BF_1	1.26 ^{F}	1.70 ^{EF}	2.03 ^{FGH}	2.46 ^{EF}	2.80 ^{EFG}
13	BF_2	2.33 ^{C}	2.93 ^{C}	3.60 ^{D}	4.30 ^{C}	5.16 ^{C}
14	BF_3	1.53 ^{E}	1.86 ^{E}	2.33 ^{F}	2.50 ^{EF}	3.03 ^{E}
15	BSF_1	2.70 ^{A}	3.66 ^{A}	4.23 ^{AB}	5.26 ^{A}	5.93 ^{A}
16	MF_1	1.13 ^{F}	1.43 ^{F}	1.73 ^{H}	2.03 ^{G}	2.26 ^{H}
17	MF_2	2.56 ^{AB}	3.40 ^{AB}	4.53 ^{A}	5.33 ^{A}	6.10 ^{A}
18	MF_3	2.70 ^{A}	3.56 ^{A}	4.46 ^{A}	5.33 ^{A}	6.10 ^{A}
19	MF_4	1.30 ^{F}	1.76 ^{EF}	2.23 ^{FG}	2.50 ^{EF}	2.83 ^{EF}

upto seven days, growth of these organisms was comparatively faster. On the seventh day, the hyphal growth of these organisms was above 5cm and were ranked superior to others. Hence, they were preliminarily selected out for subsequent experiments.

4.2.2. Performance of fungi in liquid pectin culture

The hyphal mass of the fungi (selected based on the performance in solid medium) in liquid culture five days after inoculation and turbidity of the culture broth are given in Table 3. The fungi *Trichoderma harzianum* and BSF_1 recorded comparatively higher hyphal mass (473.7mg and 462.3mg respectively) and were ranked superior to others. The culture broths of these fungi were also less turbid compared to others (Plate 5). So the fungi BSF_1 and *Trichoderma harzianum* were selected for assessing their capacity to produce pectinase.

4.2.3 Performance of bacteria in solid pectin medium

The number of colonies produced by different bacteria inoculated on pectin medium is presented in Table 4. The bacterial isolate BB_1 and *Pseudomonas fluorescens* exhibited better growth performance and produced 14.33 and 13.33 colonies on an average respectively at 24 hours after incubation. The colony number increased to 19 and 17.66 respectively on second day and on third day the colonies were found to be merged. So further observations could not be recorded, and the bacteria BB_1 and *Pseudomonas fluorescens* were selected out for further studies.

In total, two fungal isolates (BSF_1 and *Trichoderma harzianum*) and two bacterial isolate *viz.*, BB_1 and *Pseudomonas fluorescens* were screened out through preliminary screening studies.

4.3. SECOND STAGE SCREENING OF MICROORGANISMS TO PRODUCE PECTINASE

4.3.1 Screening in dye containing media

The daily hyphal growth of two fungi (selected through preliminary screening) recorded on pectin media containing different dyes is presented in Table 5

Table 3. **Performance of fungal isolates in pectin liquid culture**

Sl. No	Name of the fungal isolates	Fungal mass Dry wt (mg)	Turbidity
1	PF_3	281.0 ^{C}	++
2	JF_3	318.3 ^{B}	+++
3	BF_2	321.3 ^{B}	++
4	BSF_1	462.3 ^{A}	+
5	MF_2	330.7 ^{B}	++
6	MF_3	324.7 ^{B}	++
7	*Trichoderma viride*	329.0 ^{B}	+
8	*Trichoderma harzianum*	473.7 ^{A}	+

Turbidity of the medium

\+ - Less turbid

++ - Turbid

+++ - Highly turbid

Table 4.**Growth of bacterial isolates in pectin medium**

Sl. No	Name of the bacterial isolate	Colony forming units (cfu/g) Days after inoculation		
		1	2	3
		X 10^5	X 10^5	X 10^5
1	PB_1	4.66 ^{CD}	5.33 ^{E}	6.33 ^{BC}
2	JB_1	3.66 ^{D}	5.66 ^{E}	Colony merged
3	JB_2	5.66 ^{CD}	8.66 ^{CD}	10.00 ^{ABC}
4	AB_1	8.66 ^{B}	11.67 ^{B}	16.33 ^{A}
5	AB_2	6.66 ^{C}	9.66 ^{BC}	12.33 ^{ABC}
6	BB_1	14.33 ^{A}	19. 00 ^{A}	Colony merged
7	MB_1	5.33 ^{CD}	7.00 ^{DE}	11.33 ^{ABC}
8	MB_2	6.33 ^{C}	9.66 ^{BC}	13.67 ^{AB}
9	MB_3	5.66 ^{CD}	9.66 ^{BC}	13.67 ^{AB}
10	*Pseudomonas fluorescens*	13.33 ^{A}	17.66 ^{A}	Colony merged
11	*Azotobactor* sp.	5.66 ^{CD}	8.33 ^{CD}	11.33 ^{ABC}

Table 5. **Growth of selected fungi in pectin medium modified with dyes**

Sl. No	Name of the fungal isolates	Hyphal length (cm)											
		Days after inoculation											
		MR medium				BCR medium				BPB medium			
		3	4	5	6	3	4	5	6	3	4	5	6
1	BSF $_1$	3.90	4.20	4.60	4.90	3.80	4.40	4.70	5.60	3.40	3.90	4.40	4.90
2	*T harzianum*	3.10	3.40	3.90	4.20	3.40	3.90	4.20	4.90	3.20	3.70	4.30	4.80
3	*A foetidus* 115	3.30	3.90	4.40	4.80	3.20	3.80	4.20	4.90	3.10	3.60	4.20	4.90

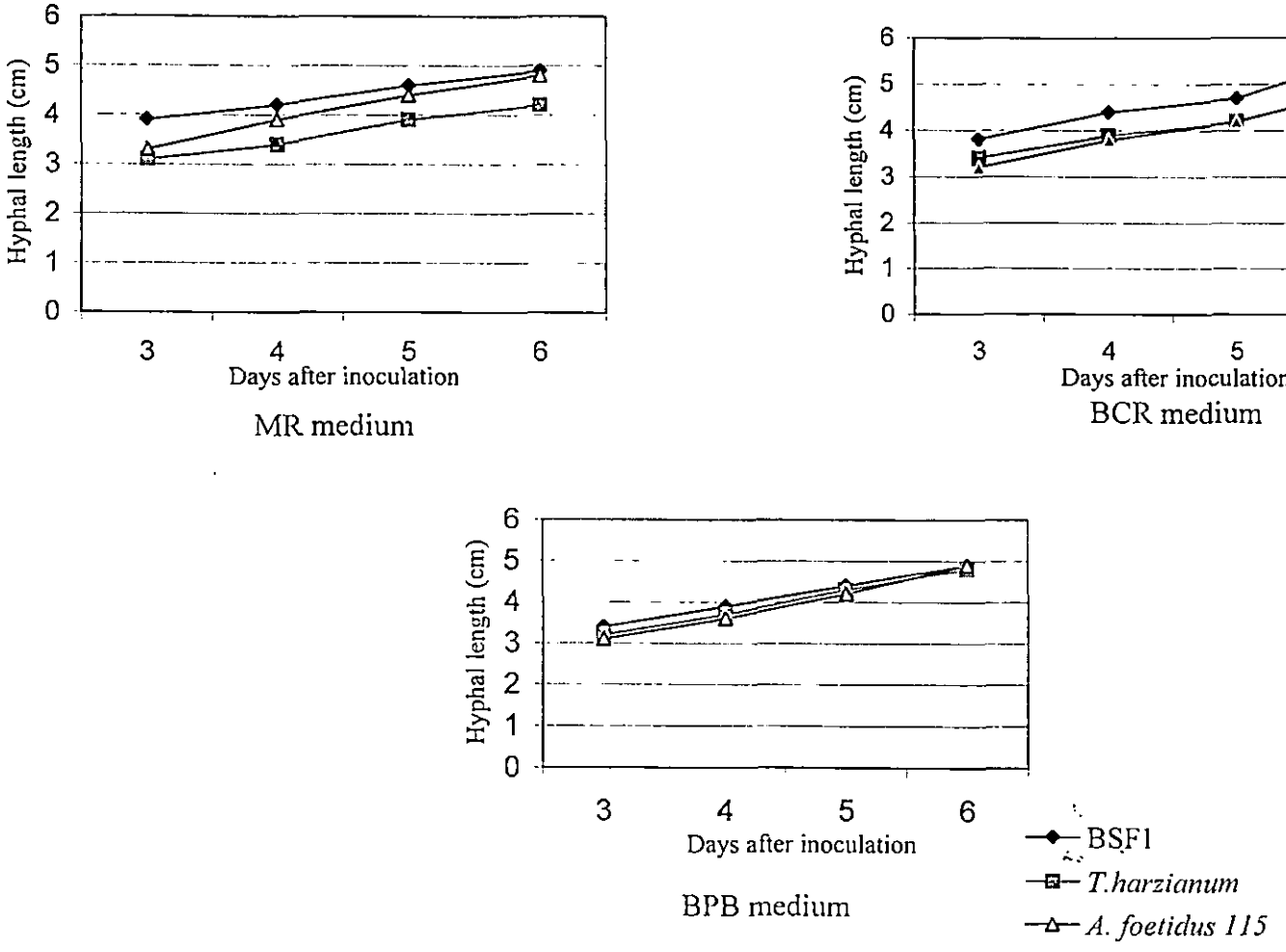


Fig 1. Growth of the fungi in dye containing pectin medium

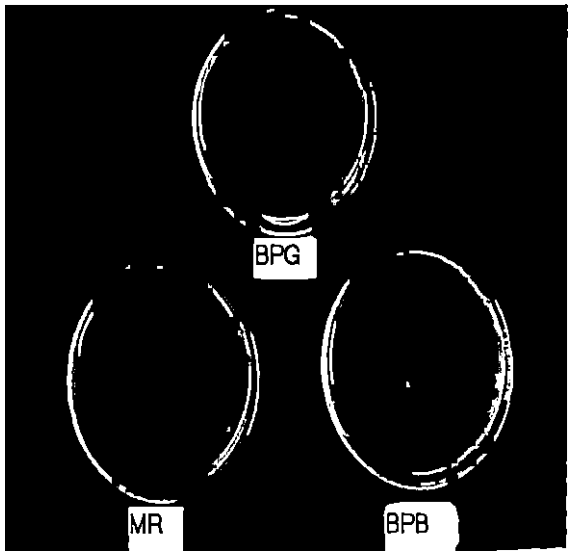


Plate 4. **Growth of BSF_1 (*Aspergillus* sp.) in pectin medium with dyes**

BPG – Bromo Cresol Green
MR - Methyl Red
BPB- Bromo Phenol Blue

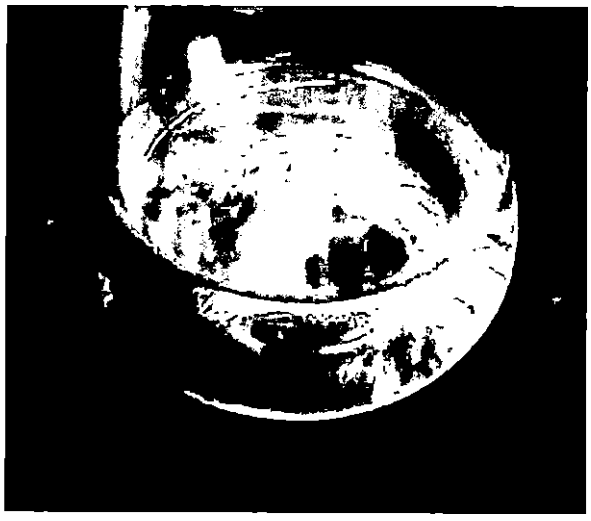


Plate 5. **Liquid pectin medium inoculated with *Trichoderma harzianum* (5 days after inoculation)**

along with hyphal growth of standard culture (*Aspergillus foetidus* 115) on same medium.

All the fungi recorded increase in hyphal length in different media. In MR and BCR medium the fungi BSF_1 recorded better growth (Plate 4), where as in BPB medium the growth of different fungi were almost same (Fig. 1). None of the fungi showed colour change in the pectin medium containing different dyes. So the observations on hydrolysed zone were not recorded.

4.3.2. Screening of microorganisms in media containing different quantities of pectin

4.3.2.1. Fungi

The growth parameters of different fungi included in the study are presented in the Table 6. and in Fig. 2.

The fungus *Aspergillus foetidus* 115 recorded no growth in any media except in medium C, when observed on third day after inoculation (Plate 6). In medium C it recorded a hyphal elongation of 1.50 cm when observed on third day after inoculation and on seventh day the hyphae elongated to an average length of 4.45 cm. In all other media the growth was slow.

The result was almost same in the case of fungus BSF_1. The growth was comparatively fast in medium C and the hyphal elongation was 5.60 cm after seven days after inoculation.

In the case of *Trichoderma harzianum*, medium D and C were comparatively better. The hyphae grew to a length of 4.80 and 5.05 cm in medium C and D respectively when observed seven days after inoculation.

No hydrolysed zone was observed in the different media inoculated with the different fungi included in the experiment.

4.3.2.2.Bacteria

The number of colony forming units recorded for *Pseudomonas fluorescens* and BB_1 in different media are presented in Table 7 and in Fig. 3. In the case of *Pseudomonas fluorescens* the medium A, C and E recorded better colony formation

Table 6. **Growth of fungi in media containing different quantities of pectin**

Medium	Hyphal growth (cm)														
	Days after inoculation														
	BSF_1					*Trichoderma harzianum*					*Aspergillus foetidus* 115				
	3	4	5	6	7	3	4	5	6	7	3	4	5	6	7
A	2.40^{B}	3.40^{BC}	4.20^{BC}	5.05^{A}	5.30^{B}	1.60^{B}	2.70^{B}	3.30^{BC}	3.65^{C}	4.25^{C}	0	1.40^{C}	2.10^{D}	2.70^{B}	3.30^{C}
B	2.90^{A}	3.65^{A}	4.35^{B}	4.85^{B}	5.30^{B}	1.65^{B}	2.25^{C}	2.60^{C}	3.10^{D}	3.60^{D}	0	1.11^{D}	2.00^{C}	2.75^{B}	3.60^{B}
C	2.90^{A}	3.75^{A}	4.50^{A}	5.05^{A}	5.60^{A}	2.20^{A}	2.90^{A}	3.40^{B}	4.00^{AB}	4.80^{B}	1.50^{A}	2.06^{A}	2.70^{A}	3.65^{A}	4.45^{A}
D	2.65^{B}	3.35^{BC}	4.05^{C}	4.65^{C}	5.30^{B}	2.30^{A}	2.95^{A}	3.60^{A}	4.25^{A}	5.05^{A}	0	1.50^{B}	2.30^{B}	2.60^{C}	3.25^{C}
E	1.50^{C}	3.50^{B}	2.80^{D}	3.15^{D}	3.40^{C}	1.50^{C}	1.80^{D}	2.25^{D}	2.30^{E}	2.55^{E}	0	1.50^{B}	2.25^{B}	2.60^{C}	3.20^{CD}

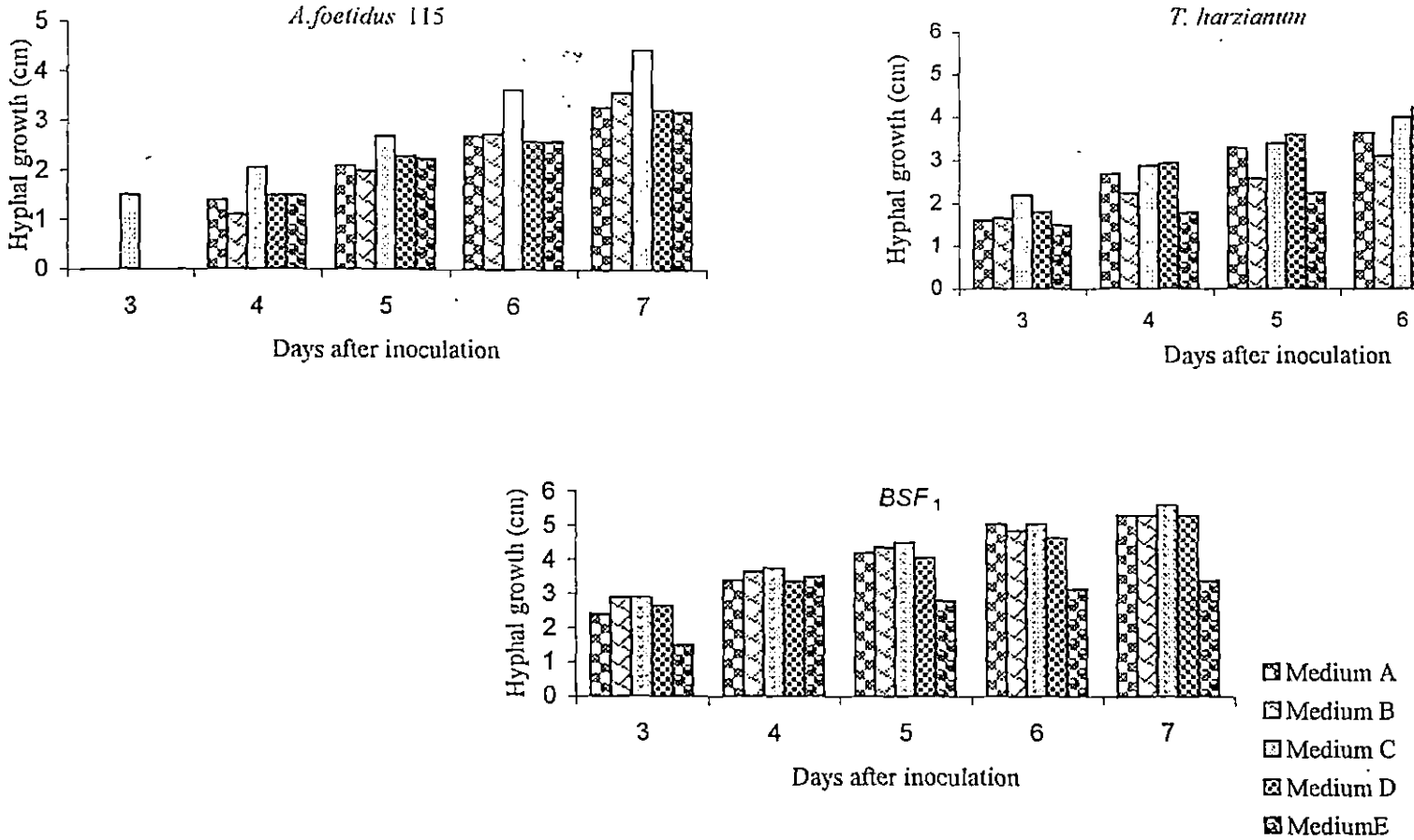


Fig. 2 Growth of fungi on the media containing varying quantities of pectin

Table 7. **Growth of bacteria in media containing different quantities of pectin**

Medium	Number of colony forming units/ml					
	Days after inoculation					
	Pseudomonas fluorescens (X 10^5)			BB_1(X 10^5)		
	1	2	3	1	2	3
A	7.00 ^{A}	7.50 ^{C}	8.00 ^{C}	6.00 ^{C}	8.00 ^{C}	10.00 ^{C}
B	5.00 ^{C}	6.50 ^{D}	6.50 ^{D}	5.50 ^{D}	6.50 ^{D}	7.00 ^{DE}
C	7.00 ^{A}	9.50 ^{A}	10.50 ^{A}	5.50 ^{D}	10.00 ^{A}	11.50 ^{A}
D	6.00 ^{B}	7.50 ^{C}	8.00 ^{C}	7.00 ^{B}	8.00 ^{C}	8.00 ^{D}
E	7.50 ^{A}	8.00 ^{B}	8.50 ^{B}	8.50 ^{A}	9.00 ^{B}	11.00 ^{A}

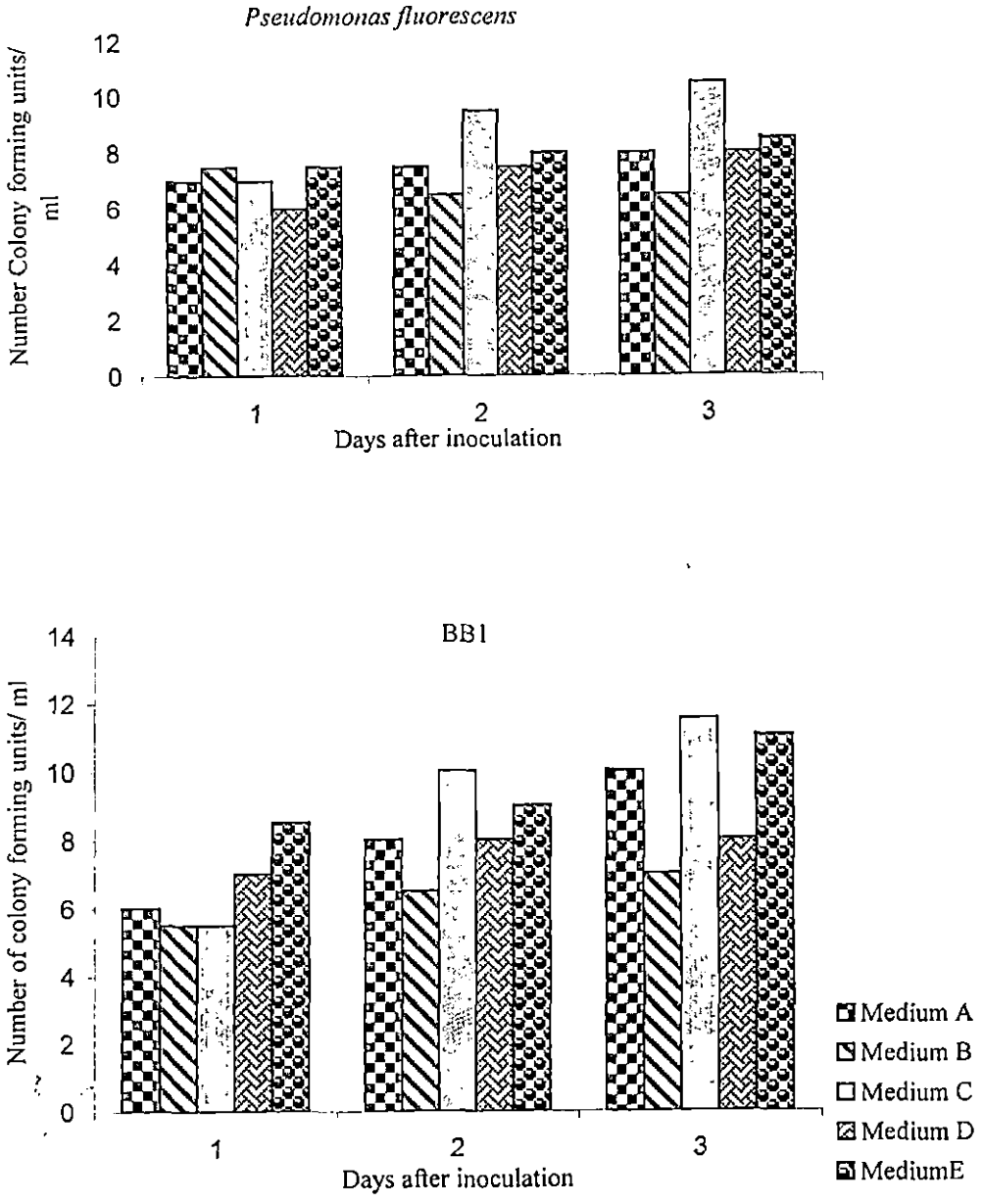


Fig 3. Growth of bacteria on the media containing varying quantities of pectin

at initial stage of observation. However further growth was slow in two media viz., A and E. At final stage of observation (third day) a total of 10.5 colonies were observed on an average in the medium C, which was comparatively high.

For BB_1 initially the colony formation was high in the medium E. When observed on second and third day medium E and C were found to support better colony formation. On third day the number of colonies in the medium C and E were 11.50 and 11.00 respectively.

4.4 PERFORMANCE OF SELECTED MICROORGANISMS IN SSF

4.4.1 Fungi

The hyphal elongation recorded daily for different fungi included in the experiment are given in the Table 8.

Compared to other microorganisms, the standard culture *Aspergillus foetidus* 115 recorded fast growth on all media (Fig. 4). It is also evident that the performance of *Trichoderma harzianum* was better compared to BSF_1 on all fruit wastes included in the study. On grape waste medium it recorded a hyphal growth of 4.20 cm on third day after inoculation, which extended to 6.00 cm on sixth day. In media containing banana waste the hyphae of *T. harzianum* recorded a length of 7.40 cm on sixth day after inoculation, which was not significantly different from that recorded for *A foetidus* 115 at this stage. On cashew apple and pineapple waste media the hyphal length recorded for *T. harzianum* were 6.50 and 6.40 cm respectively.

4.4.2 Bacteria

The number of colonies developed by different bacteria included in the study is given in Table 9 and Fig. 5

The colony formation by the different bacteria was very less on different media containing fruit wastes. There was no increase in number of colonies from third day after inoculation onwards.

Table 8. **Performance of fungal isolates on fruit wastes**

Name of the isolate	Hyphal length (cm)															
	Days after inoculation															
	Grape waste				Banana waste				Cashew waste				Pineapple waste			
	3	4	5	6	3	4	5	6	3	4	5	6	3	4	5	6
BSF_1	3.80	4.20	5.10	5.80	4.20	4.90	5.40	6.40	2.90	3.70	4.30	5.40	3.30	3.90	4.90	5.50
T.harzianum	4.20	4.80	5.30	6.00	4.70	5.80	6.40	7.40	4.60	5.30	5.90	6.50	3.00	4.00	4.80	5.40
A.foetidus 115	4.40	5.90	7.20	8.30	4.70	6.10	6.80	7.60	5.10	6.10	6.60	7.50	4.40	5.30	6.00	6.70

Table 9. **Performance of bacterial isolates on fruit wastes**

Name of the bacterial isolate	Number of colony forming units / ml of broth											
	Days after inoculation											
	Grape waste ($X 10^5$)			Banana waste ($X 10^5$)			Cashew waste ($X 10^5$)			Pineapple waste ($X 10^5$)		
	1	2	3	1	2	3	1	2	3	1	2	3
Pseudomonas fluorescens	3.50	4.50	5.00	1.50	2.00	3.50	3.50	4.50	4.50	2.50	3.50	4.00
BB_1	3.00	3.50	4.50	2.00	3.00	3.00	2.50	3.50	4.00	2.50	3.00	3.50

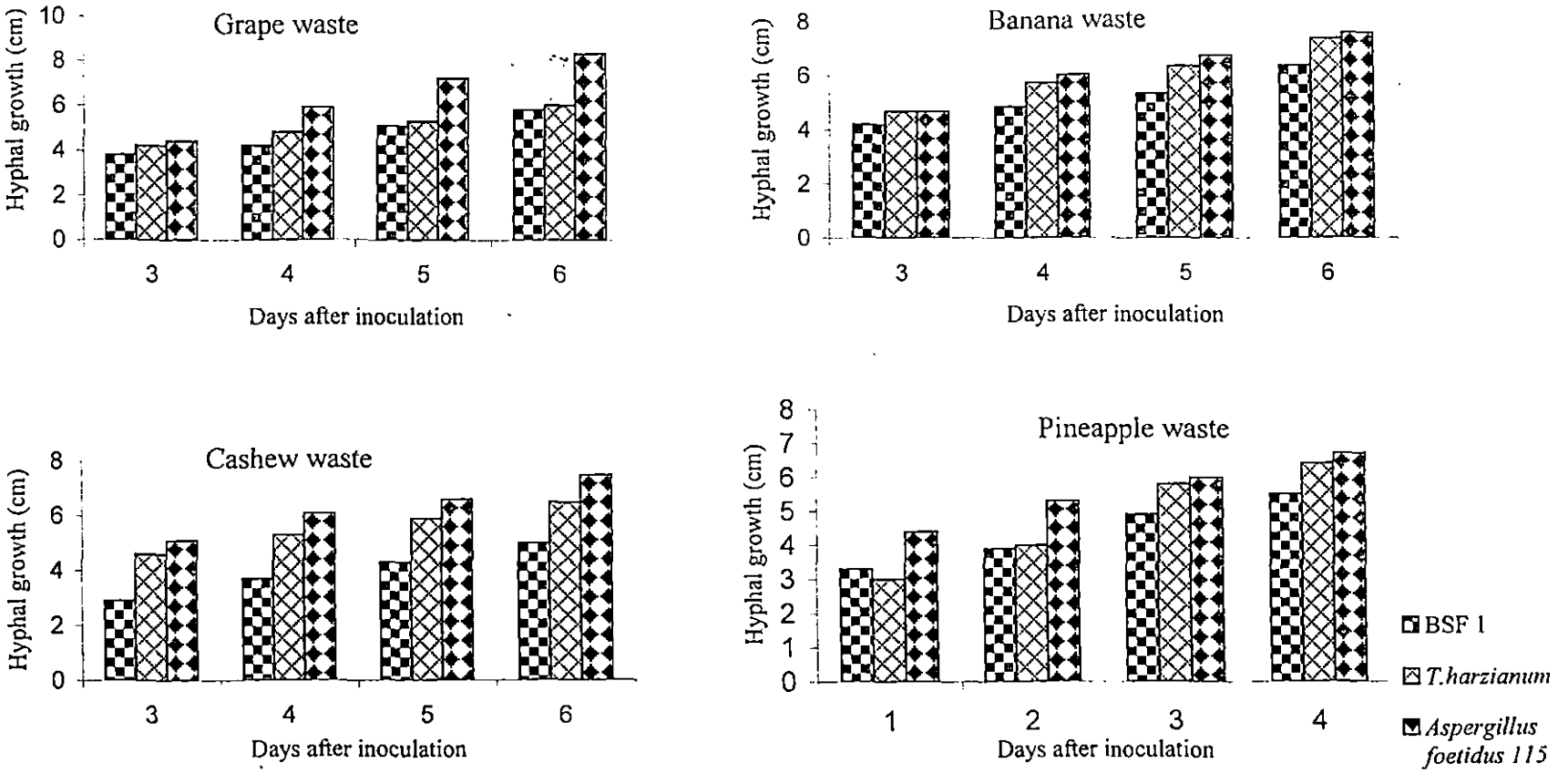


Fig 4. Growth of selected fungi on fruit waste medium

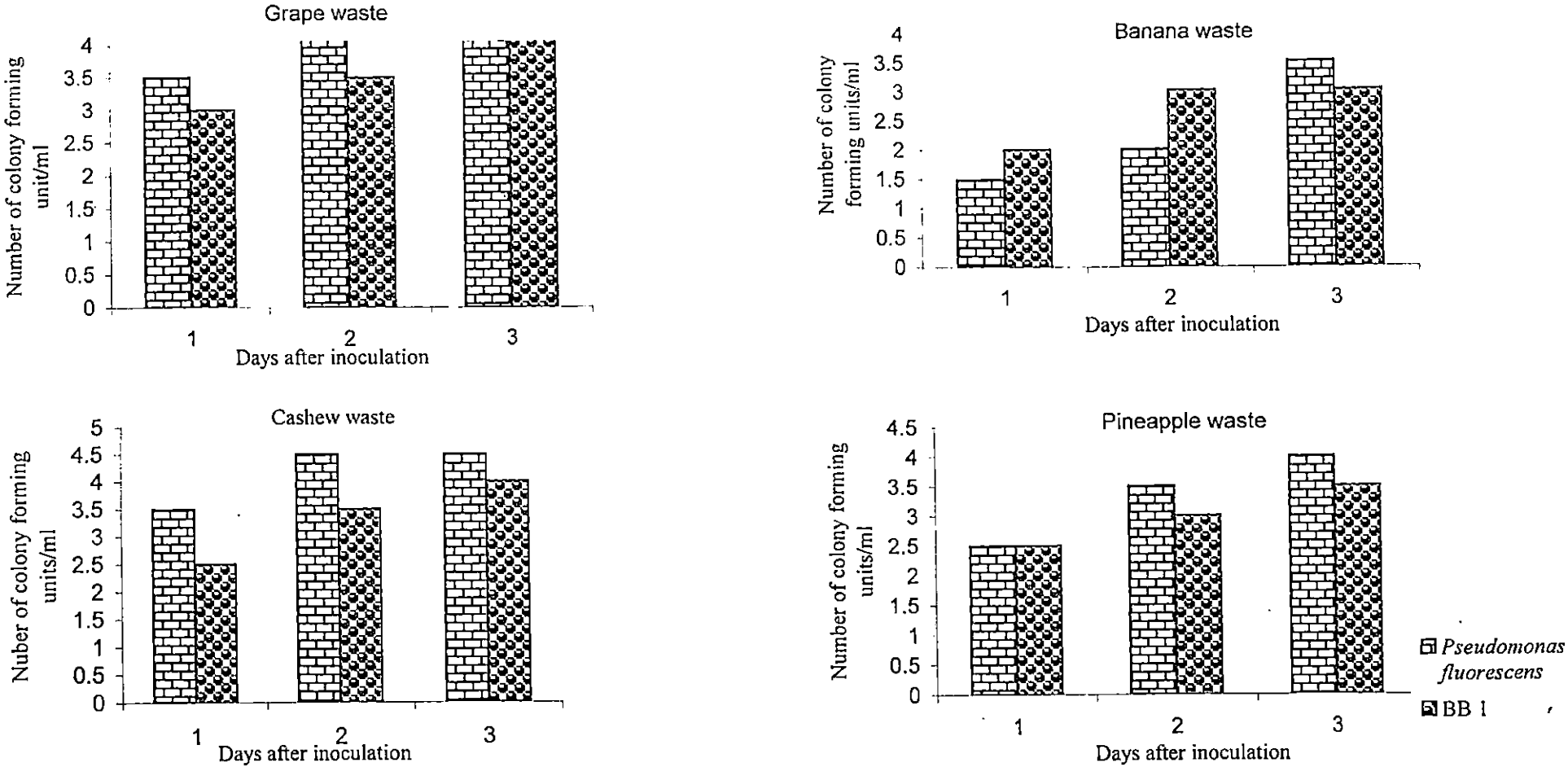


Fig 5. Growth of selected bacteria on fruit waste medium

As the efficiency of different bacteria to grow on fruit wastes were found very less they were not included in further studies. Hence only three fungi were selected for further studies.

4.4.3. Test for pectinase production

4.4.3.1 Submerged culture

The quantity of clarified juice obtained from banana pulp by adding crude enzyme extract prepared from wastes are given in Table 10. The volume of clarified juice produced was comparatively high when extract prepared from all fruit wastes under smf using *Aspergillus foetidus* 115 was added to banana pulp. The extract from grape waste which was subjected to smf using this microorganism when added to banana pulp gave 33ml of clarified juice per 100g of pulp. Similarly the extracts from cashew apple under smf gave 25ml clarified banana juice.

Next to *Aspergillus foetidus* 115, more efficiency to clarify banana pulp was exhibited by extracts of different wastes fermented by *Trichoderma harzianum*. In this case also, the extract from grape waste under smf gave maximum clarified banana juice (21ml) followed by that from cashew apple (19ml) and pineapple (18 ml). The performance of BSF_1 was more or less on par with *Trichoderma harzianum* on cashew apple waste.

4.4.2.2 Solid culture

The results of adding crude enzyme extract prepared from wastes (subjected to ssf using three fungi) on banana pulp are given in Table 11. Maximum clarified juice was obtained when extract made from different wastes under ssf using *Aspergillus foetidus* 115 was added to banana pulp. The extract from grape waste and cashew apple waste fermented using *Aspergillus foetidus*115 added to banana pulp produced maximum clarified juice of 38ml (Plate 7). The effect of different isolates *viz.*, BSF_1 and *Trichoderma harzianum* differed on different wastes.

The extract from cashew apple waste subjected to ssf using BSF_1 and *Trichoderma harzianum* produced comparatively more quantity of clarified banana juice (29 ml). The fungus *Trichoderma harzianum* was found to be more efficient in

Table 10. **Quantity of clarified banana juice obtained in *smf***

Fungi / waste	Volume of clarified banana juice per 100g pulp (ml)			
	Grape	Banana	Cashew apple	Pineapple
BSF_1	17	17	18	15
T. harzianum	21	17	19	18
A. foetidus 115	33	23	25	20

Table 11. **Quantity of clarified banana juice obtained *in ssf***

Fungi / waste	Volume of clarified banana juice per 100g pulp (ml)			
	Grape	Banana	Cashew apple	Pineapple
BSF_1	22	25	29	19
T.harzianum	24	21	29	26
A.foetidus 115	38	36	38	36

pectinase production from grape and pineapple waste as evidenced from the production of more clarified banana juice. The extract from grape waste and pineapple waste under ssf when added to banana pulp produced 24ml and 26ml clarified juice respectively. For ssf of banana waste, the microorganism BSF_1 was found better. The extract from ssf of banana pulp using this microorganism when added to banana pulp produced 25ml clarified juice.

4.4 Identification of pectinase producing microorganism

The microorganism BSF_1 was identified as that belonging to genus *Aspergillus*. using the standard keys available (Plate 8).

4.5 STANDARDISATION OF MEDIA FOR SOLID STATE FERMENTATION

4.5.1 Grape waste

The growth of different fungi on medium containing 5g grape waste and other nutrients in varying composition is given in Table 12.

The growth of BSF_1 (*Aspergillus* sp) was comparatively more (4.00 cm) on G_3 media, when recorded at all stages of observation.

In the case of *T harzianum* the initial growth was almost same in all media except in G_4. Medium G_4 presented comparatively poor growth. When observed on sixth day after inoculation, the growth was comparatively more in medium G_3 (7.15 cm).

The growth of *A. foetidus* 115 was more in medium G_3 at the first stage of observation. The superiority of this medium was continued and the hyphal elongation recorded was 8.8cm on sixth day after inoculation. At final stage of observation the growth of this fungi in medium G_1 and G_2 were on par with medium G_3 (Plate 9).

When the growth of different fungi alone is considered, it was observed that *A. foetidus* 115 grow fast in grape waste medium, followed by *T. harzianum* (Fig 6a).

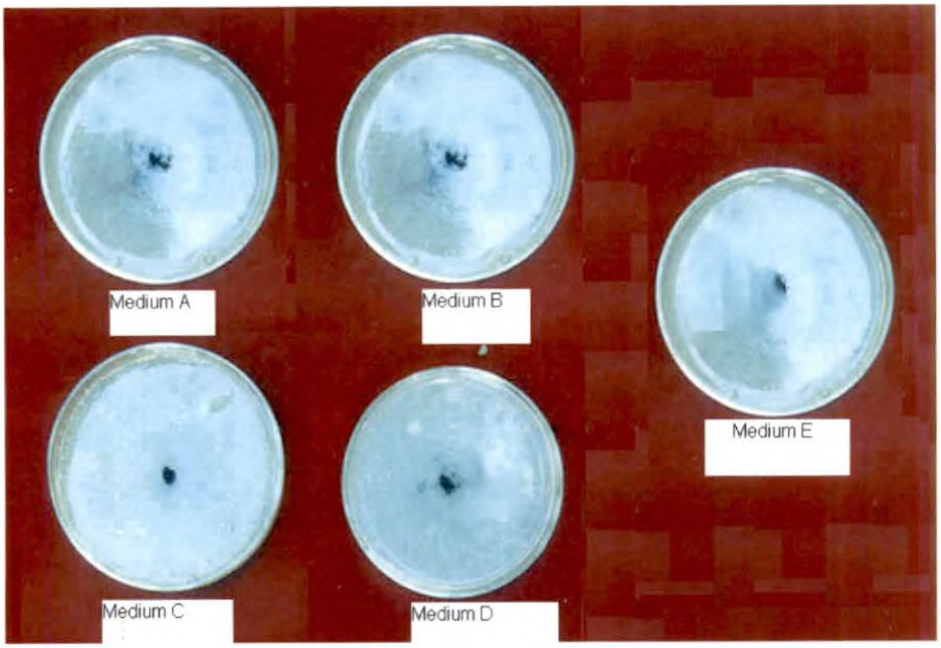


Plate 6. **Growth of *Aspergillus foetidus* 115 on media containing varying quantities of Pectin**

Plate 7. **Banana juice clarified by crude enzyme extract**

Table 8. **Sporangia of *Aspergillus* sp. (BSF_1)**

4.5. 2 Banana waste

The growth of all the fungi in terms of hyphal elongation is given in Table 13. The growth the fungus BSF_1 (*Aspergillus* sp.) was comparatively more in B_3 medium at all stages of observation. In the case of *T. harzianum* also the growth recorded was more in B_3 medium except that recorded on fourth day after inoculation. Only at this stage the growth was more in B_2. For *A foetidus* also B_3 medium was better. Even though initial growth was almost same in all the media, the medium B_3 proved its superiority by presenting comparatively fast growth at all stages of observation.

When the growth of different fungi on banana waste is considered, pooling the effect of different media, *A. foetidus* and *T. harzianum* were found to record almost same growth throughout the period of observation (Fig 6b)

4.5.3 Cashew apple waste

The growth parameters of different fungi in media containing cashew apple waste are presented in Table 14.

For the fungi BSF_1 (*Aspergillus* sp.) and *T. harzianum* the medium C_3 was found comparatively better. For BSF_1, the hyphal length recorded was 4.85 cm on third day and 7.60 cm on sixth day after inoculation. In the case of *T. harzianum* hyphal length was 4.80 cm and 7.40 cm respectively when observed at third and sixth day after inoculation.

Irrespective of media, the fungus *A. foetidus* 115 grew fast on medium C_2 and C_3 at all stages of observation followed by *T. harzianum* (Fig 6c).

4.5.4 Pineapple waste

The growth of the selected fungi on different media containing pineapple waste is presented in Table 15.

The medium P_3 was found better for the growth of all the three fungi. The growth was comparatively more in this medium at a l stages of observation. When the growth of microoragnisms alone is considered, irrespective of the media composition, *A. foetidus* 115 recorded fast growth, followed by *T harzianum* (Fig 6d).

Table 12. **Growth of fungi in grape waste media**

Medium	Hyphal length (cm)											
	Days after inoculation											
	BSF 1				*Trichoderma harzianum*				*Aspergillus foetidus* 115			
	3	4	5	6	3	4	5	6	3	4	5	6
G_1	3.30 ^{B}	4.05 ^{B}	5.00 ^{AB}	5.75 ^{B}	4.15 ^{A}	4.80 ^{B}	5.75 ^{A}	6.25 ^{B}	4.85 ^{AB}	6.30 ^{AB}	8.00 ^{A}	8.65 ^{A}
G_2	3.30 ^{B}	3.90 ^{B}	4.40 ^{B}	4.80 ^{B}	4.05 ^{A}	4.80 ^{A}	5.50 ^{A}	6.15 ^{B}	4.55 ^{BC}	5.60 ^{B}	7.10 ^{AB}	8.50 ^{A}
G_3	4.00 ^{A}	4.70 ^{A}	5.40 ^{A}	6.25 ^{A}	4.05 ^{A}	5.00 ^{A}	6.20 ^{A}	7.15 ^{A}	5.05 ^{A}	6.80 ^{A}	7.90 ^{A}	8.80 ^{A}
G_4	3.20 ^{B}	4.05 ^{B}	4.85 ^{AB}	5.65 ^{B}	3.15 ^{B}	4.00 ^{B}	4.75 ^{B}	5.65 ^{B}	4.30 ^{C}	5.45 ^{B}	6.30 ^{B}	7.20 ^{B}

Table 13. **Growth of fungi in banana waste media**

Medium	Hyphal length (cm)											
	BSF 1				*Trichoderma harzianum*				*Aspergillus foetidus* 115			
	3	4	5	6	3	4	5	6	3	4	5	6
B_1	4.60 ^{B}	5.05 ^{B}	5.65 ^{C}	6.40 ^{B}	4.80 ^{B}	6.35 ^{AB}	6.60 ^{B}	7.40 ^{B}	4.80 ^{A}	5.90 ^{A}	6.75 ^{AB}	7.20 ^{B}
B_2	4.80 ^{AB}	5.35 ^{B}	6.05 ^{B}	6.70 ^{B}	5.00 ^{AB}	6.50 ^{A}	6.15 ^{C}	7.15 ^{B}	4.90 ^{A}	5.90 ^{A}	6.40 ^{B}	7.40 ^{B}
B_3	5.15 ^{A}	6.30 ^{A}	7.20 ^{A}	8.10 ^{A}	5.20 ^{A}	6.15 ^{B}	7.15 ^{A}	8.12 ^{A}	5.20 ^{A}	6.30 ^{A}	7.30 ^{A}	8.80 ^{A}
B_4	4.10 ^{C}	4.90 ^{B}	5.50 ^{C}	6.40 ^{B}	4.45 ^{C}	5.30 ^{C}	6.15 ^{C}	7.15 ^{B}	4.55 ^{A}	5.30 ^{B}	6.30 ^{B}	7.30 ^{B}

Table 14. **Growth of fungi in cashew apple waste media**

Medium	Hyphal length (cm)											
	Days after inoculation											
	BSF_1				*Trichoderma harzianum*				*Aspergillus foetidus 115*			
	3	4	5	6	3	4	5	6	3	4	5	6
C_1	3.15 ^{B}	3.75 ^{B}	4.20 ^{B}	4.65 ^{C}	3.55 ^{A}	4.65 ^{B}	5.35 ^{B}	6.05 ^{B}	4.95 ^{A}	6.05 ^{A}	6.45 ^{B}	7.70 ^{B}
C_2	2.95 ^{B}	3.75 ^{B}	4.50 ^{B}	5.25 ^{B}	3.65 ^{B}	4.25 ^{BC}	5.00 ^{C}	5.70 ^{BC}	5.15 ^{A}	6.15 ^{A}	7.30 ^{A}	8.80 ^{A}
C_3	4.85 ^{A}	5.70 ^{A}	6.40 ^{A}	7.60 ^{A}	4.80 ^{A}	5.70 ^{A}	6.75 ^{A}	7.40 ^{A}	5.15 ^{A}	6.15 ^{A}	7.20 ^{A}	8.80 ^{A}
C_4	3.00 ^{B}	3.60 ^{B}	4.45 ^{B}	5.30 ^{B}	3.15 ^{B}	3.80 ^{C}	4.75 ^{C}	5.40 ^{C}	4.60 ^{A}	5.70 ^{A}	6.60 ^{B}	7.75 ^{B}

Table 15. **Growth of fungi in pineapple waste media**

Medium	Hyphal growth (cm)											
	Days after inoculation											
	BSF_1				*Trichoderma harzianum*				*Aspergillus foetidus 115*			
	3	4	5	6	3	4	5	6	3	4	5	6
P_1	3.25 ^{B}	3.95 ^{B}	4.90 ^{B}	5.60 ^{B}	3.20 ^{B}	4.00 ^{B}	5.30 ^{B}	6.15 ^{A}	4.35 ^{C}	5.30 ^{BC}	6.00 ^{B}	6.55 ^{B}
P_2	3.40 ^{B}	4.00 ^{B}	4.80 ^{B}	5.75 ^{B}	3.05 ^{B}	3.45 ^{B}	4.50 ^{C}	5.35 ^{B}	4.75 ^{B}	5.55 ^{B}	6.05 ^{B}	6.85 ^{B}
P_3	4.40 ^{A}	5.20 ^{A}	6.15 ^{A}	7.15 ^{A}	4.70 ^{A}	5.50 ^{A}	6.50 ^{A}	7.60 ^{A}	5.20 ^{A}	6.30 ^{A}	7.25 ^{A}	8.05 ^{A}
P_4	3.10 ^{B}	3.95 ^{B}	4.85 ^{B}	5.55 ^{B}	3.25 ^{B}	4.15 ^{B}	5.05 ^{B}	5.80 ^{B}	4.30 ^{C}	5.20 ^{C}	5.85 ^{B}	6.45 ^{B}

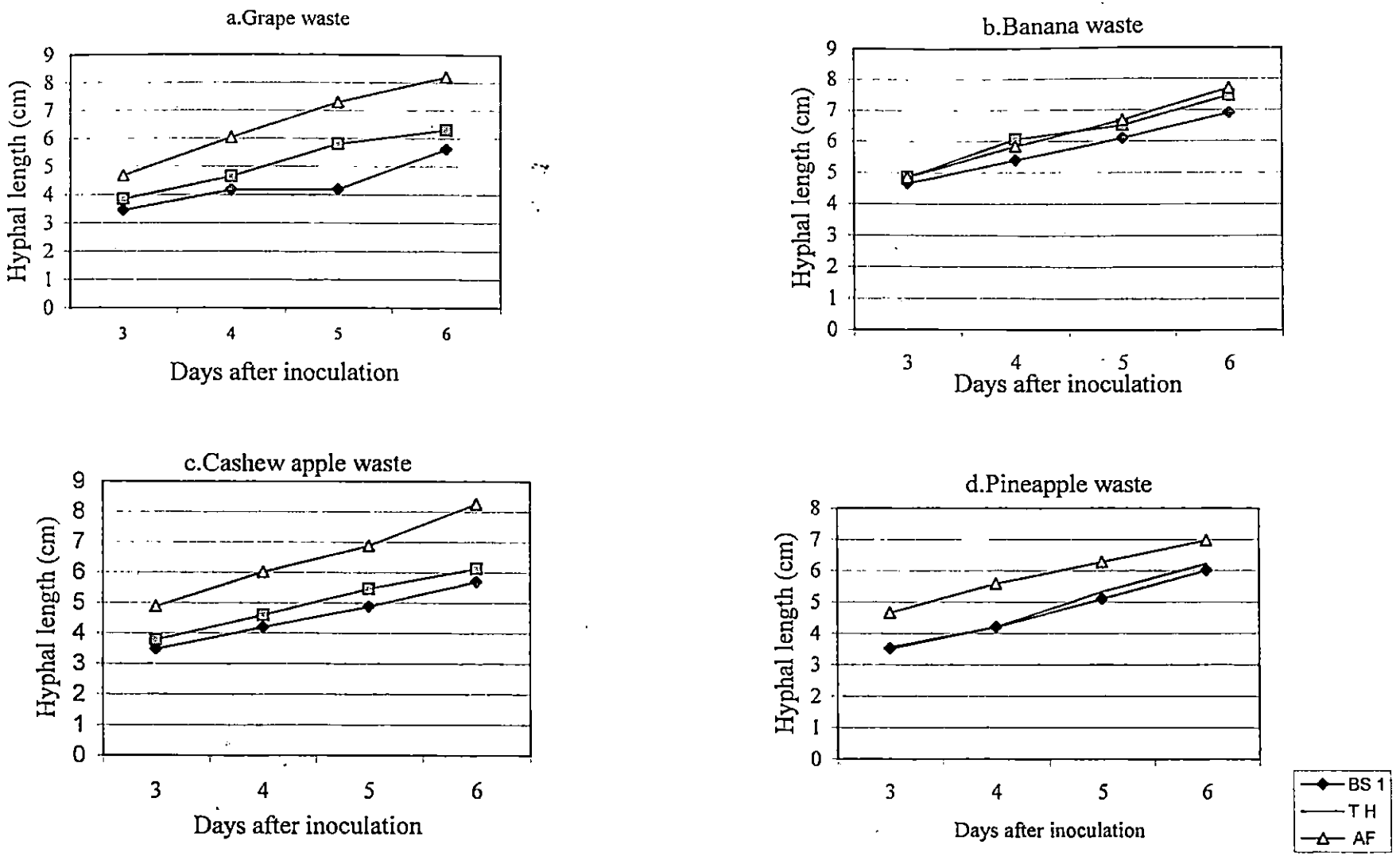


Fig 6. Growth of selected fungi in medium with different fruit waste

4.6 PECTIN ESTERASE ACTIVITY

The activity of pectin esterase recorded in the crude enzyme extracts (Plate 10) taken using two extractants (calcium chloride and distilled water) from different fruit wastes subjected to ssf using three fungi (BSF_1, *Trichoderma harzianum* and *Aspergillus foetidus* 115) at varying time (five and eight days after inoculation) and temperature (25, 30 and 40° C) are given in Table 16, 17, 18, 19, 20 and 21.

In general, the pectin esterase activity was high when the wastes were subjected to fermentation using *Aspergillus foetidus* 115. The efficiency of other two microorganisms was found varying in relation to the wastes and conditions provided. Similarly keeping all the media at higher temperature *viz.*, 40°C was found better compared to the other two temperature studied. The extracts from all the media containing different wastes kept at 40°C recorded high value for PE activity (Fig. 7).

Among the two extractants tried *viz.*, $CaCl_2$ and distilled water, distilled water was found better for extracting enzymes from the fermented media (Fig 8). The ideal extractant, time and temperature for effecting fermentation was not found varying with respect to each microorganisms. Hence the pectin esterase activity exhibited by three fungi at 40°C, eight days after inoculation extracted with distilled water alone was compared.

For the grape waste fermentation and production of pectinase , the microorganism BSF_1(0.26 milli equivalents of NaOH consumed min^{-1} ml^{-1}) was found better compared to *Trichoderma harzianum* (0.20 milli equivalents of NaOH consumed min^{-1} ml^{-1}). The *Aspergillus foetidus* 115 recorded an activity of 0.35 milli equivalents of NaOH consumed min^{-1} ml^{-1} in the same medium under same condition.

The pectin esterase activity in crude enzyme extract from fermented banana waste was observed and results presented in Table 16. The enzyme activity in crude enzyme extract of banana waste fermented by *T.harzianum* abd BSF_1were 0.20 and 0.19 milli equivalents of NaOH consumed min^{-1} ml^{-1} respectively. The enzyme

Table 16. **Pectin esterase activity in fermented grape waste medium**

Fungus	Pectin esterase activity											
	Days after inoculation											
	5						8					
	25° C		30° C		40° C		25° C		30° C		40° C	
	DW	CC	DW	CC	DW	CC	DW	CC	DW	CC	DW	CC
BSF_1	0.08^{A}	0.03^{C}	0.11^{BC}	0.04^{BC}	0.17^{B}	0.10^{AB}	0.15^{B}	0.06^{BC}	0.21^{B}	0.08^{BC}	0.26^{B}	0.12^{BC}
T.h	0.11^{AB}	0.06^{B}	0.14^{B}	0.06^{A}	0.17^{B}	0.09^{B}	0.18^{AB}	0.08^{B}	0.18^{C}	0.09^{B}	0.20^{C}	0.10^{B}
A.f	0.18^{A}	0.11^{A}	0.22^{A}	0.10^{A}	0.24^{A}	0.13^{A}	0.23^{A}	0.17^{A}	0.30^{A}	0.21^{A}	0.35^{A}	0.23^{A}

Table17. **Pectin esterase activity in fermented banana waste medium**

Fungus	Pectin esterase activity											
	Days after inoculation											
	5						8					
	25° C		30° C		40° C		25° C		30° C		40° C	
	DW	CC	DW	CC	DW	CC	DW	CC	DW	CC	DW	CC
BSF_1	0.03^{C}	0.03^{B}	0.08^{BC}	0.06^{B}	0.11^{C}	0.07^{B}	0.09^{C}	0.04^{BC}	0.13^{C}	0.09^{B}	0.19^{C}	0.11^{B}
T.h	0.09^{B}	0.03^{B}	0.10^{B}	0.05^{B}	0.14^{B}	0.04^{C}	0.16^{B}	0.06^{B}	0.18^{B}	0.06^{C}	0.20^{B}	0.09^{C}
A.f	0.14^{A}	0.11^{A}	0.21^{A}	0.10^{A}	0.26^{A}	0.14^{A}	0.24^{A}	0.16^{A}	0.26^{A}	0.16^{A}	0.28^{A}	0.16^{A}

DW – Distilled water
CC – Calcium chloride

Pectin esterase activity given is in terms of milli equivalents of NaOH consumed min^{-1} ml^{-1} of crude enzyme

T.h – *Trichoderma harzianum* ; A.f - *Aspergillus foetidus* 115

Table 18. **Pectin esterase activity in fermented cashew apple waste medium**

Fungus	Pectin esterase activity											
	Days after inoculation											
	5						8					
	25° C		30° C		40° C		25° C		30° C		40° C	
	DW	CC	DW	CC	DW	CC	DW	CC	DW	CC	DW	CC
BSF_1	0.03^{C}	0.03^{B}	0.04^{C}	0.03^{B}	0.09^{B}	0.05^{BC}	0.04^{C}	0.03^{B}	0.06^{C}	0.04^{B}	0.10^{BC}	0.06^{B}
T. h	0.06^{B}	0.03^{B}	0.08^{B}	0.04^{B}	0.10^{B}	0.03^{B}	0.09^{B}	0.03^{B}	0.10^{B}	0.03^{BC}	0.12^{B}	0.06^{B}
A.f	0.15^{A}	0.11^{A}	0.19^{A}	0.11^{A}	0.19^{A}	0.11^{A}	0.24^{A}	0.16^{A}	0.24^{A}	0.16^{A}	0.29^{A}	0.19^{A}

Table 19. **Pectin esterase activity in fermented pineapple waste medium**

Fungus	Pectin esterase activity											
	Days after inoculation											
	5						8					
	25° C		30° C		40° C		25° C		30° C		40° C	
	DW	CC	DW	CC	DW	CC	DW	CC	DW	CC	DW	CC
BSF_1	0.04^{C}	0.02^{C}	0.06^{C}	0.04^{B}	0.07^{C}	0.04^{BC}	0.08^{C}	0.02^{C}	0.10^{C}	0.07^{B}	0.11^{C}	0.07^{BC}
T.h	0.08^{B}	0.03^{B}	0.09^{B}	0.04^{B}	0.12^{B}	0.05^{B}	0.15^{B}	0.06^{B}	0.16^{B}	0.06^{B}	0.19^{B}	0.09^{B}
A.f	0.15^{A}	0.11^{A}	0.21^{A}	0.11^{A}	0.22^{A}	0.13^{A}	0.21^{A}	0.15^{A}	0.28^{A}	0.20^{A}	0.30^{A}	0.21^{A}

DW – Distilled water
CC – Calcium chloride

Pectin esterase activity given is in terms of milli equivalents of NaOH consumed min^{-1} ml^{-1} of crude enzyme

T.h – *Trichoderma harzianum* ; A.f - *Aspergillus foetidus* 115

Table 20. **Pectin esterase activity in fermented mango peel medium**

Fungus	Pectin esterase activity											
	Days after inoculation											
	5						8					
	25° C		30° C		40° C		25° C		30° C		40° C	
	DW	CC	DW	CC	DW	CC	DW	CC	DW	CC	DW	CC
BSF_1	0.04^{C}	0.03^{B}	0.05^{C}	0.03^{C}	0.09^{BC}	0.04^{B}	0.05^{C}	0.03^{C}	0.08^{C}	0.05^{B}	0.19^{B}	0.06^{C}
T.h	0.07^{B}	0.03^{B}	0.08^{B}	0.05^{B}	0.11^{B}	0.04^{B}	0.10^{B}	0.06^{B}	0.11^{B}	0.04^{BC}	0.13^{C}	0.08^{B}
A.f	0.14^{A}	0.11^{A}	0.18^{A}	0.12^{A}	0.20^{A}	0.12^{A}	0.21^{A}	0.13^{A}	0.22^{A}	0.14^{A}	0.26^{A}	0.17^{A}

Table 21. **Pectin esterase activity in fermented jackfruit rind waste medium**

Fungus	Pectin esterase activity											
	Days after inoculation											
	5						8					
	25° C		30° C		40° C		25° C		30° C		40° C	
	DW	CC	DW	CC	DW	CC	DW	CC	DW	CC	DW	CC
BSF_1	0.04^{C}	0.03^{B}	0.06^{C}	0.05^{B}	0.10^{BC}	0.06^{B}	0.05^{C}	0.03^{BC}	0.09^{C}	0.06^{B}	0.11^{C}	0.06^{C}
T.h	0.08^{B}	0.03^{B}	0.09^{B}	0.05^{B}	0.12^{B}	0.03^{C}	0.11^{B}	0.04^{B}	0.12^{B}	0.05^{B}	0.15^{B}	0.09^{B}
A.f	0.15^{A}	0.12^{A}	0.17^{A}	0.12^{A}	0.23^{A}	0.13^{A}	0.22^{A}	0.15^{A}	0.23^{A}	0.15^{A}	0.25^{A}	0.13^{A}

DW – Distilled water
CC – Calcium chloride

Pectin esterase activity given is in terms of milli equivalents of NaOH consumed min^{-1} ml^{-1} of crude enzyme

T.h – *Trichoderma harzianum* ; A.f – *Aspergillus foetidus* 115

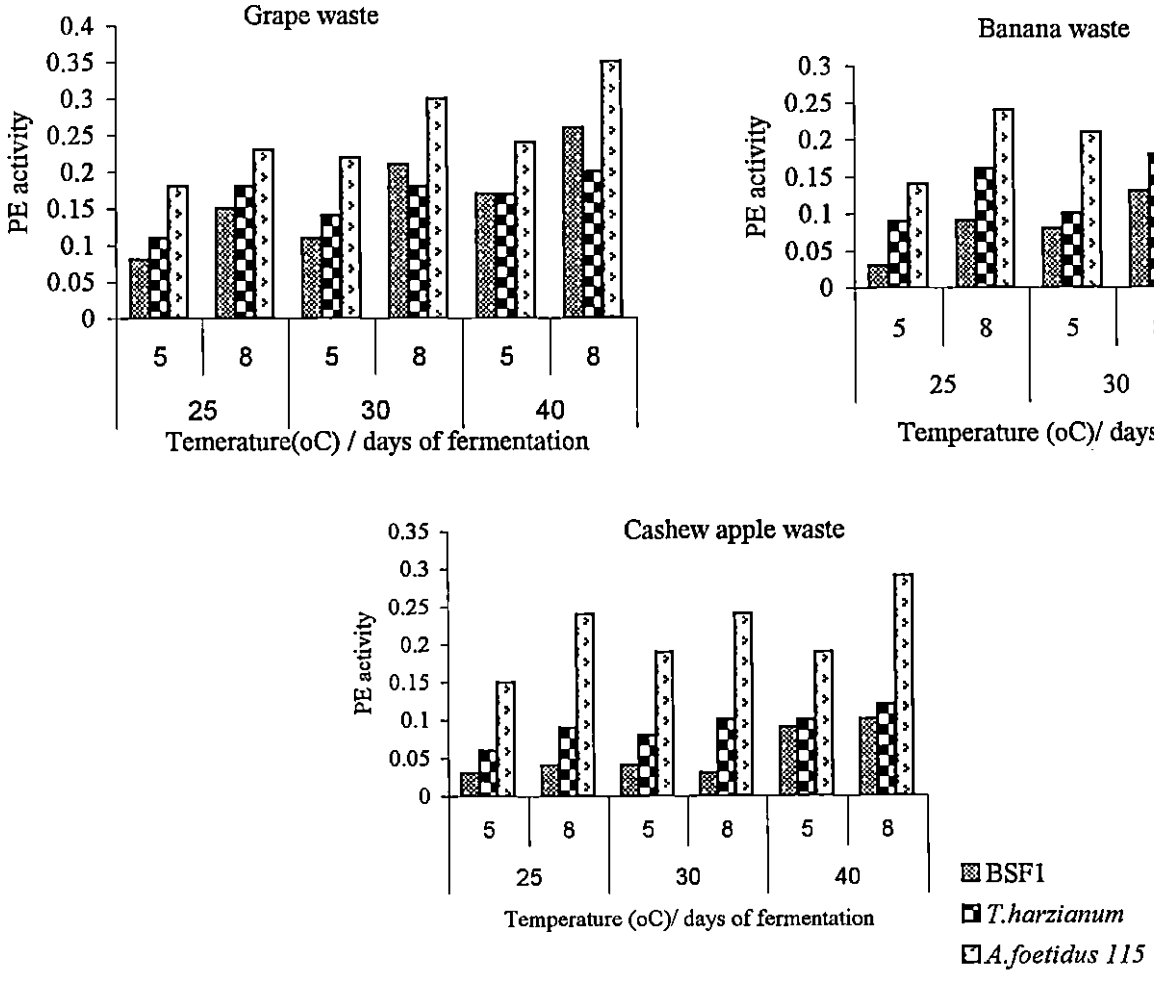


Fig. 7. Activity of pectin esterase in relation to time and temperature

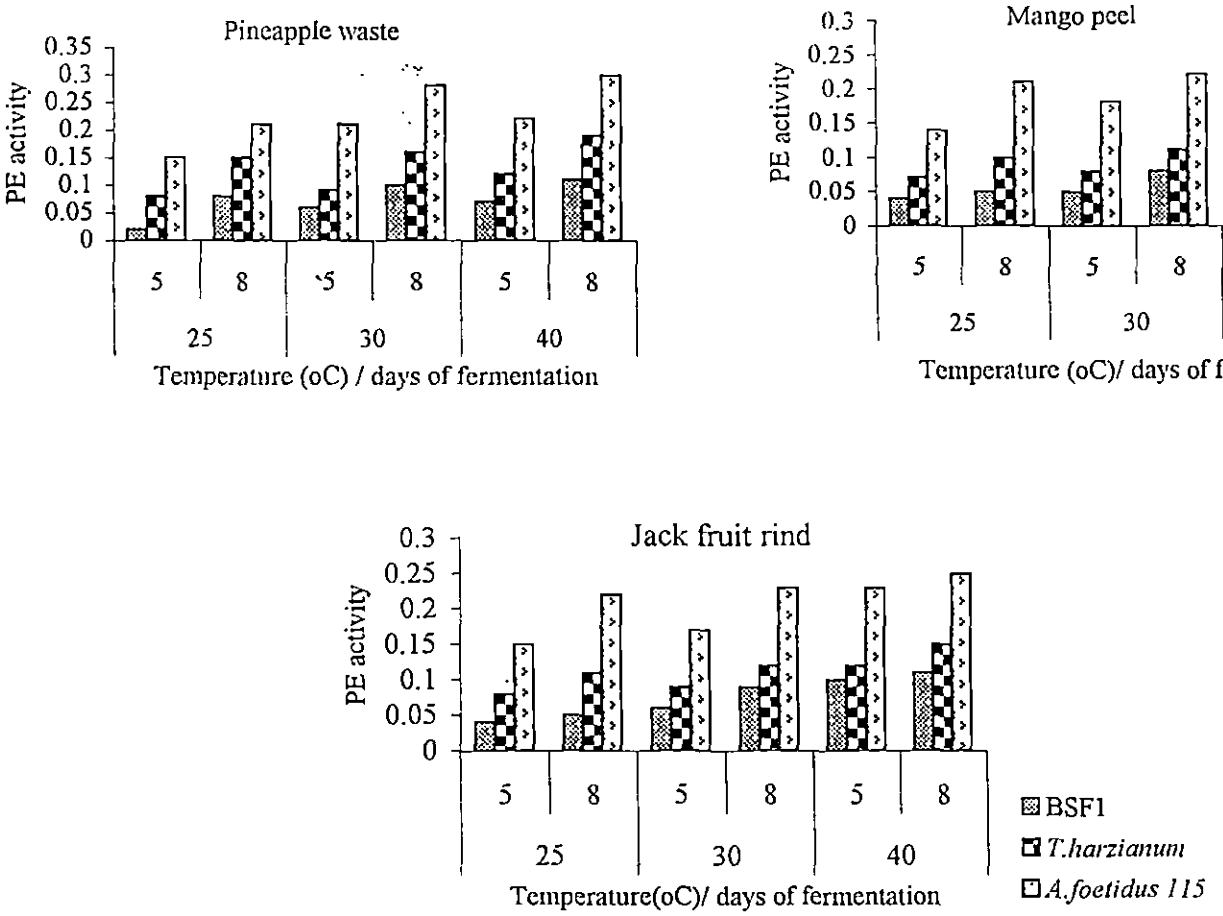


Fig. 7. (contd.) Activity of pectin esterase in relation to time and temperature

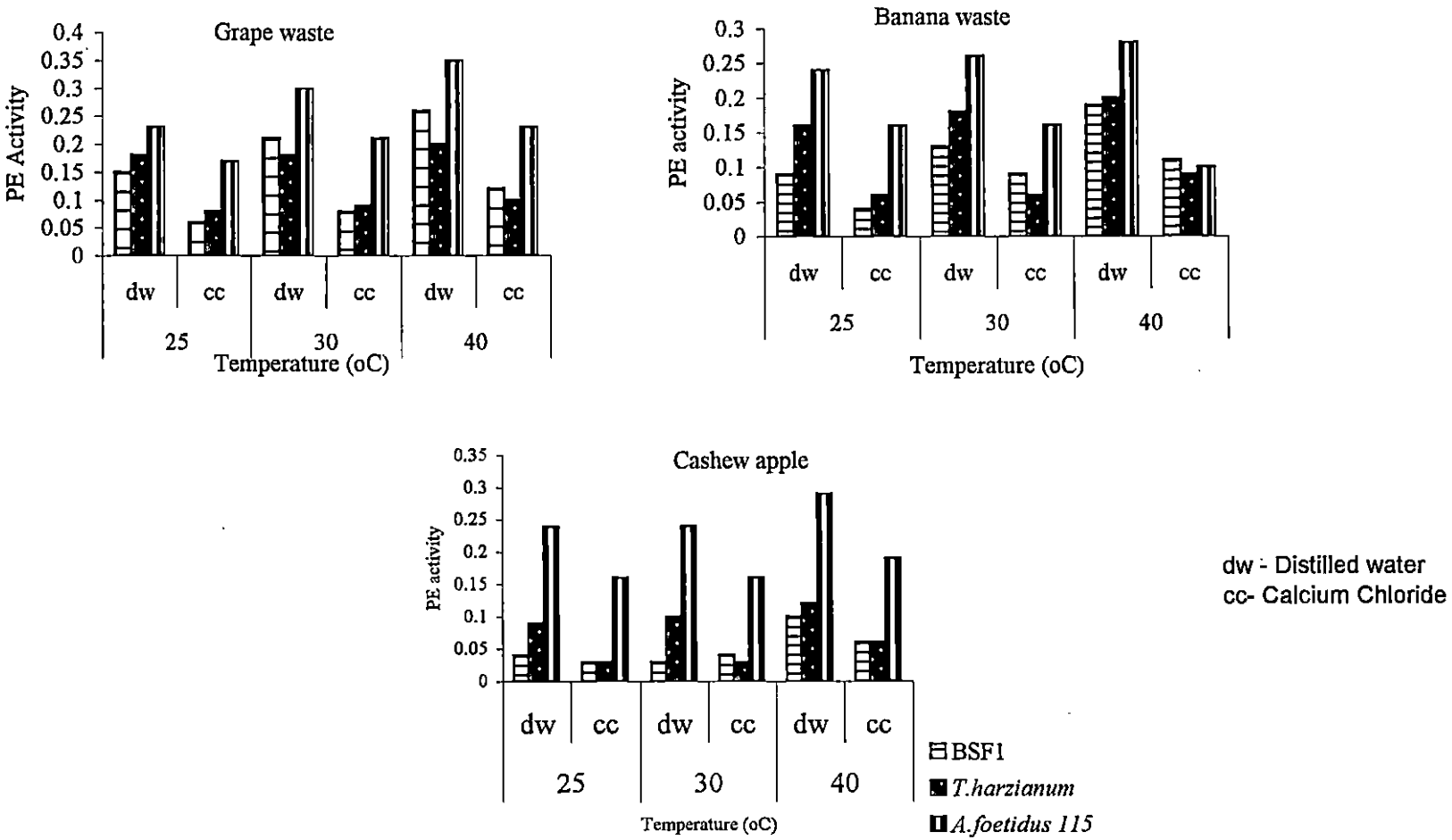


Fig. 8. Activity of pectin esterase in relation to the extractant

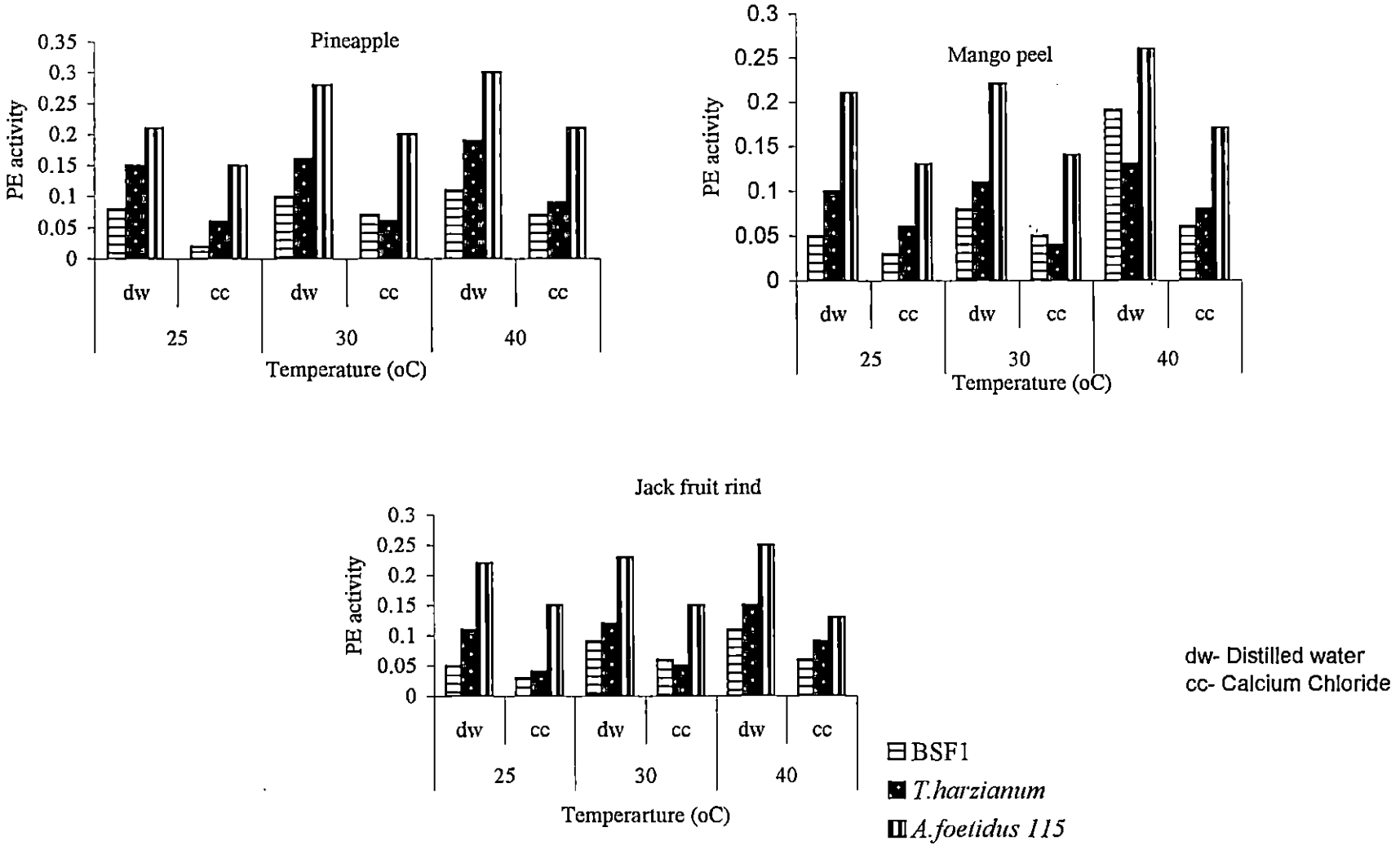


Fig.8 (Contd.). Activity of pectin esterase in relation to the extractant

activity recorded for *Aspergillus foetidus* 115 under the same condition was 0.28 milli equivalents of NaOH consumed min^{-1} ml^{-1}. There was no significant difference in pectin esterase activity when cashew apple waste was subjected to ssf using BSF_1 or *Trichoderma harzianum* (0.10 milli equivalents of NaOH consumed min^{-1} ml^{-1} and 0.12 milli equivalents of NaOH consumed min^{-1} ml^{-1} respectively). The activity recorded for both were lower than that recorded for *Aspergillus foetidus* 115.

The PE activity of enzyme extract of mango peel fermented by BSF_1 was higher (0.19 milli equivalents of NaOH consumed min^{-1} ml^{-1}) compared to *T. harzianum* (0.13 milli equivalents of NaOH consumed min^{-1} ml^{-1}). In the case of jack fruit rind *Trichoderma harzianum* produced better results (Activity 0.15 milli equivalents of NaOH consumed min^{-1} ml^{-1} compared to 0.11 milli equivalents of NaOH consumed min^{-1} ml^{-1} for BSF_1)

4.6.1 Test for pectinase production

The quantity of clarified juice obtained when crude enzyme extracts from media containing different wastes (fermented by efficient microorganisms) were added to the banana pulp is given in Table 22.

The enzyme extract from grape waste fermented by *A. foetidus* 115 gave maximum clarified banana juice (47.2ml), followed by that from banana (38.4ml) and pineapple (38.2ml). The enzyme extract from different wastes fermented by *T.harzianum* could be ranked second with respect to efficiency to clarify banana pulp (Plate 11) The enzyme extract from grape waste fermented by BSF_1 produced more clarified juice followed by *Aspergillus foetidus* 115.

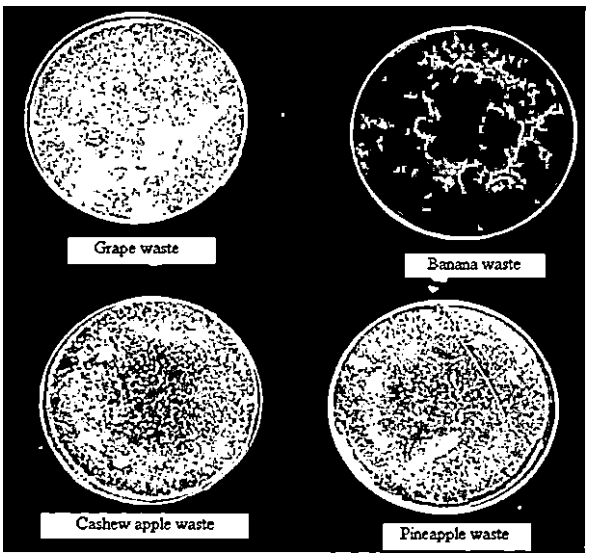


Plate 9. **Growth of *Aspergillus foetidus* 115 in ssf of fruit wastes (6 th day after inoculation)**

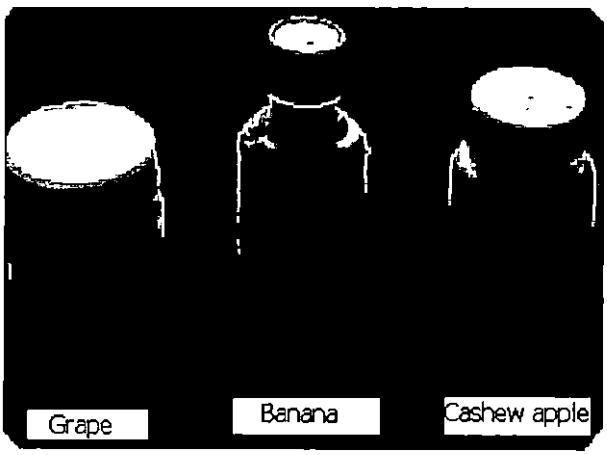


Plate 10. **Crude enzyme extract from fruit wastes**

Plate 11. **Banana juice clarified with enzyme extract from fermented wastes**

Table 22. **Effect of crude enzyme extract on banana pulp clarification**

Sl. No	Waste	Effective microorganism	Volume of clarified banana juice (ml)
1.	Grape	*Aspergillus foetidus* 115	47.20
		BSF_1	28.60
2.	Banana	*Aspergillus foetidus* 115	38.40
		T. harzianum	27.70
3.	Cashew apple	*Aspergillus foetidus* 115	37.80
		T. harzianum	28.80
4.	Pineapple	*Aspergillus foetidus* 115	38.20
		T. harzianum	28.70
5.	Mango peel	*Aspergillus foetidus* 115	29.50
		T. harzianum	19.00
6.	Jackfruit rind	*Aspergillus foetidus* 115	28.00
		T. harzianum	19.5

Discussion

5. DISCUSSION

Enzymes are high value, low bulk commodities isolated and characterised from various microbial, animal and plant sources. Among the various industrial uses of enzymes, the major exploitation is confined to the food industry (Lonsane and Ramakrishna, 1989). The enzymes are used in various food and fermentation industries such as starch, dairy, distillery, brewery, milling, baking etc., for involving wide range of effects. They include control of texture, appearance, nutritive value and generation of desirable flavours and aromas, or their precursors. Their functions also include reduction of viscosity, improve extraction, enhance separation, develop functionality, synthesis chemicals and carry out bio conversions (Norttingham University, 1997 and Singh, 2000). However the use of enzymes in food industries in India is too meagre probably due to the cost factor (Lonsane and Ramakrishna, 1989). This also reflects on the poor growth of enzyme producing industries in India.

Considering wide spectrum of uses of enzymes, intensive research efforts are being bestowed at present on the development of indigenous technologies for their production and optimal application in food processing.

The commercial enzymes are obtained from plants, animals and microbial source. Some of them such as papain and chyrmosin are conventionally recovered from plants (Singh, 1990). Rennet is an example of enzyme from animal source (Singh, 2000). However, owing primarily to the economics and ease in manufacturing, microbial sources are generally preferred for the production of most of the commercial enzymes due to the several advantages such as purity, economy, consistency, ease in process modification and availability of cheaper substrate as well as technology. (Rose, 1980 and Mcneil and Harvey, 1990). The microbes while subjecting the medium, on which they are grown for fermentation, induce production of enzymes in medium. Both smf and ssf techniques are employed for enzyme production. However, ssf is more popular compared to the other (Aidoo *et al.*, 1982). The enzymes from microbial sources are estimated to account 80 per cent of the total

enzyme production (Godfrey and Richelt, 1983 and EnzymeTech, 2001). They also reported that even some of the enzymes obtained from plants and animal sources are being replaced by microbial enzymes, mainly due to the limited availability of plant and animal tissues. Screening of large number of microorganisms is an important step in selecting a highly potent culture for developing efficient fermentation process for enzyme production (Boccas *et al.*, 1994).

Among the different enzymes used in fruit and vegetable processing industries pectinase occupy prime position (Wiley, 1977 and Enzyme Tech, 2001). They are a group of enzymes mainly used by processing industries for fruit juice extraction and clarification. (Joshi *et al.* 1991., Patil and Pai, 1998., Singh, 2000 and Teren *et al.*, 2001). They are also used in natural clouding agents and depectinisation of juice. In tea and coffee processing industries pectinase find application in viscosity reduction, detoxification and polyphenol extraction (Sanderson and Coggon 1977., Shamel and Zongbi, 1993. and Landbo and Meyer, 2001). It is estimated that pectinase market for various kind of industrial process is about 1000 million pound per annum (Enzyme Tech, 2001). The huge demand for enzyme highlights the necessity of evolving technologies for increasing their production in an economic way.

In this context, the present study was formulated to isolate efficient microorganisms capable of producing pectinase through solid state fermentation of fruit wastes.

5.1 ISOLATION OF MICROORGANISMS FROM FRUIT AND VEGETABLE WASTES

Almost all the fruit wastes contain pectin (Srivastava and Kumar, 1994) which provide structural integrity and stability to them. Decaying of fruit wastes happens due to pectin degradation, which follows production of pectinase as inducible enzyme (Forgarty and Kelly, 1983). Naturally the process of decaying fruit waste is also associated with numerous microorganisms (Mehrotra, 2000). The various fungus

various fungus associated with the fruit wastes include *Penicillum* sp., *Mucor* sp. and *Aspergillus* sp. (Asheh and Dejack, 1995., Gomez *et al.* 2000., Jecu 2000 and Verma *et al.* (2001). So the possibility of producing pectinase by any of the microrganisms growing on them cannot be neglected (Babitskaya *et al.* 1993 and Mehrotra, 2000). Hence in the present study, large number of microorganisms were isolated from decaying fruit and vegetable wastes and analysed for their efficiency to produce pectinase.

As disposal of fruit and vegetable wastes is a problem to the processing industries, the development of technologies to utilise them for production of valuable substance will be much appreciated (Joshi and Joshi, 1990). Wastes will also serve as cheap resource for production of valuable byproducts.

In the present study, microbial colonies were isolated on large scale from different fruit and vegetable wastes. Then they were pure cultured to separate the different microorganisms in the colony. Finally 19 different types of fungi and 9 bacteria were isolated from various fruit and vegetable wastes and carried over for further studies.

The fruit wastes from which the microorganisms isolated are reported to be rich source of pectin by Madhav (2001). Many organisms synthesis enzyme in the presence of an inducer in culture media. This inducer may be the substrate or the modification of the substrate for the enzyme. Thus, the pectin in fruit wastes may serve as the inducer substrate for organisms and pectinase may be induced enzyme. Pectinase production from various pectin media is reviewed adequately by many scientists (Babistkaya *et al.* 1993 and Dahm *et al.* 1999).

5.2 PRELIMINARY SCREENING OF MICROORGANISMS

The fungi and bacteria isolated from fruit wastes were cultured on media containing pectin as sole carbon and energy source. Boccas *et al.* (1994) reported this as an efficient method of screening microorganisms for pectinase production. Any

material, which can supply the inducer to the growing organisms, reduces catabolite repression and results in enhanced enzyme yield. Most of the pectinase are inducible enzymes and hence substrate rich in pectin must be added to the medium to stimulate enzyme production. In addition to the inducing properties, pectin also enhances the release of pectic enzymes into the fermentation medium. Pandey (1992) also observed the inducement of enzyme by substrate.

The growth of the microorganisms in the solid pectin media was recorded in the present study. Boccas *et al.*, (1994) recorded growth of *Aspergillus* sp. on coffee pulp and correlated the growth with pectinase production. Hours *et al.* (1988) reported that pectinase synthesis from apple pomace subjected to ssf is directly related to growth of microorganism *Aspergillus foetidus* on the medium. Similarly Soccal *et al.* (1994) observed that the growth of *Rhizopus* sp. on raw cassava is directly related with glucoamylase production in the medium. The growth of microorganisms on a substrate is directly related with its capacity to utilise that substrate for which production of enzyme and subsequent degradation of substrate is a must (Boccas *et al.*, 1994).

In the present study the hyphal elongation exhibited by different fungi in pectin media were recorded and those recorded higher values were selected out, assuming that they have capacity to utilise pectin source, degrading it through production of pectinase. The solid pectin media inoculated with different fungi were continuously monitored for production of hydrolysed zone as reported by Antier *et al.* (1992) and Boccas *et al.* (1994).

None of the microorganisms produced hydrolysed zone and hence based on the growth performance on the pectin media, six fungal isolates (*viz.*, PF_3, JF_3, BP_2, BSF_1, MF_2 and MF_3) and two bacterial isolates BB_1 and *Pseudomonas fluorescens* were selected for further studies. A further selection was made among the fungi based on their performance in liquid pectin medium. The fungi selected based on the performance in solid pectin medium were inoculated in liquid pectin medium together with two fungi *viz.*, *Trichoderma viridae* and *T. harzianum*.

Apart from the hyphal length recorded, turbidity of culture broth was also taken into account. The liquid pectin medium is generally translucent in nature. If pectin degradation happens, it becomes transparent. (Doner, 1986). Less turbidity and more transparency of the medium can be taken as a positive sign of pectinase production (Doner, 1986). Hence, in this experiment, turbidity of the liquid culture medium inoculated with eight fungi were ranked and hyphal mass recorded. Accordingly two fungi, *viz.*, BSF_1 and *Trichoderma harzianum*, which produced more hyphal mass and made the culture broth less turbid, were selected out.

5.3 SECOND STAGE SCREENING OF MICROORGANISMS

Second stage screening of microorganisms was done on pectin media modified with different dyes as well as on media containing varying quantities of pectin. Media modified with different dyes like methyl red, bromo cresol blue and bromo phenol blue were used, as they will express vivid colour change at lower pH (Jain, 1995) as given in Expt 3.3. There is possibility of lowering pH of the medium through pectinic acid production by the way of, pectin degradation by the microorganisms (Doner, 1986). Except for a visible colour change expressed by BSF_1 in MR medium one month after inoculation (Plate 12) no encouraging results were obtained in this study.

Media with varying quantities of pectin were also tried to avoid the possibilities of pectin becoming a limiting factor for growth of microorganisms. It was expected that more pectin degradation may result in more pectinic acid production and hydrolysis of media. Even though hydrolysis of media happened as evidenced by the growth of microorganisms, visible hydrolysed zone could not be observed.

As the microorganisms were found to grow on both types of media the observations on growth parameters were recorded. No selection for microorganisms made at this stage, as there was no scope for that based on the study.

The pectin medium (4g pectin, 0.1g urea, 0.15g ammonium sulphate and 20g agar added with 1000ml distilled water *viz.*, medium C) was found to support better growth of all fungi and bacteria included in the study. The fungus *Trichoderma harzianum* exhibited fast growth also on medium containing lower quantities of pectin, urea and even without ammonium sulphate. So it can be assumed that for growth of *Trichoderma harzianum* the media requirement is comparatively simple. All fungi were capable of growing on pectin medium.

When compared with the growth of fungus BSF_1 (the isolate included in preliminary screening) on standard pectin medium (Table 2) and medium C (Table 6), it can be inferred that this fungus is also capable of growing fast on medium containing pectin without other nutrients. This shows its efficiency to degrade pectin and make the pectin medium more transparent.

Based on the observations of the study, medium C can be recommended as a better medium for growth of all selected microorganisms. Even though, BSF_1 and *Trichoderma harzianum* exhibited growth on standard pectin medium. The growth of *Aspergillus foetidus* 115 was very poor on it (Table 6). The Institute of Microbial Technology has specified the media for the growth of *Aspergillus foetidus* 115, which includes carbon, nitrogen and other nutrients. The poor growth exhibited by *Aspergillus foetidus* 115 may be due to non availability of the required nutrients (Hornecka *et al.*, 1994 and Jecu, 2000).

5.4 PERFORMANCE OF SELECTED MICROORGANISM IN SSF

Performance of selected fungi and bacteria on solid state fermentation of fruit wastes was analysed in the study in comparison with that of *Aspergillus foetidus* 115 obtained from IMTECH, Chandigarh.

The growth performance of different organisms on solid medium containing wastes *viz.*, grape, banana, cashew apple and pinapple was analysed first. The wastes selected for the study are reported to contain considerable quantities of pectin in

them. Madhav (2001) reported that the banana peel and pineapple contain adequate quantities of dry pectin. Mohammed and Hassan (1995) reported that pineapple peel and banana peel are good source of pectin. Similarly Jindal (1980) and Srivastava and Kumar (1994) opined that wastes from grape contain the pectin (0.4 %) and can be used for pectin extraction.

The fungi could be ranked according to their efficiency for growing on media containing fruit wastes. Irrespective of the media the fungus reported as standard pectinase producing one *viz.*, *Aspergillus foetidus* 115 exhibited fast growth on all media containing waste. This observation also supports the views expressed by different scientists (Nair *et al.*,1995., Kollar, 1998. and Laurant *et al.*,2001). The growth of pectinase producing microorganisms has direct correlation with their capacity to utilise pectin and to produce pectinase. It can also be assumed that the wastes subjected to fermentation in this study are capable of supporting growth of *Aspergillus foetidus*, by providing required nutrients prescribed for its growth (IMTECH, 2002).

The fungus *Trichoderma harzianum* and BSF_1 could be ranked second and third respectively for their efficiency to grow on fruit wastes. The fungus screened from banana stalk *viz.*, BSF1 it was identified as that belonging to *Aspergillus* sp. (plate 8) using the standard keys available (Pandey and Trivedi, 2002).

Boccas *et al.* (1994) and Maheswari (2001) reported the production of pectinase using *Aspergillus niger* in ssf of coffee pulp and banana respectively, both containing high quantities of pectin. They opined that the microorganisms capable of degrading pectin containing sources may produce pectinase and such microorganisms are to be screened out scientifically to exploit them for enzyme production. Work done by on Asheh and Dejack (1995)., Gomez *et al.* (2000)., Jecu (2000) and Verma *et al.* (2001) resulted in screening of efficient microbes for pectinase production.

The growth recorded by different bacteria on media containing different fruit wastes was not encouraging. They exhibited a poor growth initially and three days after inoculation, no growth was recorded. This revealed their inefficiency to utilise

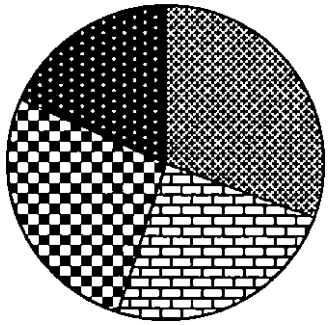
the metabolites contained in fruit wastes. Hence they were not carried over for trying in ssf of fruit wastes.

5.5 BANANA JUICE CLARIFICATION - TEST FOR PECTINASE PRODUCTION

The test for pectinase production was carried out by adding the extract taken from fermented fruit waste medium (crude enzyme extract) to banana pulp and measuring the quantity of clarified juice obtained (Fig 9). Maheswari (2001) reported that this serves as a good test for presence of pectinase in the extract taken from fermented media. As banana pulp is highly consistent due to the presence of high quantity of pectin, it cannot be pressed easily to extract juice from it. Preparation of banana juice and juice based products thus depend on enzyme pectinase as reported by Shanmugavelu *et al.* (1992) and Biocon (2002). The enzyme will degrade pectin and will yield clarified juice from pulp.

Adding the crude enzyme extract (0.5 %) to the banana pulp yielded valuable information. The enzyme extract from all culture media under this experiment was found to clarify banana pulp revealing the presence of pectinase in the extract (Plate 11). The efficiency to clarify the pulp was found differed depending on the waste and microorganisms used for fermentation. The production of juice was comparatively high when the *Aspergillus foetidus* 115 was used as the fungus (Fig 10). This revealed its high capacity to produce pectinase in ssf of fruit wastes. So this microorganisms reported to produce pectinase from different pectin source (IMTECH, 2002) can explicitly used for pectinase production from different wastes included in the study through ssf technology. The variation in pectinase production efficiency expressed by *Trichoderma harzianum* and BSF_1 (*Aspergillus* sp) point out the necessity of selecting them with respect to each waste. For ssf of cashew apple waste both the organisms were found good. For pineapple waste *Trichoderma harzianum* and for banana BSF_1 (*Aspergillus* sp.) were better.

a.Volume of juice by adding extract from smf



b. Volume of juice by adding extract from ssf

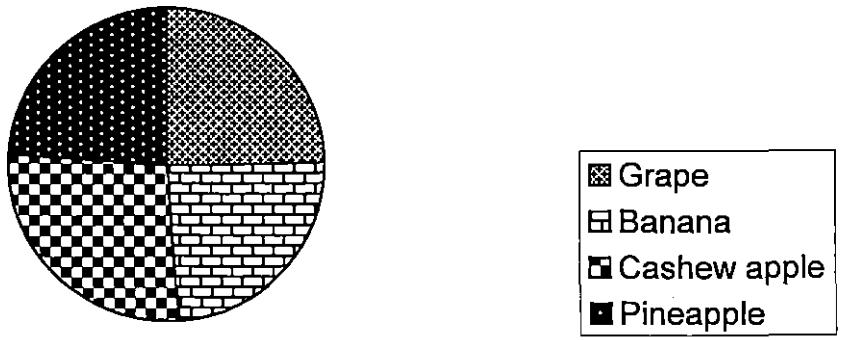


Fig 9. Volume of clarified juice (%) due to addition of crude enzyme

The study also revealed the superiority of ssf technology in enzyme production from fruit wastes. For all microoragnisms, ssf was better compared to smf for pectinase production (Table 21 and Fig 10). Similar results were obtained by Aidoo *et al.* (1982) and Mcneil and Harvey (1990). As solid state fermentation is reported to have many advantages over submerged state fermentation (Aidoo *et al.*, 1982), ssf was adopted for trying enzyme production from different wastes.

5.6 STANDARDISATION OF CONDITIONS FOR SSF

Hours *et al.* (1988) reported that pectic enzyme production through ssf largely depend upon the media and the different conditions *viz.*, temperature, pH, duration of fermentation, moisture level in the medium and microorganism used. In the present study attempts were made to standardise the ideal medium for pectinase production with respect to each waste included in the study. Keeping the quantity of the waste as constant (5g) the quantities of other ingredients *viz.*, urea and ammonium sulphate added to the medium was varied.

Analysis of the results revealed that among the all media tried, medium containing 5g urea and 0.3g ammonium sulphate was ideal for growth of all microorganisms. They exhibited fast growth on this medium. Navaratnam *et al.* (1995) and Arasaratnam *et al.* (2001) reported that enzyme production not only depends on carbon and nitrogen in the medium but also the other nutrients provided for their growth. The enzyme production not solely depended on the N/C ratio (Pandey *et al.*, 1994), but also on the total amount of nitrogen used. When the nitrogen content of the media was increased by using two nitrogen sources *viz.*, urea and ammonium sulphate, enzyme yield was enhanced. Arasaratnam *et al.* (1997) also observed that increased nitrogen content in fermenting medium enhanced enzyme production. Based on the results of the present study it can be asssumed that for efficient pectinase production through ssf of different wastes, media containing 0.025g urea and 0.3g ammonium sulphate for every 5g of fruit waste is necessary.

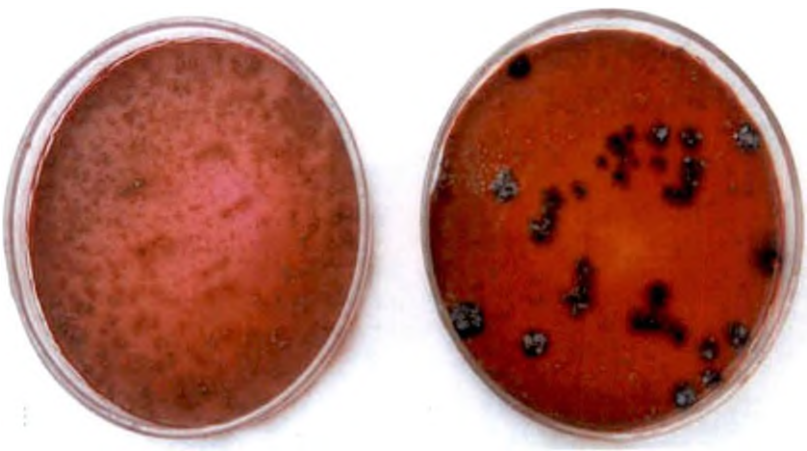


Plate 11. **Colour change of pectin medium modified with Methyl Red dye.**

Moisture content of the media was standardised through a preliminary trial. Addtion of water at the rate of 50ml per 100g of media was found enough to moisten it. Reducing or increasing water from that level was found to have adverse effect on growth of microorganisms. Increase in moisture content of medium above 60 per cent delayed the time taken for enzyme production, which was similar to that generally observed in the submerged fermentation. Increase in moisture level would have lead to decrease porosity of medium, loss of particle structure, reduction of gas volume, decreased gas exchange and lowered oxygen transfer due to decreased diffusion (Jansz *et al.*, 1977 and Ramadas *et al.*, 1996). Nishio *et al.*, 1979 and Lonsane *et al.*, 1985 observed maximum glucoamylase activity in media containing moisture levels between 35 and 50 per cent. Similarly low moisture level also leads to suboptimal growth, lower degree of substrate swelling, reduced solubility of nutrient present in the solid substrate and high water tension (Wang *et al.*, 1974). Optimum moisture level for ssf of different organisms around 55 per cent has been reported by Ramesh and Lonsane 1990., Battiaglino *et al.*, 1991 and Tao *et al.*, 1997. The results obtained in the present study are in agreement with these reports.

5.7 STANDARDISATION OF IDEAL EXTRACTANT, TEMPERATURE AND DURATION OF FERMENTATION

Pectinase are a group of enzymes which consist of pectin esterase, pectin lyase and polygalacturonase (Rose *et al.*, 1980). Analysis of the activity of these different components is possible through biochemical methods. Talboys and Bosch (1970) reported that analysis of PE activity represents the activity of pectinase in the medium. So the crude enzyme extract from the media kept under varying temperature for different period of fermentation were extracted using two extractant and analysed for PE activity in the present study. Based on the activity the ideal temperature, duration of fermentation and extractant were standardised.

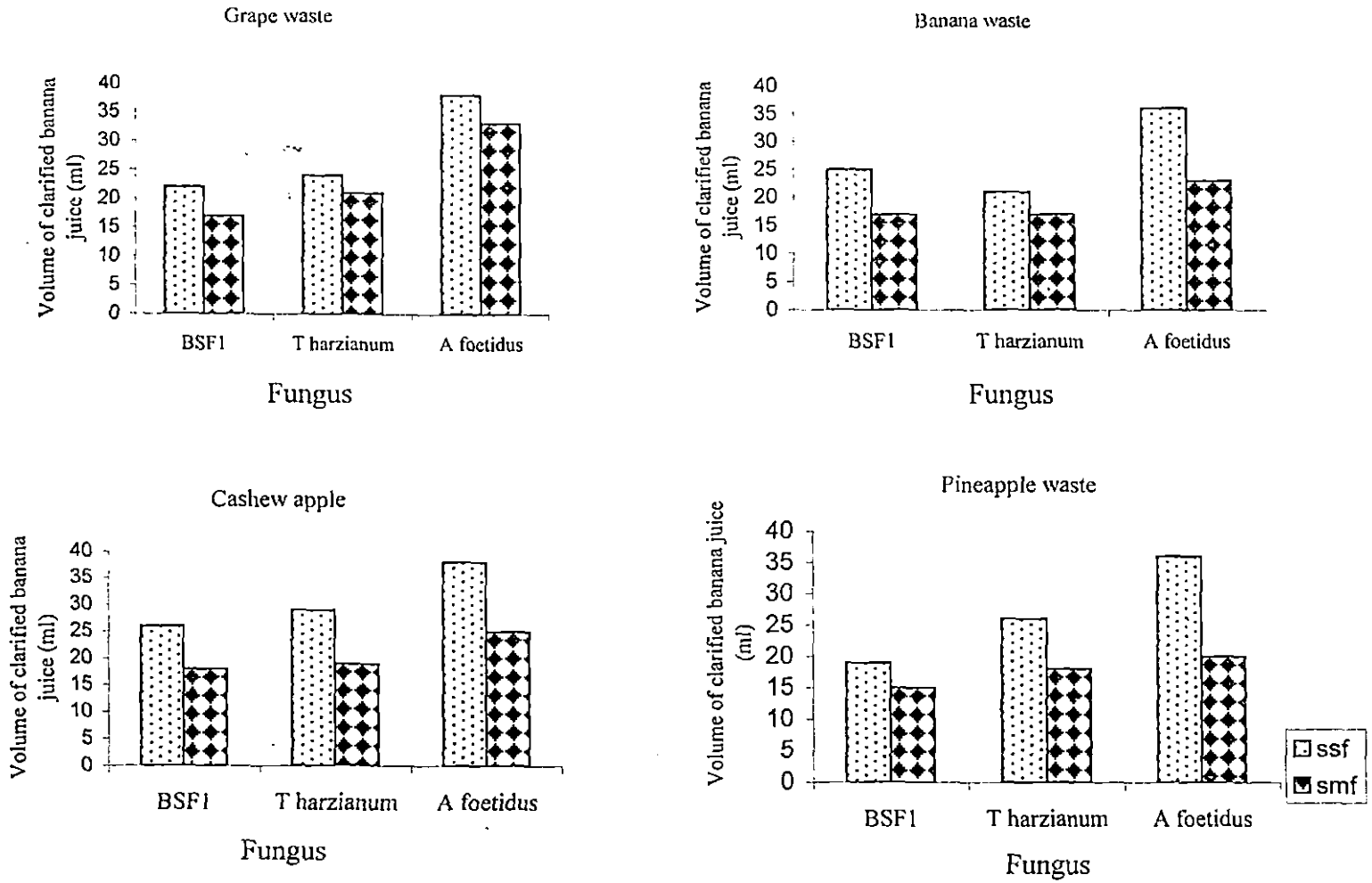


Fig 10. Volume of clarified juice obtained due to addition of crude enzyme extract from from wastes to banana pulp

For extracting culture medium to get crude enzyme extract two extractats *viz.*, calcium chloride and distilled water were used. The selection of extractant for enzyme depends upon the enzyme depending upon the type of fermenting medium and the enzyme to be extracted (Maheswari, 2001). Among two extractant tried distilled water proved its superiority over calcium chloride irrespective of the waste, microorganism, temperature and duration of fermentation (Fig 8). The result was in confirmation to the finding of Schwimmer (1981). He reported that recovery of lipase from ssf was more when water was used as the extractant.

Higher level of temperature and duration of fermentation included in the study were selected through preliminary trial. For maximum enzyme production the ideal temperature in which fermentaion to be carried out was observed to be 40°C for al. wastes irrespective of microorganisms. Higher temperature beyond this level was not tried as in many cases denaturation of enzyme at temperature beyond 40°C is reported. (Pandey, 1992). At higher temperature, build up of large amount of metabolic heat happen in the media, resulting in increase of the temperature of fermenting media and subsequent enzyme denaturation. High temperature also causes evaporative water loss and reduction in vegetative growth of microorganisms. Controlled evaporation with continuous water replacement is reported to promote heat dissipation and assuring productive vegetative growth of microorganism for enzyme production (Tengerdy, 1985).

The ideal duration for which the medium to be kept for fermentation was identified as eight days after inoculation. Probably during period below this, the fermentation would not be completed resulting in lower enzyme production. The maximum enzyme production is reported in the log phase of the fungal growth, where as at its stationary phase enzyme production is practically nil (Pelczer, 1996). In the present study, when observed on eight days after fermentation, the media were found to be completely covered with thick mycelial layer. This can be considered as the log phase of the microoragnism as maximum enzyme production was obtained at this stage.

Several enzymes are now traded as commandity products on the world market because there exsist clear large scale need for enzymes by different industries. The great demand has reflected the rise in number of enzymes available on a industrial scale at relatively decreasing cost. The present study on use of fruit waste on pectinase production using selected microorganisms will introduce a novel strategy for production of enzymes. Development of technologies in this line not only will help to reduce the cost of enzyme, but also serve as an efficient method for waste disposal.

The microrganisms selected through the present study may express for pectinase production, foolproof technology could be evolved through further study. The results of this study will serve as a guideline to achieve final goal of technology standardisation for enzyme production from fruit wastes. Studies on fermentation of the enzymes extracted from fermented wastes are also necessary. Only purified enzyme will express the full potential when they are introduced for use by different industries.

Summary

SUMMARY

The project entitled "**Fruit waste utilisation for pectinase production through solid state fermentation (ssf)**" was carried out in the Department of Processing Technology, College of Horticulture, Vellanikkara during 2001 – 2003 period. The major objective was to analyse the possibilities of pectinase production through controlled fermentation of different fruit wastes using potent microorganisms.

The isolation of microorganisms from decaying fruit and vegetable wastes and their pure culturing yielded 19 different types of fungi and nine bacteria.

Preliminary screening of microorganisms for the efficiency to produce pectinase both on solid and liquid pectin medium resulted in selection of two fungi *viz.*, BSF_1 (*Aspergillus* sp.) and *Trichoderma harzianum* and two bacteria *viz.*, BB_1 and *Pseudomonas fluorescens.*

None of the microorganisms produced hydrolysed zone in dye containing media and media with different quantities of pectin in second stage screening.

The medium with composition 4g pectin, 0.1g urea, 0.15g ammonium sulphate and 20g agar dissolved in 1000ml distilled water was found to support better growth of all microorganisms.

Based on the efficiency to grow on different fruit wastes in ssf, standard culture *Aspergillus foetidus* I 15 ranked first followed by *Trichoderma harzianum* and BSF_1.

The selected bacteria didn't exhibit good growth on media containing fruit wastes in ssf and were excluded due to their inability to utilize the pectin in fruit wastes.

The crude enzyme extract made from fruit wastes subjected to ssf and smf using different fungi were found to be effective for clarification of banana pulp. This served as ideal tool for identifying pectinase producing microorganisms and the

media for their growth. Among the fungi *Aspergillus foetidus* 115 ranked first with respect to pectinase production from all wastes. The enzyme extract from the different media subjected to both ssf and smf using these microorganisms produced maximum clarified banana juice.

The ideal medium for enzyme production for *Aspergillus foetidus* 115 was grape waste in smf and cashew apple waste in ssf.

Trichoderma harzianum was found to be more efficient for enzyme production from all wastes compared to BSF_1.Grape waste served as the best medium.

In ssf the efficiency of *Trichoderma harzianum* and BSF_1 varied with respect to waste. For grape and pineapple waste fermentation, *Trichoderma harzianum* was found to be more efficient, where as, for banana waste BSF_1 was better. Both the fungi were equally good for ssf of cashew apple waste for enzyme production.

The composition of the best fruit waste medium observed for enzyme production in ssf was - 5g waste, 0.75g urea, 0.3g ammonium sulphate and 2g agar in 100ml distilled water. The conditions for maximum enzyme production in ssf was standardised by analyzing the pectin esterase activity in different media.

The ideal condition for maximum enzyme production was identified as keeping the cultures under a temperature of 40°C for duration of eight days after inoculation.

Similarly distilled water was found to be the best extractant for extracting pectinase from fermented solid fruit media, compared to calcium chloride.

Maximum pectinase production was recorded when grape waste subjected to ssf using *Aspergillus foetidus* 115 under standardised conditions of media, temperature, duration of incubation and extractant for enzyme, which was evident through banana juice clarification.

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FRUIT WASTE UTILISATION FOR PECTINASE PRODUCTION THROUGH SOLID STATE FERMENTATION

By

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ABSTRACT OF THE THESIS

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ABSTRACT

Enzymes are proteinaceous biocatalysts known for their high selectivity and specificity and are active under mild temperature and pressure conditions, which enable their effective use in food processing to modify, alter and enhance quality of food. Among the different enzymes used in fruit and vegetable processing industries, pectinase occupy prime position. The present study "Fruit waste utilisation for pectinase production through solid state fermentation" was taken up for analysing the possibilities of producing pectinase from fruit and vegetable wastes.

Isolation, pure culturing and initial screening of microorganisms for their efficiency for pectinase production on media containing pectin resulted in selection of two fungi (*Trichoderma harzianum* and BSF_1) and two bacteria (BB_1 and *Pseudomonas fluorescens*). The ideal solid pectin medium for better growth of selected microorganisms was standardised.

The efficiency of bacteria to grow on media containing different wastes was found poor, where as the fungi grew fast. The fungus *Aspergillus foetidus* 115 exhibited maximum growth and production of pectinase on all waste media in solid state fermentation as well as in submerged state fermentation. The activity of this microorganism was high when inoculated on grape waste medium, which was evidenced through banana juice clarification studies. Among *Trichoderma harzinum* and BSF1, the formar found to have better efficiency for fermentation of wastes for pectinase production.

The ideal fruit waste medium (5g waste, 0.75g urea, 0.3g ammonium sulphate) and optimum condition for fermentation (temperature 40°C, extraction at eight days after inoculation using extractant distilled water) for ssf of fruit wastes for maximum enzyme production were standardised, through analysing the pectin esterase activity in crude enzyme extract taken from different media.