IMPACT: International Journal of Research in Applied, Natural and Social Sciences (IMPACT: IJRANSS)
ISSN(P): 2347-4580;ISSN(E): 2321-8851
Vol. 4, Issue 6, Jun 2016, 75-82

STUDIES ON PECTINOLYTIC BACTERIA USEFUL IN FRUIT JUICE INDUSTRY

A. PADMAVATHI[1] & M. RAGHU RAM[2]

[1]Department of Microbiology, CH. S. D. St. Theresa's College for Women, West Godavari Dt. Andhra Pradesh, India

[2]Department of Botany & Microbiology, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India

ABSTRACT

Capturing and Exploitation of diversified microorganisms from different habitats and their optimum utilization in industrial sector is the need of the hour. Soil samples collected from commercial crops (maize and banana) of West Godavari district A.P. were screened for the production of Polygalacturonase, using citrus pectin as carbon source. Two potential Polygalacturonase producing bacteria, one from each crop field soil which produced maximum zone of hydrolysis on pectin agar were selected. The bacterial strains were identified as *Bacillus subtilis* MRRP129 (KF621016) from banana, *Bacillus axarquiensis* MRRP128 (KF621022) from maize fields by 16S rRNA sequencing. Maximum production of the enzyme was observed at 32^0 C temperature and pH 7, at 72 hours of incubation, when 1% pectin was used in static conditions, for both the strains. The isolates in this study produced good amount of polygalacturonase activity at neutral pH; hence, they can be useful in juice industry to increase the yield of banana, grape, or apple juice.

KEYWORDS: Polygalacturonase, Pectin Agar, Enzyme Production, *Bacillus Subtilis, B. Axarquiensis*

INTRODUCTION

Microorganisms have been endowed with vast potentials. They produce an array of enzymes which have been exploited commercially over the years. The increasing energy demands have focussed worldwide attention on the utilization of renewable resources particularly agricultural and forest residues, the major components of which are cellulose, starch, lignin, xylan and pectin. These materials have attracted considerable attention as an alternative feed stock and energy source. Since they are available abundantly several microbes are capable of using these substances as carbon and energy sources by producing a vast array of enzymes in different environmental niches (Antranikian,1992). Pectic substances are abundant in plant biomass. Pectins are heterogenous group of high molecular weight complex acidic polysaccharides that are made largely of D- galacturonic acid.

The enzymes that hydrolyse pectic substances are broadly known as pectinolytic enzymes or pectinases. These enzymes were some of the first enzymes to be used in homes and one of the upcoming enzymes of the commercial sector. Primarily these enzymes are responsible for the degradation of the long and complex molecules called pectin that occur as structural polysaccharides in the middle lamella and the primary cell walls of young plant cells (Kashyap, 2000).

The pectinases are required for extraction and clarification of fruit juices and wines, extraction of oils, flavours and pigments from plant materials, preparation of cellulose fibres for linen, jute and hemp manufacture, coffee and tea fermentations and novel applications in the production of oligogalacturonides as functional food components (Urmila phutela, 2005).

Pectinases from food and food by-products processed waste alone account to a total of one third quarter of world's food enzyme production. The continuous search for pectin hydrolyzing enzymes from biomass of fruit industry waste is deriving increasing attention. By products or waste obtained from orange, apple, grapes, pine apple, papaya, lemon juice manufacturing industries are used as source of the enzyme production. Soil samples obtained from fruit processed area are found to be an appreciable reservoir of pectinolytic bacteria. These enzymes are mainly synthesized by plants and microorganisms. Pectinases are classified under three headings ac-cording to the following criteria: whether pectin, pectic acid or oligo-D-galacturonate is the preferred substrate, whether pectinases act by trans-elimination or hydrolysis- and whether the cleavage is random (endo-, liquefying of depolymerising enzymes) or endwise (exo- or saccharifying enzymes). The three major types of pectinases are as follows Pectinesterases (PE) (3.1.1.11), De-polymerising enzymes (3.2), Protopectinase. The present study is on one of the depolymerising enzymes- ExoPolygalacturonases.

Intensive research is being pursued to isolate these enzymes from new sources for large scale production and application because the organisms that produce such enzymes in high titres are limited. There are only few reports on Polygalacturonase production by bacteria. The aim of present study is to isolate and characterise Polygalacturonase producing bacteria useful in fruit juice industry.

MATERIALS AND METHODS

The Micro Organism and the Enzyme Production: Soil samples were collected from commercial crops like maize and banana of W.G.Dt. in A.P. Pectinase producing bacteria were isolated by growing them on pectin agar medium. Pectin agar medium was used with the following composition 1% citrus pectin, 0.14% NH_2 SO_4, 0.6% K_2HPO_4, 0.2% KH_2PO_4 and 0.01%Mg S O_4 $7H_2O$, Agar agar 2% pH-6.0(Sanjay patel, 2015) was autoclaved for 15min at 121◦C.

Screening of Potential Pectinase Producing Bacteria: From the bacteria showing pectinase activity potential pectinase producing bacteria were screened (Ouattara,2008) based on the diameter of zone of hydrolysis. Pectinase activity was tested by adding 1gm of iodine, 5gms of potassium iodide to 330 ml of water.

Polygalacturonase Production: Bacterial strains producing >2cm clearance zones around the colonies were used for enzyme production essay. Liquid medium containg1% citrus pectin, 0.14% (NH4)2SO4, 0.6%K2HPO4, 0.2%KH2PO4 AND 0.01%Mgso47H20,% pH-6.0 was autoclaved for 15 min at 121◦C.After cooling the medium was inoculated with 1ml of bacterial suspension. Cultures were grown in 125ml erlenmeyer flasks with 25ml of medium in a rotary shaker at150rpm, at 30◦c for 72hrs. Biomass was separated at 10,000rpm for 20min at 4◦c.Enzyme activities were measured in the cell free supernatant.

Enzyme Assay: Polygalacturonase activity was assayed spectrophotometrically at 540nm by measuring the release of reducing groups from citrus pectin using 3, 5 dinitrosalicylic acid (DNS) reagent assay (Miller,1959) Galacturonic acid monohydrate was used as standard. The reaction mixture containing 1ml of 1%pectin, 1ml of enzyme in 0.1mM acetate buffer of pH5 was incubated at 40◦c, 20min. The blank was prepared in a similar way except the crude enzyme.3ml of DNS reagent was added and heated at 100◦c for 20min. After that 1ml of sodium potassium tartarate was added. One unit of enzymatic activity (U) was defined as 1 µmol of galacturonic acid released per minute.

Fermentation Conditions for Polygalacturonase Production: Different parameters like carbon, nitrogen, pH, Temperature, incubation time, metal ions and pectin concentration were optimized for *maximum production of Enzyme*

activity.

- **Effect of Incubation Time on Enzyme Activity:** The activity of the enzyme was studied at different incubation periods-24hrs, 48hrs, 72hrs, 96hrsand 120hrs. Enzyme assay was carried out at different incubation periods.
- **Effect of pH and Temperature on Enzyme Activity:** The activity of the enzyme was studied at different pH-3, 5, 7, 9 &11and temperatures-10◦c,20◦c,30◦c,40◦c,50◦c then enzyme assay was carried out.
- **Effect of Pectin Concentration on Enzyme Activity:** The activity of the enzyme was studied by using different concentration of pectin- 0.05%, 0.5%.1%, 1.5%, 2%.
- **Effect of Carbon and Nitrogen Sources on Enzyme Activity:** The activity of the enzyme was studied by using different carbon sources(natural and synthetic at a concentration of 1%) and inorganic nitrogen sources like NH_4Cl, NaNO3, NaNo2, (NH4)2SO4 and organic beaf extract yeast extract and malt extract at a concentration of 0.1%.

RESULTS AND DISCUSSIONS

Screening of Pectinase and Potential Pectinase Producing Bacteria

In the present study 29 different soil samples belonging to maize (16) and banana (13) fields were collected. To know the bacterial diversity10^{-6} dilution of each soil sample was inoculated on to nutrient agar plates. Triplicates were maintained for each soil sample. Total number of bacteria and number of different types of bacteria were counted. The different types of bacteria were plated on pectin agar media to isolate pectinase producers. A total number of 893 colonies (744 from maize and 149 from banana) were found to be pectinase producers (Table-1)

Table 1: Pectinase Producers from Different Soil Samples

S. No.	Name of the Soil Sample	No. of Fields from which the Soil Sample is Collected	No. of Pectinase Producers
1	Maize	16	744
2	Banana	13	149

Screening of Potential Pectinase Producing Bacteria

The pectinase producing bacteria were screened for potential pectinase producing bacteria based on the diameter of zone of hydrolysis (›2cm).179 from maize and 75 from banana were found to be potential pectinase producing bacteria(Table-2).From these two efficient strains showing largest zone (MZ501-5CM,B1303-4CM)were selected for further study. The bacteria were identified as Bacillus sps *Bacillus axarquiensis* MRRP128 (KF621022) from maize, *Bacillus subtilis* MRRP129 (KF621016) from Banana fields. (Plate-1)

Plate: 1 A. *Bacillus Axarquiensis* MRRP128 (KF621022), B. *Bacillus Subtilis* MRRP129 (KF621016)

Table 2: Potential Pectinase Producers from Different Soil Samples

S.No.	Name of the Soil Sample	No. of Fields from which the Soil Sample is Collected	Total no. of Colonies	No. of potential Pectinase Producers (›2cm).
1.	Maize	16	3213	179
2.	Banana	13	2130	75

Growth Pattern

The growth pattern was observed at every 4hrs interval up to 120hrs of incubation. There is gradual increase in growth till 72 hrs of incubation after that both the isolates entered in to stationary and later phase of decline. There is gradual increase in growth from 24-72hrs for *Bacillus axarquiensis* and from 12- 72hrs for *Bacillus subtilis*) (Figure-1).

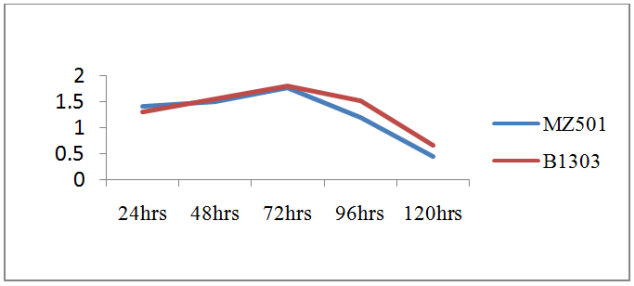

Figure 1.Growth Curve of KF621022 & KF621016

Polygalacturonase Enzyme Assay

Polygalacturonase activity of the two isolates was studied using DNS method (Miller 1959). The two isolates were selected to study optimum conditions. The enzyme production is growth associated. Upto 72 hrs there is increase in enzyme production (Table-3) later there is gradual decrease in enzyme activity (Figure2).The enzyme production is growth associated.

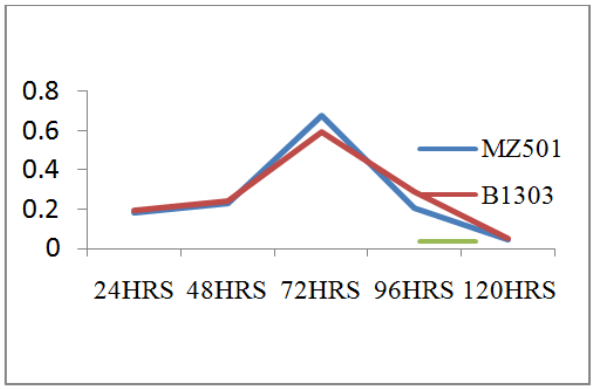

Figure 2: Enzyme Production by the Two Isolates at Different Incubation Periods

Table 3: Maximum Enzyme Production at 72hrs of Incubation

S. No	Colony Type	Incubation Time At Which Maximum Enzymatic Activity	Enzyme Units µmol/L
1	Control	72hrs	0.0
2	MZ501	72hrs	1415
3	B1303	72hrs	1320

Effect of Incubation Time on Polygalacturonase Activity: Growth and enzyme activity was observed at 4hrs interval The enzyme production of *Bacillus axarquiensis* isolated from maize field is growth associated. The enzyme production increased gradually from 12-48hrs and from then onwards there is enhanced enzyme production up to 72hrs.Later there is gradual decrease in enzyme activity. The OD value increased from 26.7% to 34.4% within 48hrs and then reached 45% with 12more hrs of incubation.

Polygalacturonase produced by *Bacillus subtilis*is, also growth associated. The enzyme production increased from 12 to60hrs, and then there is tremendous increase in enzyme production. The OD value increased from 32% to40% within 48hrs of incubation and then to 45% at 60hrs of incubation. Finally increased up to 72hrs of incubation. Later there is gradual fall in enzyme activity. (Table-4)

Table 4: Effect of Incubation Time on Polygalacturonase Production

Bacterial Isolate	12hrs	24 hrs	36 hrs	48 hrs	60 hrs	72 hrs	84 hrs	96 hrs	108 hrs	120 hrs
Mz501	0.153	0.181	0.193	0.233	0.306	0.677	0.306	0.206	0.161	0.047
B1303	0.131	0.189	0.202	0.236	0.270	0.590	0.487	0.288	0.155	0.048

Effect of pH and Temperature on Enzyme Activity

Temperature and pH are the two very important physical factors influencing the activity of an enzyme. In the present study maximum enzyme activity occurred at pH 7.0 for both the isolates Most of the bacillus sps produce high amt of pectinase between pH 7-9(Kobayashi, 1999).The enzyme production increased from 6.8-7 and then the enzyme lost its activity. The enzyme activity of the selected isolates was studied at different temperatures like10◦C,20◦C,30◦C,40◦C and50◦C.Optimum temperature for *Bacillus axarquiensis* was found to be 32◦C and from then onwards increase in temperature decreased enzyme activity even *Bacillus subtilis*is showed the same. Many bacillus sps needs 32-37◦C for better pectinase production (Soriano et, al, 2005) (Figure 3 & 4)

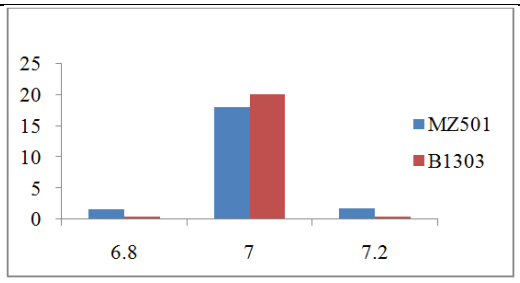

Figure 3: Effect of pH on Enzyme Production by the Bacterial

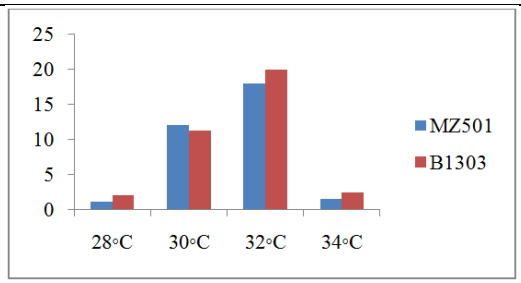

Figure 4: Effect of Temperature on Enzyme Production by isolate the Bacterial Isolates

Effect of Substrate Concentration: To enhance the enzyme production different concentrations of the substrate pectin was used.The best concentration was found to be 1% pectin for both the isolates. Figure 5-1% pectin supported good growth and enzyme production of *Bacillus axarquiensis* where as 1% concentration was found be good for *Bacillus subtilis*is.After that increase in pectin concentration had feedback inhibition.The polygalacturonase activity of KF621022 at 1% concentration was found be1412U/lit and of KF621016 was1415 at 72hrs of incubation.

Table 5: Effect of Pectin Concentration on Polygalacturonase Activity

S.No	Pectin conc%	Mz501u/lit	B1303u/lit
1.	CONTROL	47	47
2.	0.05	47	94
3.	0.5	94	849
4.	1	1412	1415
5.	1.5	839	1084
6.	2	47	179

Effect of Carbon and Nitrogen Sources: The polygalacturonase activity of the culture filtrate of the selected isolates was determined using various commercial (ribose, glucose, fructose, sucrose and starch) and natural (rice bran, wheat bran, orange peel, banana peel, mango peel, sugarcane baggase and aqua waste) sources along with citrus pectin 1%.All the commercial carbon sources supported good growth of the two isolates except sucrose and starch which had negative impact (Figure5). Even natural carbon sources supported good growth of of the isolates except wheat flour which had feedback inhibition (Figure6). The best carbon source was found to be ribose (1.5%) concentration **(**Table 6). The amount of enzyme produced by KF621022 was 2122U/lit and of KF621016 was 2358U/lit. Pectin was found to be the right carbon source for bacterial strain for higher production of pectinase than glucose due to feedback inhibition (Namasivayam et. al.2011)

Among the various inorganic nitrogen sources tested the isolates grew best with $(NH_4)_2SO_4$ using pectin as carbon source. The effect of different organic nitrogen sources on growth and enzyme production was given in Table 7

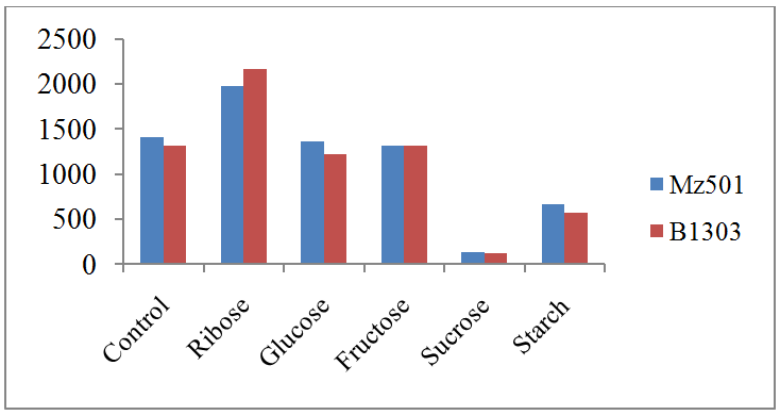

Figure 5: Effect of Commercial Carbon Sources on Polygalacturonase Production

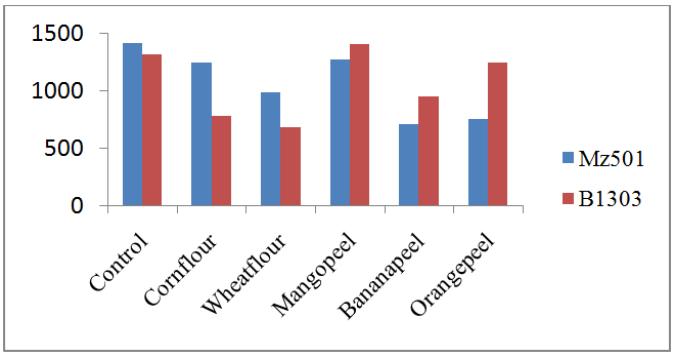

Figure 6: Effect of Natural Carbon Sources on Polygalacturonase Production

Table 6: Effect Different Concentrations of Ribose on Polygalacturonase Activity µmol/L

Ribose Concentration	M z501U/lit	B1303U/lit
0.5%	613	566
1.0%	2075	2169
1.5%	2122	2358
2.0%	330	707

Table.7.The Effect of Different Organic Nitrogen Sources on Polygalacturonase Activity

Microorganism	Malt extractU/lit	Yeast extractU/lit	Beaf extracU/litt
MZ501	47	141	1520
B1303	58	740	1459

CONCLUSIONS

The bacterial isolates showed maximum enzyme activity at 72hrs of incubation, 32◦c, pH-7 with ribose and beaf extract as carbon and nitrogen sources. As the optimum pH is 7 can be used for fruit juice clarification. This clearly shows that Soil samples obtained from fruit/crop processed area are found to be an appreciable reservoir of pectinolytic bacteria. There is more number of pectinase producers as well as potential pectinase producers from maize fields than banana fields. Even there is rich Bacterial diversity and so the pectinase producers.

ACKNOWLEDGEMENTS

The author A. Padmavathi is thankful to CH.S.D.St.Theresa's college for women (A) Eluru for their constant encouragement and support and Head, Department of Botany & Microbiology, Acharaya Nagarjuna University for providing the laboratory facilities.

REFERENCES

1. Abdel-Mohsen SaberIsmail, Utilization of orange peels for the production of multienzyme complexes by *Aspergillus. nigerA-2O,* process Biochemistry, (645-650)-31, 1996.
2. Aisha M.H.Al.Rajhi, purification and characterization of an extracellular Polygalacturonase from *Rhizocctonia solani Kuhn* (AG2-2)(476-484)-4, 2013.
3. Aminzadeh et. al,, Isolation and characterization of Polygalacturonase produced by *Tetracocco sporium sp* Iran J Chem Chem.Eng., (47-54)-26, 2007.
4. Arotupin et. al, Effect of carbon and nitrogen sources on Polygalacturonase production by *Trichoderma. viride BITRS-1001* isolated from tar sand in ondo state,Nigeria, Malaysian journal of Microbiology (153-158)-(7)3, 2011.
5. Blandino.A. et. al, Utilization of whole wheat flour for the production of extracellular pectinases by *Aspergillus. awamori, Rhizopus.stolonifer,* process Biochemistry,(497-503)-37, June 2001.
6. Chawanit sittidilokratna et.al, Screning of Pectinase producing Bacteria and their efficiency in Biopulping of paper mulberry bark, Science Asia,(131-135)-33, 2007.
7. Danielle Biscaro Pedrolli et. al, studies on productivity and characterization of Polygalacturonase from

Aspergillus. giganteus submerged culture using citrus pectin and Orange waste,Appl Biochem Biotechnol, (191-200)-144, Oct 2007.

8. Elangovan Namasivayam et. al.,production of extracellular pectinase by *Bacillus cereus* isolated from market solid waste,Bioanalysis and biomedicine,(070-075)-(3)3,2011.
9. Guneet Kaur et. al. Production, characterization and application of a thermostable Polygalacturonase of a thermophilic mould *Sporotrichum thermophile* Apinis,Bioresource technology, (239-243)-94, March 2004.
10. HonoreG.Ouattara., et.al., Implication of bacillus sps in the production of pectinolytic enzymes during cocoa fermentation,world journal of microbial biotechnol, (1753-1760)-24, feb 2008.
11. Lizu-ming et.al, Production and characterization of ThreeAlkaline Endo Polygalacturonases from a Newly isolated *Bacilli.gibsonii*,The Chinese Journal of Process engineering,(768-773)-(8)4, Aug 2008.
12. Maria.F.S.Teixeira et.al, carbon sources effect on pectinase production from *Aspergillus.japonicus586*, Brazilian journal of Microbiology, (286-290)-31, Nov 2000.
13. Maria.S., et.al., purification and characterization of a novel pectinase from *Acrophialophora nainiana* with emphasis on its physicochemical properties, Journal of biotechnology (33-42)-123, Oct 2006.
14. Miller.GL. Analytical Biochemistry31, 1959, 426.
15. Nagapadma. P. & K. Anuradha, Bacterial isolates for Polygalacturonase Production from diverse sources, International journal of scientific progress and Research, (110-113)-(10)3, 2015.
16. Nicemol Jacob et.al, purification and partial characterization of Polygalacturonase from *Streptomyces lydicus*, Bioresource technology, (6697-6701)-99, Nov 2008.
17. Tohru kobayashi et.al, purification and properties of galacturonic acid releasing exo Polygalacturonase from a strain of *Bacillus*, Bioscience, Biotechnology and Biochemistry (842-847)-65(4), 2001.