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Evaluation and Optimization of Pectinase Production by Endophytic Fungi *Talaromyces* sp. isolated from *Calophyllum inophyllum*

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ABSTRACT

Enzyme technology is one of the most promising tools in the field of biotechnology as applied biocatalysts. Most of the extracellular enzymes involved in polymer degradation in degradation of plant material have been attributed to fungi. Fungal pectinases account for 25% of the global food and industrial enzyme sales. Pectinases or pectinolytic enzymes which hydrolyze pectic substances belong to a group of extracellular enzymes that break down pectic polysaccharides of fruits into simpler molecules like galacturonic acids and help in improved extraction of juices with higher yield. They have the functional and technical applications in food processing industries in decreasing the viscosity of fruit juices by clarification of juice and liquefaction of pulps. Apart from these they have the biological applications in plant–fungal interactions. Considering these facts, fifty endophytic fungi isolated from the medicinal plants *Aplinia calcarata, Bixa orellana, Calophyllum inophyllum* and *Catharanthus roseus* were screened for extracellular pectinase on solid media. Among them 62 % of endophytic fungi screened were able to produce pectinase enzyme. Among them 12 positive isolates with maximum pectinase production were further subjected for quantitative screening of the enzyme in liquid media. In the present investigation, the isolate Ci-1 identified as *Talaromyces* sp. exhibited maximum extracellular pectinase activity both in soild and liquid media. Environmental conditions such pH, temperature are known to alter the exoenzyme expression and activity. Hence influence of various physical factors such as pH of the culture media and incubation temperature, and nutritional factors such as different carbon and nitrogen sources on production of pectinase were studied. The maximal pectinase production was observed at pH 6.0 of the cultural media and at 35 ^{o} C of incubation temperature. Among the various carbon sources screened, wheat bran powder at concentration of 1.5% revealed maximum pectinase production. Among different nitrogen sources dihydrogen ammonium phosphate (0.3%) was found to be optimum for pectinase production.

Key words: Endophytic fungi, Extracellular enzyme, Pectinase activity

INTRODUCTION

Fungal enzymes have been widely used for the industrial production due to advantages such as cost effectiveness, consistency, less time and space required for production and ease of process modification and optimization. Apart from bacteria, fungi are considered to be the best sources for the same. In nature, fungi play a central role in the degradation of plant biomass. Plant-biomass-degrading fungi produce an extensive set of carbohydrate-active enzymes specifically dedicated to degrade plant polysaccharides, like starch, cellulose, hemicelluloses, and pectin which are the main components of plant cell walls representing up to 70% of the biomass (Jorgensen *et al*., 2007). Pectin is present mainly in primary cell walls and middle lamella by binding the cells together to form a gel like matrix (Voragen *et al*., 2009). Pectinolytic enzymes are widely distributed in higher plants and fungi (Patel *et al.*, 2017). Pectinases are responsible for the degradation of the long and complex molecules of the pectic substances, present mostly in plants (Alkorta *et al*., 1998). They breakdown the complex of polysaccharides to monomers such a D-galacturonic acid, with rhamnose residues in the main chain and arabinose, galactose and xylose in side chain. The hydrolysis of pectin backbone is by the synergistic action of several enzyme, including pectin methylesterase (EC.3.1.11.1),

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Polygalactouranase PGase (Exo-PGase- EC3.2.21.67 and EndoPGase-EC4.2.2.15), exo-pectate Lyase (EC4.2.2.9) and endopectin lyase (4.2.2.10) (Siddiqui *et al*.., 2013).

Pectinase production occupies about 10% of the overall manufacturing of enzyme preparations in the world market and their demand is increasing in the years to come (Pedrolli and Carmona, 2014). Almost all the commercial pectinolytic enzymes or pectinases used in the food industry are of fungal sources as they are the potent producers of pectic enzymes (Sin *et al.,* 2002). There is huge demand for pectinase in the various industries to improve the clarification of concentrated fruit juices to increase the yield (Almeida *et al*., 2005; Silva *et al*., 2002). The cloudiness in the juices is mainly caused by the presence of polysaccharides such as pectin and starch. Therefore, pectinase treatment is an effective way to reduce the pectin in the fruit juices because it has the ability to hydrolyze pectin and many studies have shown that it increases the storage stability (Mieszczakowska-Frąc *et al*., 2012)

Crude enzyme preparations has extensive commercial importance have been widely used in wine making (Mojsov *et al.,* 2010, Sieiro *et al.,* 2012), oil extraction (Perez, *et al.,* 2013, Mortabit, *et al.,* 2014), fermentation of tea, coffee and cocoa (Murthy and Naidu *et al.,* 2011).

Screening is often the first step to select microorganisms with characteristics intended for industrial applications which allows the characterization and selection of fungal strains with optimal production of enzymes. The reason behind to choose endophytic fungi for the pectinase production is mainly due two reasons one is, as they occupy a relatively unexplored site and can represent a new source with potentialities. The production of extracellular enzymes from endophytic fungi was explored on solid media in our previous paper (Sunitha *et al*., 2013) as penetration and limited colonization of selected plant cell is a common trait of endophytic fungi. Endophytes occupy the same ecological niche as most pathogens, therefore, it can be assumed that they utilize the same strategy employed by pathogens for the penetration of the host tissues (Petrini *et al*., 1992). At the beginning of colonization process, endophytic fungi have to achieve at least partial degradation of cell wall which consists of pectin.

Due to the potential and wide applications of pectinases, there is a need to highlight recent developments on several aspects related to their production, once the potential source is identified. Great amounts of agroindustrial wastes rich in polysaccharides, such as pectic substances are produced at worldwide. These substances can be investigated as carbon and energy sources for pectinase production (Kashyap *et al.,* 2001). Industrially important enzymes have traditionally been obtained from submerged fermentation (SmF) because of the ease of handling and greater control of environmental factors such as temperature and pH (Gangadharan *et al*., 2006).

The present study was carried out to quantify the production of pectinase from the previous studies and once the potential source is identified optimization of the media was evaluated to find the optimum conditions for pectinase production

MATERIALS AND METHODS

Detection of pectinase production on solid media

Twenty two isolates of *A. calcarata*, twenty isolates from *C. inophyllum*, four isolates from *B. orellana* and *C. roseus* respectively were screened for pectinase. The functional role of pectinase by fungal endophytes was assessed by growing them on Potato Extrose Agar for 6-7 days and placing 5mm mycelial plugs on Pectin Agar medium (Sunitha *et al*., 2013). After incubation for 3-7days at room temperature, the plates were flooded with 1% aqueous solution of hexadecyl trimethylammonoium bromide the zone of pectinolytic activity surrounding the fungal colony was measured in mm. The uninoculated plate served as control.

Production and evaluation of pectinase in liquid media

Twelve endophytic fungi which revealed zone above then 10mm in primary screening were selected for the production in liquid media. Six fungi from *A. calcarata* viz., *Cylindrocephalum* sp. (Ac7), *Phoma* sp. (Ac11), *Aspergillus fumigates* (Ac18), *Myrothecium* sp. (Ac22), *Fusicoccum* sp. (Ac26) and *Aspergillus* sp. (Ac 32), five from *C. inophyllum* viz., *Talaromyces* sp. (Ci1), *Fusarium oxysporum* (Ci16), *Fusarium chlamydosporum*

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(Ci19) *Acremonium implicatum* (Ci20), *Nigrospora sphaerica* (Ci21) and one isolate from *B. orellana*, *Colletotrichum gloeosporoides* (Bo26) were grown in 25 mL of CZB supplemented with 1.5% pectin in 150 mL Erlenmeyer flasks and autoclaved at 121°C, 15 lbs for 15min. After sterilization, the flasks were cooled to room temperature and 0.1mL spore suspension of the fungal strain was added and incubated at 25°C for 7 days.

Colorimeteric assay for enzymes

The culture broth was filtered using Whatman filter paper No.1, the filtrate was centrifuged at 8000rpm for 10 min. at 4°C and the supernatant was used for enzyme assay. The denatured culture filtrate served as control.

Determination of fungal biomass

The biomass of fungal culture was expressed as dry weight by drying the mycelium in hot air oven at 80°C for 16 hrs. The uninoculated flask served as control.

Biochemical characterization of the enzyme

The enzyme activity was assayed over independent physical parameter keeping others as constant and incorporated it at the optimized level in the next experiment. The enzyme activity was evaluated at different temperature (25, 30, 35, 40, 45, 50 and 55°C), incubation time of the reaction mixture (10, 20, 30, 40 and 50mins.) and effect of pH ranging from 3.0-11.0 using 0.1M sodium acetate (pH 3.0-5.0), 0.1M citrate phosphate (pH 5.0-7.0) 0.1M Tris-Hcl (pH 7.0-8.5) and 0.1M glycine-NaOH (pH 9.0-11.0). The optimized assay procedure was henceforth adopted in the subsequent experiments.

Pectinase assay

The pectinase activity was determined by measuring the release of reducing groups by modified 3,5-dinitrosalycilic acid (DNS) method (Miller, 1959). The reaction mixture contained 0.5 mL of the culture filtrate and 0.1% of pectin (in 0.1 M sodium acetate buffer pH 5) incubated at 40°C for 30 min. The enzyme activity was expressed as the amount of galacturonic acid released mL^{-1} $minute^{-1}$ under the above conditions. One unit of enzyme activity was described as the amount of one µmol of galacturonic acid released per minute under standard assay conditions.

Optimization of cultural conditions for the production of pectinase enzyme

The factors such as temperature, pH, incubation time, sources of carbon affecting production of pectinase were optimized by varying conditions. The experiments were conducted in 150 mL Erlenmeyer flask containing 20 mL of production medium (0.1% -NH_4NO_3, 0.1% $(NH_4)_2$ HPO_4 and 0.1% $MgSO_4 7H_2O$) and supplemented with 1.5% (w/v) pectin and the pH of the media was maintained at 6.0. After sterilization by autoclaving, the flasks were cooled and inoculated with 1mL of spore suspension (aliquots of 10^6 spores/mL) of *Talaromyces* sp. and incubated at different temperature viz., 15, 25, 30 and 40°C for 7days to study the effect of temperature on pectinase production.

Similarly the effect of pH on pectinase production was studied, by varying the pH of the culture medium from 3.0-7.0. Great amounts of agroindustrial wastes rich inpolysaccharides, such as pectic substances are produced at worldwide. As the basic aim of the study was to use organic waste for the cost-effective pectinase production, different waste byproducts viz., corn flour (COF), rice bran powder (RBP), wheat bran powder (WBP), mandarin peel powder (MPP), Banana peel powder (BPP) 1.5% (w/v) were used as carbon source in place of purified pectin and compared with pectin. Except COF all the substrates were dried in hot air oven at 80°C, blended in mixer and were sieved through 2mm mesh to get uniform powder. For the production medium 2.5gm of each substrate, was added and the pH of the media was maintained at 6.0. After sterilization, 1mL of aliquots of 10^6 spores/mL of *Talaromyces* sp. was inoculated and incubated at 35°C for 7 days. To the 2.5g of wheat bran 0.1 % of various nitrogen sources viz., ammonium nitrate (AN), sodium nitrate (SN), ammonium-dihydrogen phosphate (ADP), triammonium citrate (TAC) and L- Asparagine was suspended in 20mL distilled water. The pH of the media was adjusted to 6.0. After sterilization, spore suspensions were inoculated and the flasks were incubated at 35°C for 7 days.

The enzyme was extracted by adding 10 mL sterile distilled water to the cultured flasks, followed by vigorous shaking and filtration. The filtrate was used as crude enzyme.

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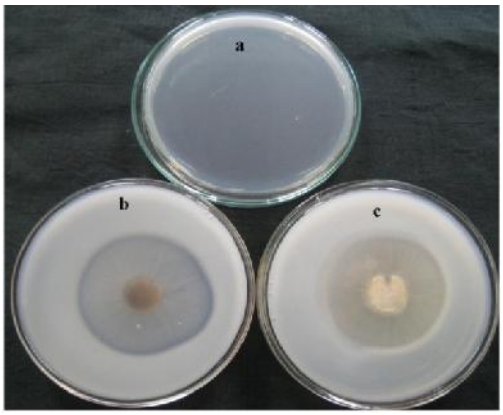
Statistical analysis

All the experiments were performed in triplicates and the means were analyzed statistically with the software, SPSS program version 20.0. Statistical significance was tested by one-way analysis of variance (ANOVA). The significant differences between the means have been presented as Duncan's Multiple range Test (DMRT) in the form of probability ($p \leq 0.05$). (Duncan, 1995).

RESULTS

Pectinase production on solid media

Among the endophytic fungi screened for pectinase enzyme 62 % of were able to produce pectinase (Fig.1) Fourteen isolates from *A. calcarata* and thirteen isolates of *Calophyllum inophyllum* were positive for pectinolytic activity, three isolates from *B. orellana* and *C. roseus* isolates none of them were positive for pectinase enzyme. Maximum pectinase activity (17mm zone) was observed in *Talaomyces* sp. (Ci1), followed by *Fusarium oxysporum* (Ci16) of *C. inophyllum*. Among *A. calcarata* isolates, *Fusicoccum* sp. (Ac26), *Myrothecium* sp. (Ac22), and *Cylindrocephalum* sp. (Ac7) were the significant producers of pectinolytic activity. *Colletotrichum gloeosporioides* (Bo4) of *Bixa orellana* also exhibited significant pectinase activity. Among the *C. roseus* isolates the moderate pectinase activity was observed by *Drechsclera* sp. (Sunitha *et al.*, 2013). Overall twelve isolates with zone 10mm and above were subjected for quantitative analysis of pectinase enzyme Elsababty *et al.*, 2015 also reported a zone of 20mm on solid media by three *Talaromyces* sp. *Talaromyces barcinensis*, *T. ucrainicus* and *Talaromyces trachyspermus*



a: Control

b: Positive

c: Negative

Fig 1: Pectinolytic activity on Pectin Agar medium

Production of extracellular pectinase in liquid media

Among the 12 endophytic fungi *Talaomyces* sp. (Ci1) from *C. inophyllum* was able to produce maximum pectinase activity (Fig 2), followed by *Fusicoccum* sp. (Ac 26), *Myrothecium* sp. (Ac22), and *Cylindrocephalum* sp. (Ac7) from *A. calcarata.* The biomass production also coordinated in case of *Talaomyces* sp. and *Fusicoccum* sp. However there was variation in the biomass production for the few of the isolates, though the biomass production was high in case of *Cylindrocephalum* sp. (Ac7) and *Colletotrichum gloeosporioides* (Bo26) the pectinase activity was low (Fig. 2). The least pectinase activity was observed in case of *Nigrospora sphaerica* (Ci23) although there was moderate production of biomass and also it revealed moderately better activity in the preliminary screening on solid media. This may be due to the variation in the culture medium as the utilization of the substrates varies from one fungus to the other.

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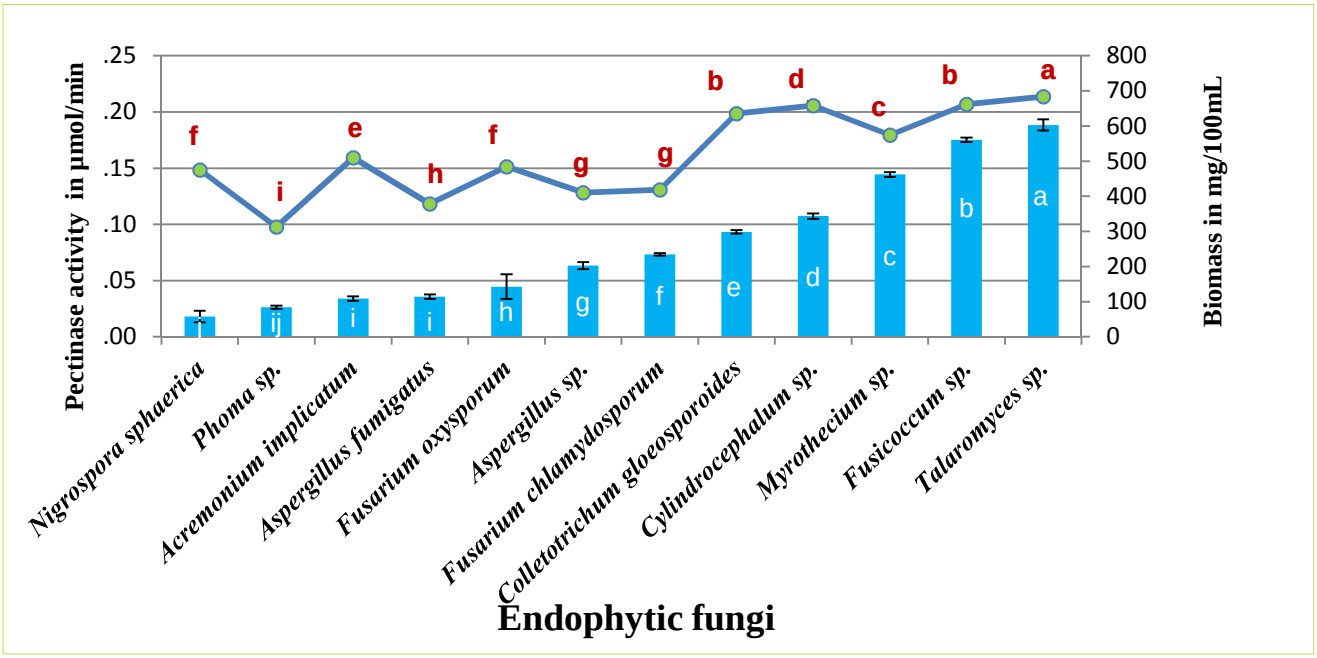

Fig 2: Production of extracellular pectinase in liquid media

Values followed by the same lower case alphabets in the each graph are statistically equivalent (P<0.05) according to the Duncan multiple range test.

OPTIMIZATION OF CULTURAL CONDITIONS FOR THE PRODUCTION OF PECTINASE ENZYME

Effect of incubation temperature on pectinase production

Studies on the effect of temperature showed that 35°C was optimum incubation temperature for the production of pectinase below and above that there was decrease in the production (Fig3).

Effect of pH of the culture media pectinase production

An increase in pectinase production was observed from the pH 3.0 to 6.0 and at pH 7.0 the production decreased. The optimum pH for the pectinase production was at 6.0 and the biomass yield also correlated with the pectinase production (Fig4).

Effect of carbon source on pectinase production

Pectinase production was significantly high in wheat bran powder, followed by RBP, BPP and COF (Fig5).

Effect of nitrogen source on pectinase production

Dihydrogen Ammonium phosphate (ADP) showed maximum pectinase production followed by triammonium citrate (TAC), ammoiniunm nitrate (AN), L-asparagine and least in sodium nitrate (Fig6).

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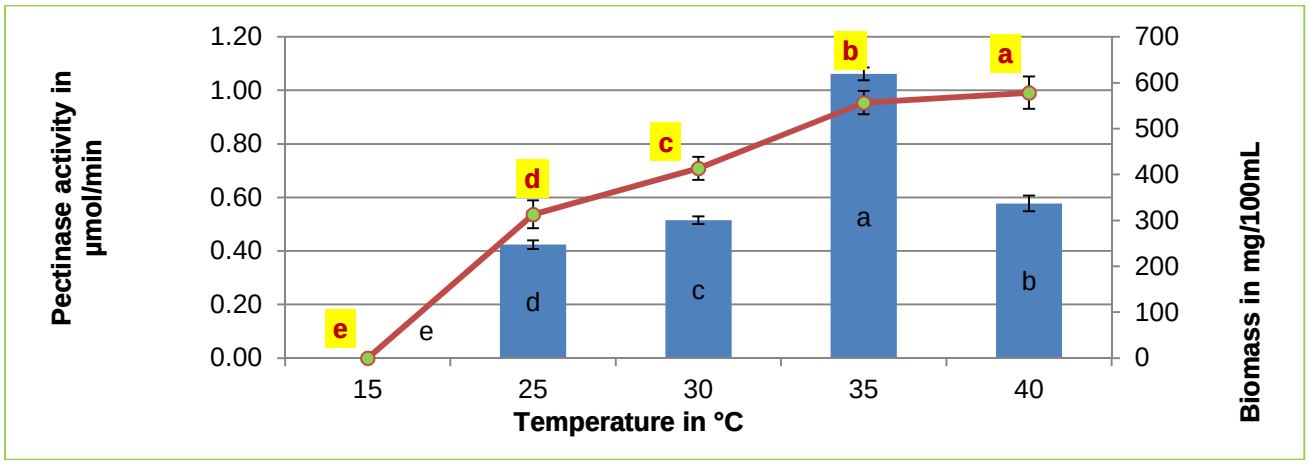

Fig 3: Effect of incubation temperature on pectinase production

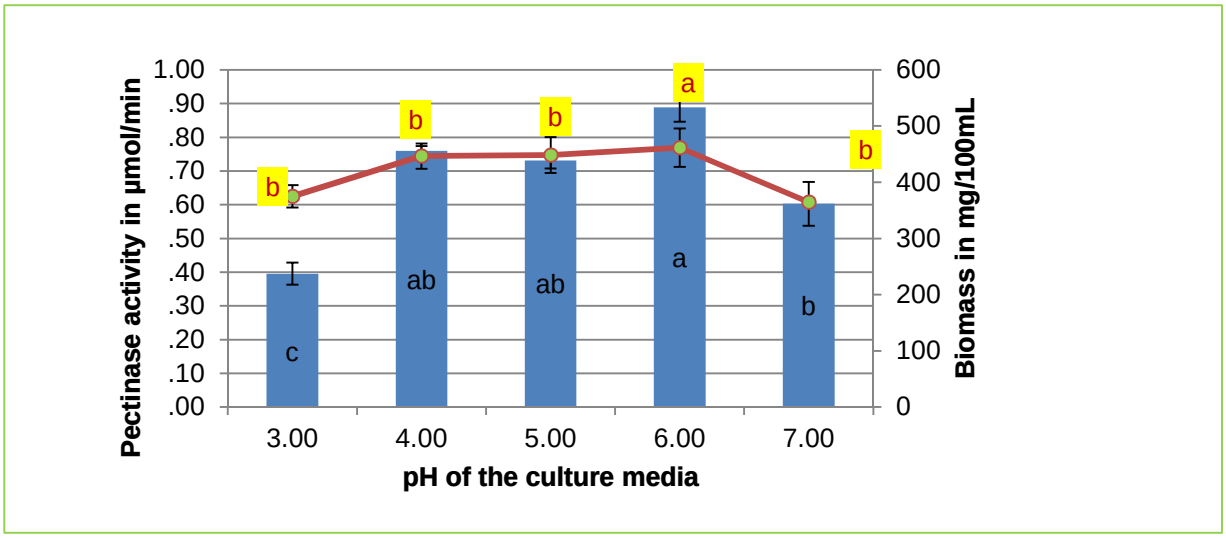

Fig 4: Effect of pH of the culture media pectinase production

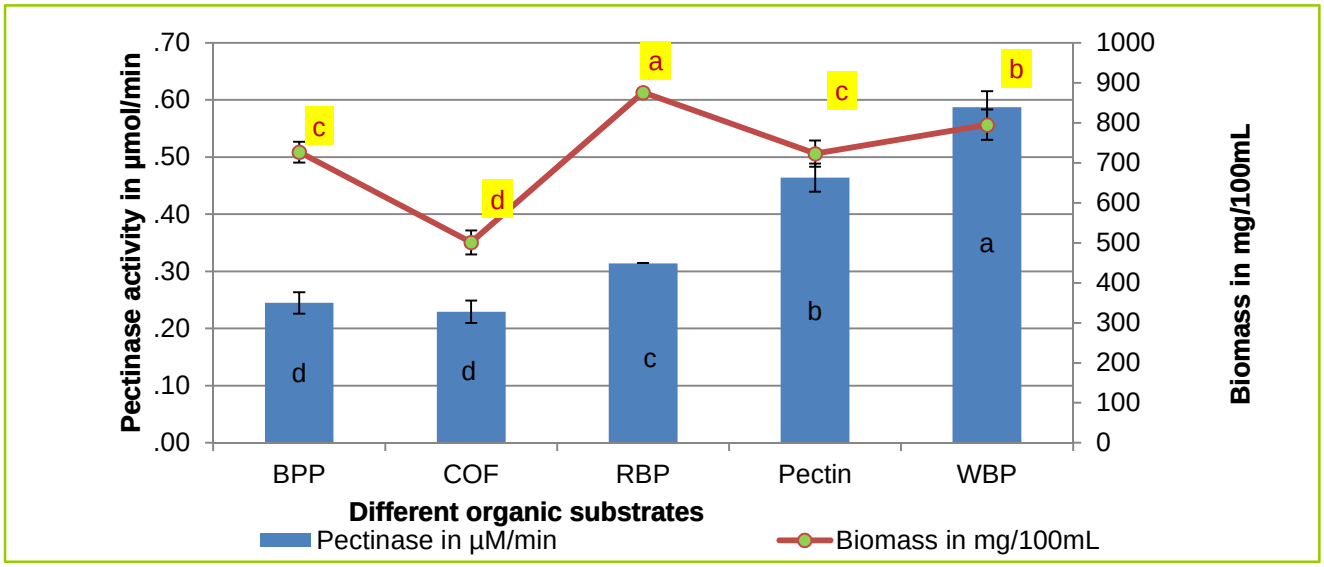

Fig 5: Effect of carbon source on pectinase production

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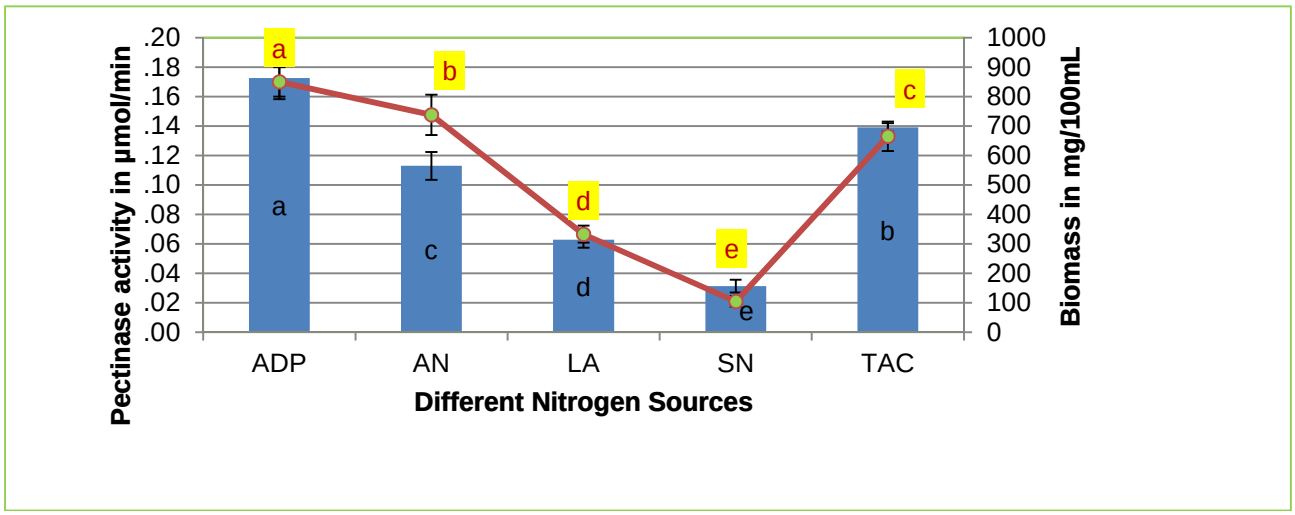

Fig 6: Effect of nitrogen source on pectinase production

Values followed by the same lower case alphabets in the each graph are statistically equivalent (P<0.05) according to the Duncan multiple range test.

DISCUSSION

From an industrial perspective, being able to rapidly identify potential isolate for the production of enzyme is most important followed by quantification of the enzyme production. The most significant inference from this study is degree of variability in the secretion of enzyme by the endophytic isolates. Pectic enzymes are induced in the presence of pectic substances by both pathogenic and endophytic fungi. In endophyte host systems pectic enzymes may well be of secondary importance and are synthesized by all types of endophyte when required for example for the passage of hyphae through cell walls (Perombelon and Hadley, 1964). Microbial pectinases are important in the phytopathologic process, plant-microbe symbiosis, and in the decomposition of dead plant material (Gummadi and Panda, 2003). Degradation of host tissue by phytopathogens generally begins with the production of pectinolytic enzymes, which are the major enzymes involved in plant attack. (Hoondal *et al*., 2002). If an endophyte can degrade pectic substances, this implies that the fungus is likely to be a latent pathogen (Choi *et al*., 2005).

Schulz and Boyle (2006) hypothesize that the fungal endophyte-plant host interaction is characterized by equilibrium between fungal virulence and plant defence, if this balance is disturbed by either a decrease in plant defence or an increase in fungal virulence, disease develops.

In the present study, the optimum pH for the production of pectinase was found to be pH 6.0, similar to the findings of El-Batal *et al*., (2013), for the production of polygalacturanase (PGase) by *Penicillium citrinum.* Torres *et al*., (2006) and Banu *et al*., (2010) reported pH 6.0 and pH 6.5 optima for the production of pectinase from *A. niger* and *Penicillium chrysogenum* respectively. Similarly Rajendran *et al*., (2011) and Patil and Chaudhari (2010) reported same pH optima in case of *Penicillium* sp. The increase or decrease in the pH of the culture media beyond the optimum value show decline in enzyme production. However, the mechanism by which the pH acts on the enzyme production is not known, it may be because of maximum availability of nutrients at that particular pH, as reported by Joshi *et al*., 2006. Low pH (3-5) is favourable for the production of endo PGase and high pH (5.0- 5.8) for exo PGase (Pedrolli *et al*., 2009).

Among the various substrates used for the production of pectinase wheat bran powder revealed excellent production and there was no growth in mandarin peel powder (MPP). There was neither growth nor pectinase production in mandarin peel powder (MPP). Contrary to our results many authors have reported the maximum yield in citrus based substrates. However Silva *et al*., (2002) reported orange bagasse and wheat bran gave

high yields of PGase by *P. viridicatum* RFC3. Contradictory to our results Mrudula and Anitharaj (2011) reported that the orange bagasse was the most significant for the pectinase production in *A. niger*. Similar to our results, Buyukkilecli *et al*., (2011) reported that the orange peel had a negative effect on the exoPGase activity in *Aspergillus sojae*. It may be due to increase in the crystallinity and decrease in the lignin contents after treatment, so the substrate becomes more acceptable for the microorganisms. Padma *et al*., (2012) also reported low yield from BPP by *Aspergillus awamori* MTCC 9166 which was similar to our findings. However Sethi *et al*, (2016) reports claimed enhanced production of pectinase by *Aspergillus terreus* NCFT 4269.10 using banana peels as substrate. Barman *et al*, 2015 reported optimsation of pectinase production by *Aspergillus niger* using banana (Musa balbisiana) peel as substrate. In the present study though the biomass was higher in RBP the enzyme activity was significantly low. This could be of two reasons; one is as the substrates were not pretreated therefore the organism was not able to degrade the complex material, second the media was viscous inhibiting the aeration for the growth of the organism.

Khan *et al*., 2012 reported that the combination of wheat straw and mosambi bagasse gave higher yields than the wheat straw, mosambi peel, lemon peel amd mosambi baggase substrates optimum pectinase production in 65% moisture with the wild strain *Aspergillus niger* ATCC16404 30^0C for 7 days of incubation in solid state fermentation. The optimum incubation temperature for the production of pectinase was slightly higher in our studies. Cheng *et al.,* 2016 reported a novel acid-stable endo-polygalacturonase from *Penicillium oxalicum* CZ1028 which had excellent stability over a broad acidic pH range (2.2–7.0) and good thermostability at 55°C.

The present study, revealed ammoniumdihydrogen phosphate (ADP) as best source for the production of pectinase from *Talaromyces* sp. Similarly, Joshi *et al*., (2006) also reported ADP as the best nitrogen source for the growth and production of pectinase by *A.niger*. Sethi *et al*, (2016) reports claimed about three-fold increase in pectinase activity with ammonium persulfate. Galiotou-Panayotou and Kapantai (1993) has reported inhibitory effects for the production PGase by *A. niger* NRRL -364 with ammonium nitrate and potassium nitrate. Akhter *et al*., (2011) reported as ammonium sulphate (1.69%) as best nitrogen source for pectinase production in *Penicillium chrysogenum*. Kumar *et al*., (2010) reported maximum production of pectinase by *A. niger* at pH 5.0 and at 30°C incubation temperature on leaves of *Ficus religiosa* with 0.3% ammonium sulphate. Mandhania *et al*., (2010) observed enhanced pectinase (PME) production by *Aspergillus heteromorphus* in ADP and peptone.

Crotti *et al.,* 1999 reported *Talaromyces flavus* as good producer extracellular pectinesterase and polygalacturonase after 24 h in submerged culture lemon pulp pellets. Elsababty *et al.,* 2015 reported modearate pectinolytic activity from *Talaromyces trachyspermus*, *T. barcinensis* and *T. ucrainicus* isolated from soil samples of cultivated fruits and vegetables might aid in the spoilage of fruits.

The production of the agro-waste is expanding rapidly since last two decades because of cheap and easily available substrate. These agricultural wastes disposal has become major issue in our country hence an effort was made to use pectin-rich agro-industrial waste for the production pf pectinase. Scrutiny of literature suggests that many authors have reported pectinase production on many agricultural waste products orange peel powder, wheat bran, orange and lemon peel, wheat bran, apple pomace, wheat bran and sugarcane bagasse, pine apple peel by *Aspergillus niger* , *Aspergillus flavus* and most of the commercially available pectinase preparations used in food processing are traditionally derived from genera *Aspergillus* (Koyani and Rajput, 2015, Janveja and Soni 2016, Anand *et al*., 2017). To the best of my knowledge this is the first report of pectinase from endophytic fungi *Talaromyces* sp. isolated from *Calophyllum inophyllum* on wheat bran media.

Conclusion

In the present study, 62 % of the endophytic fungi screened were able to produce pectinase indicating endophytic fungi are the excellent sources of pectinase. Maximum pectinase activity was observed by *Talaromyces* sp. isolated from *Calophyllum inophyllum* indicating the potential isolate for pectinase production. Optimum activity of pectinase was observed by *Talaromyces* sp. at pH 6.0 and the excellent

stability was observed at pH 4.0 and 5.0, this characteristic make the enzyme an interesting biocatalyst for juice clarification, food, paper, and textile industries. Optimum activity of pectinase was observed by *Talaromyces* sp. in the agricultural waste substrate wheat bran powder signifying the alternative carbon sources and cost effective production of the enzyme. It also contributes to decrease the pollution concern about waste accumulation in the environment. There are a lot of industrial processes which can be applied to improve the quality and the yield of the pectinases. The future efforts into pectinase research should be concentrated on elucidation of the regulatory mechanism of enzymes secretion at the molecular level and the mechanism of action of different pectinolytic enzymes towards pectic substrates.

REFERENCES

- Akhter N, Morshed MA, Uddin A, Begum F, Sultan T, Azad AK (2011) Production of pectinase by A*spergillus niger* cultured in solid state media. *Int J Biosci* 1:33–42
- Alkorta, I., Gabirsu, C., Lhama, M.J, Serra, J.L. Industrial applications of pectic enzymes: a review. *Proc Biochem* . 1998; 33: 21-8.
- Almeida, C.; Brányik, T.; Moradas-Ferreira, P.; Teixeira, J. (2005). Process Biochem., 40, 1937
- Anand, G., Yadav, S., Yadav, D., (2017) Production, purification and biochemical characterization of an exo-polygalacturonase from *Aspergillus niger* MTCC 478 suitable for clarification of orange juice. 3 Biotech 7:122
- Barman S, Sit N, Badwaik LS, Deka SC (2015) Pectinase production by Aspergillus niger using banana (*Musa balbisiana)* peel as substrate and its effect on clarification of banana juice. J Food Sci Technol 52:3579–3589
- Banu AR, Devi MK, Gnanaprabhal GR, Pradeep BV, Palaniswamy M (2010) Production and characterization of pectinase enzyme from Penicillium chrysogenum. Indian J Sci Technol 3(4):377–381
- Cheng Z., Chen D., Lu B, Wei, Y., Xian L., Li Y, Luo Z, and Huang R .,2016. A Novel Acid-Stable Endo-Polygalacturonase from *Penicillium oxalicum* CZ1028: Purification, Characterization, and Application in the Beverage Industry. *J. Microbiol. Biotechnol*. 26(6), 989–998.
- Castilho, I.R., T.L.M. Alves and R.A. Medronho, 1999. Recovery of pectolytic enzymes produced by solid state culture of *Aspergillus niger*. *Process Biochem*., 34: 181-186.
- Choi, Y.W., I.J. Hodgkiss and K.D. Hyde, 2005. Enzyme production by endophytes of *Brucea javanica*. *J. Agricultural Technol*., 1: 55-66.
- Crotti., L. B., Jabor,.,V.A. B., Chelgatti.M.A.D.C., Said.S. 1999. Studies of pectic enzymes produced by *Talaromyces flavus* in submerged and solid substrate cultures *Journal of Basic Microbiology* 39(4):227-35 ·
- Duncan, D.B., 1955. Multiple range and multiple on decomposition of the leaves of the mangrove F-tests. Biometrics., 11: 1-42.
- El-Batal A.I. , A.M. Osman, A.M.S. Ibrahim.2013, Optimization and characterization of polygalacturonase enzyme by gamma irradiated *Penicillium citrinum* J. Chem. Pharm. Res., 5 pp. 336-337
- Elsababty Z, Ali AM, Houbraken J (2015) Cellulolytic and Pectinolytic Enzymes of Some Selected Heat Resistant Fungi. J Microbiol Exp 2(2): 00042
- Gangadharan, D., S. Sivaramakrishnan, K.M. Nampoothiri and A. Pandey. 2006. α-Amylase Production by B. amyloliquefaciens, Food Technol. Biotechnol., 44(2): 269-274.
- Galiotou-Panayotou MPR, Kapantai M, Kalantzi O (1997) Growth conditions of Aspergillus sp. ATHUM-3482 for polygalacturonase production. Appl Microbiol Biotechnol 47:425–429
- Gummadi, S.N. and T. Panda, 2003.. Purification and biochemical properties of microbial pectinases: A review. Process Biochem., 38: 987-996.
- Hoondal G, Tiwari R, Tewari R, Dahiya N, Beg Q (2002) Microbial alkaline pectinases and their industrial applications: a review. Appl Microbiol Biotechnol 59:409–418
- Jabasingh, S..A, Varma, S., Garrec, .2014. Production and purification of cellulase from *Aspergillus nidulans* AJSU04 under solid-state fermentation using coir pith. Chem. Biochem Eng 28: 143-151.

- Jorgensen., H., Kristeensen , J.B., And Felby, C. 2007 Enzymatic conversion of ligncellulose into fermentable sugars: Challenges and oppuruinities. Biofuels Bioprod. bioref. 1 (2). 119-134..
- Janveja, C and Soni, S. K .2016. A Combinatorial Statistical Approach For Hyperproduction Of Pectinase From Aspergillus Niger C-5 Via Solid State Fermentation Of Wheat Bran And Its Kinetic Characterization Journal of Multidisciplinary Engineering Science Studies (JMESS) Vol. 2 Issue 11**.**
- Kashyap D, Vohra P, Chopra S, Tewari R. 2001. Applications of pectinases in the commercial sector: a review. Bioresour.Technol. 77: 215-227.
- Khan, A Sahay.,S and Rai.,N.,2012. Production and optimization of Pectinase enzyme using
- *Aspergillus niger* strains in Solid State fermentation. Research in Biotechnology, 3(3): 19-25.
- Koyani RD, Rajput KS (2015) Solid State Fermentation: Comprehensive Tool for Utilization of Lignocellulosic through Biotechnology. J Bioprocess Biotech 5: 258
- Kumar, S., Sharma, H. K. and Sarkar, B. C. 2011. Effect of substrate and fermentation conditions on pectinase and cellulase production by Aspergillus niger NCIM 548 in submerged (SmF) and solid state fermentation (SSF). Food Science and Biotechnology 20 (5): 1289- 1298.
- Miller GL. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31: 426-428.
- Mrudula, S., Anitharaj, R. 2011. Pectinase production in solid state fermentation by Aspergillus niger using orange peel as substrate. *Global J Biotechnol Biochem* 6:64–71
- Rajendran, R., S.K. Sundaram and K.U.Maheswari. 2011, J.Environ.Sci.Technol, 4: 568-578
- Siddiqui,M.A., Pande, V., and Arif . M., Production, Purification, and Characterization of Polygalacturonase from Rhizomucor pusillus Isolated from Decomposting Orange Peels. *Enzyme Research*, vol. 2012, pp. , 2012
- Mandhania S, Jain V, Malhotra SP (2010) Culture optimization for enhanced production of microbial pectin methylesterase under submerged conditions. Asian J Biochem 5(1):12–22.
- Mojsov, K., Ziberoski, J., ozinovic, Z., Petreska, M. 2010. A comparison of effects of three commercial pectolytic enzyme preparations in red wine making. *Int. J. Pure Appl. Sci. Technol*. 1,127–136.
- Mieszczakowska-Frąc M, Markowski J, Zbrzeźniak M, Pìocharski W 2012. Impact of enzyme on quality of blackcurrant and plum juices.LWT - Food Sci Technol 49:251–256.
- Miller, G.L, (1959). Use of dinitro salicyclic acid reagent for determination of reducing sugar. *Anal Chem.*, 31, 426-429.
- Mortabit, D., Mourad Zyani, M., Koraichi, S.I. 2014 Improving olive oil yield from Moroccan Picholine by bacterial enzymes extract. *IJISET* 1, 717–728.
- Murthy, P.S., Naidu, M.M. 2011. Improvement of robusta coffee fermentation with microbial enzymes. *Eur. J. Appl. Sci*. 3, 130–139.
- Patel., A.K., Singhania,R. R., Pandey, A., 2017. Biotechnology of microbial enzymes Chapter 2 – Production, Bioacatalysis and Industrial Applications, Pages 13–41.
- Patil NP, Chaudhari BL (2010) Production and purification of pectinase by soil isolate Penicillium sp. and search for better agro-residue for its SSF. Recent Res Sci Technol 2(7): 36–42
- Pedrolli, D, B, Monteiro, A ,C, Gomes, E and Carmona E C. **2009**. Pectin and Pectinases: Production, Characterization and Industrial Application of Microbial Pectinolytic Enzymes *The Open Biotechnology Journal, , 3,* 9-18
- Pedrolli., D., B and Carmona., E., C., 2014 Purification and Characterization of a Unique Pectin Lyase from Aspergillus giganteus Able to Release Unsaturated Monogalacturonate during Pectin Degradation. *Enzyme Research* Volume 2014.
- Perez, E.E., Ferna´ndez, M.B., Nolasco, S.M., Crapiste, G.H. 2013. Effect of pectinase on the oil solvent extraction from different genotypes of sunflower (Helianthus annuus L.). *J. Food Eng.* 117, 393–398.
- Perombelon, M. and Hadley, G. (1965). Production of pectic enzymes by pathogenic and symbiotic Rhizoctonia strains. New Phytol, 64(1): 144-151.

- Schulz. B & Boyle C (2006) What are endophytes? Microbial Root *ndophytes* (SchulzBJE, BoyleCJC &SieberTN, eds), pp. 1–13. Springer-Verlag, Berlin.
- Sethi,, B K NandaP K , Sahoo, S,., 2016. Enhanced production of pectinase by Aspergillus terreus NCFT
- 4269.10 using banana peels as substrate. 3 Biotech 6:36
- Siddiqui MA, Pande V, Arif M (2013) Polygalacturonase production from Rhizomucor pusillus isolated from fruit markets of Uttar Pradesh. Afr J Microbiol Res 7(3):252–259
- Sieiro, C., Garcı´a-Fraga, B., Lo´pez-Seijas, J., Silva, A.F. Da., Villa, T.G., 2012. Microbial pectic enzymes in the food and wine industry. In: B. Valdez (ed.) Food Industrial Processes – Methods and Equipment, pp. 201–218 .
- Silva D., Martins E. Da Silva. and Da Silva R., Gomes E.(2002). Pectinase production by *Penicillium viridictum* RFC3 by solid state fermentation using agricultural waste and agro-industrial by products. *Brazilian Journal of Microbiology*,33: 318 – 324
- Sin, H.N, Yusof, S., Hamid, N.S.A, Rahman, R.A. 2006 Optimization of enzymatic clarification of sapodilla juice using response surface methodology. *J Food Eng*.
- Sunitha, V. H, Nirmala Devi. D, and Srinivas, C. 2013. Extracellular Enzymatic activity of endophytic fungal strains isolated from medicinal plants. World Journal of Agricultural Sciences. 9 (1): 01-09.
- Torres EF, Sepulveda TV, Gonzalez GV (2006) ,Production of hydrolytic depolymerising pectinases.,Food. Technol. Biotechnol. 44:221-227.
- Voragen A G. J., Coenen, G., Verhoef, R P., Schols H ,A., 2009. Pectin, a versatile polysaccharide present in plant cell walls. Struct Chem 20:263–275