PRODUCTION OF PECTINASE ENZYME BY PECTINOLYTIC BACTERIA ISOLATED FROM FRUIT WASTE DUMPING SOIL SAMPLES

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ABSTRACT *The present study investigates to isolate the pectinolytic bacteria from fruit waste dumping soil and screening the pectinase producing bacteria as well as used cheaper substrate to enhance the pectinase enzyme production. Fruit wastes are one of the main sources of municipal waste and dumped into soil. The four bacteria were isolated from 4 soil samples were Bacillus subtilis, Bacillus cereus, Bacillus megaterium and Bacillus thuringiensis. The substrates used for production of pectinase enzyme were Wheat bran, pigeon peas husk and Grams husk. From these four bacteria, Bacillus cereus and Bacillus thuringiensis showed highest potential to produce pectinase enzyme. Bacillus cereus produced highest pectinase enzyme 111.10 IU/ml/min from pigeon peas husk. Bacillus megaterium produced highest pectinase enzyme 113.87 IU/ml/min from Wheat bran. The best substrate for pectinase enzyme was Wheat bran. From this result it was concluded that Bacillus megaterium produced highest pectinase enzyme from Wheat bran.*

Keywords:

Introduction :

Fruit wastes are one of the main sources of municipal waste. In the past decade, along with the rise of the middle class and fast economic growth, different varieties of fruits produced in countries are increasingly consumed. Due to the high consumption and industrial processing of the edible parts of fruit, fruit wastes such as citrus fruit skins, pineapple residues, sugarcane bagasse and other fruit residues (principally peels and seeds) are generated in large quantities in big cities. Fruit waste has become one of the main sources of municipal solid wastes (MSW), which have been an increasingly tough environmental issue. At present, the two main techniques to dispose MSW are landfill and incineration. However, inappropriate management of landfill will result in emissions of methane and carbon dioxide [1], and incineration involves the subsequent formation and releases of pollutants and secondary wastes such as dioxins, furans, acid gases as well as particulates [2], which pose serious environmental and health risks. For these reasons, there is an urgent need to seek resource and value-added use for fruit wastes. In fact, inexpensive and readily available use of agro-food industry waste is highly cost-effective and minimizes environmental impact. One of the most beneficial approaches is to recover the bioactive constituents, making full use of them in the food, pharmaceutical as well as cosmetics industry. Thus, utilization of the fruit wastes as sources of bioactive compounds may be of considerable economic benefits and has become increasingly attractive[3].

Microbial enzymes are routinely used in many environmentally friendly and economic industrial sectors. Environmental pollution is no longer accepted inevitable in technological societies. Over the past century there has been a tremendous increase in awareness of the effects of pollution, and public pressure has influenced both industry and government. There is increasing demand to replace some traditional chemical processes with biotechnological processes involving microorganisms and enzymes such as Pectinase [4][5].

A large number of strains of microorganisms such as bacteria, fungi, actinomycetes, yeast have ability to degrade pectin[6][7]. However, Bacterial strain are always preferred over fungal strain because of ease in fermentation and strain improvement which can be carried out easily in bacterial strain to improve the yield[8].

Different types of micro-organisms have been exploited for the production of enzymes. Pectinolytic enzymes have been reported to be produced by a large number of bacteria and fungi such as *Bacillus spp., Clostridium spp., Pseudomonas spp., Aspergillus spp., Monilla laxa, Fusarium spp., Verticillium spp., Penicillium spp., Sclerotinia libertiana, Coniothyrium diplodiella, Thermomyces lanuginosus, Polyporus squamosus, etc*.

Pectinases have been used in processes and industries where the elimination of pectin is essential; fruit juice processing, coffee and tea processing, macerating of plants and vegetable tissue, degumming of plant fibers, treatment waste water, extracting vegetable oil, bleaching of paper, adding poultry feed and in the textile, alcoholic beverages and food industries. Pectinolytic enzymes are commonly used during processing of fruits and vegetables for juices and wine. The pectic substances account for about 0.5–4% of the weight of fresh material. The raw pressed juice is rich in insoluble particles mainly made up of pectic substances. When the tissue is ground, the pectin is found in the liquid phase (soluble pectin) causing an increase in viscosity and the pulp particles. It is difficult to extract this juice by pressing or using other mechanical methods. With the addition of pectinases the viscosity of the fruit juice drops, the press ability of the pulp improves, the jelly structure disintegrates and the fruit juice is easily obtained with higher yields, Pectinase are produced during the natural ripening process of same fruits. This can also increase the volume of juice (increase the yield), lowers the viscosity of juice, and reduces the cloudiness of juices, which is caused by suspended pieces of cell wall [9]. Pectinase group of enzymes include polygalacturanases, pectin methyl esterase, pectin lyases. These pectinase enzymes act in different ways and on the pectins. Pectinase are extensively used in fruits juices processing (extraction and clarification) vegetable oil extraction, processing of alcoholic beverages and a variety of applications in food industries [10].

Hence keeping in mind the above problem, the present investigation was to isolate the pectinolytic bacteria from fruit waste dumping soil and screen the pectinase producing bacteria as well as used cheaper substrate to enhance the pectinase enzyme production.

Materials and Methods :

Collection of soil sample:

Soil samples were collected from 4 different locations at a depth of 7 cm from fruit waste dumping yards of Nagpur[11]. The soil samples were transported to the laboratory in sterile polythene bags. Soil sample was serially diluted and plated. [12]

Isolation of *Bacillus spp.* from soil sample by serial dilution method:

Prepared a set of 10 sterilized test tubes with 10 ml of distilled water in first test tube and 9 ml of distilled water in remaining test tubes respectively. One gram of soil sample was added in first test tube containing 10 ml distilled water, mixed it well. Further 1 ml of suspension is removed with a sterile pipette and transferred to second test tube containing 9 ml distilled water This dilution step was repeated, each time 1 ml of previous suspension was taken and transfer to the next test tube. Labelling of these tubes were done sequentially starting from 10^{-1} to 10^{-9}.

Spread Plate Technique for isolation:

To grow bacterial colonies, take three pre-prepared HiCrome Bacillus Agar plates and label them as 10^{-5}, 10^{-6}, 10^{-7}. Sample tubes 10^{-5}, 10^{-6} and 10^{-7} were vortex and pipette out 0.1 ml suspension onto each plate. This increase the dilution value, by a factor of ten. Open the plate lid and with the help of a sterilized glass spreader, spread the drop of inoculums around the surface of the agar until traces of free liquid disappears. Replaced the plate lid. Re-flame the spreader and repeat the process with the next plate, working quickly so as not to contaminate the agar with the airborne organisms. The bacterial plates were incubated. The plates were kept inverted during the incubation to prevent drops of moisture due to condensation from falling onto the agar surface. Plates were incubated in inverted position at 37^0 C for 24 hours. After 24 hrs plates were observed for colonies.

Identification of *Bacillus spp*.:

The isolated colonies were identified on the basis of morphology by performing Gram staining and motility, biochemical by testing sugar fermentation using Glucose, Lactose, Mannitol , Maltose, Sucrose, IMViC Test, Catalase test, Oxidase test, Triple Sugar Iron (TSI) test, Urease test and cultural characteristics by inoculating bacteria on Hichrome
Bacillus Agar. [1]

A. Test for the potent isolates for cellulolytic, proteolytic and amylolytic activity :

The potent pectinolytic isolates were checked for cellulolytic, proteolytic and amylolytic activities by using carboxymethylcellulose (CMC) agar medium, casein agar medium and starch agar medium through getting clearing zones.

Carboxymethylcellulose (CMC) agar medium:-

The CMC agar medium contains 1.0% peptone, 1.0% CMC, 0.2% K_2HPO_4, 1% agar, 0.03% $MgSO_4.7H_2O$, 0.25% $(NH_4)_2SO_4$ and 0.2% gelatin at pH 7[13].

- **Test for cellulolytic activity-** Pure cultures of bacterial isolates were individually transferred onto CMC agar plates. After incubation for 48 hours, CMC agar plates were flooded with 1% congo red and allowed to stand for 15 min at room temperature. One molar NaCl was thoroughly used for counterstaining the plates. Clear zones were appeared around growing bacterial colonies for 48 hours of incubation at 30^0C indicating cellulose hydrolysis[14].

Starch agar medium:-

The Starch agar medium was sterilized in an autoclave, poured in individual Petri dish and allowed to solidify[15].

- **Test for amylolytic activity**: Plates with bacterial colonies were flooded with Gram's iodine reagent (0.01M I_2-KI solution) and observed for zone of degradation of starch as revealed by forming clear and transparent zones. Of the various colonies, the one that exhibits highest degradation of starch was selected for pure culture, physico-chemical characterization and partial purification of the enzyme.

Casein agar medium:-

The plates prepared were inoculated with single colony of each isolate and incubated for 2 days at 37°C. Hydrolysis of milk protein was seen as a zone of clearing around colony.

Isolation of pectinolytic Bacteria:

Pure culture of bacterial isolates were individually transferred onto pectin agar plates. Clear zones were appeared around growing bacterial colonies for 24 hours of incubation at 37^0C indicating pectin hydrolysis.

Production of Pectinase enzyme by Fermentation:

1. **Production of Pectinase using different substrate-**
   Use of Wheat husk as a substrate: The fermentation was done using 250 ml conical flask containing 5gm wheat bran mixed with appropriate amount of mineral salt solution contain 1% KH_2PO_4, 5% NaCl, 0.1% $MgSO_4.7H_2O$, 0.1%$CaCl_2$ and 5% wheat husk, Gram husk and Pigeon pea Husk as a substrate was inoculated with 12 hours old culture. It was inocubated at 37^0C for 24 hours(1 day), 96 hours (4 days), 168 hours (7 days)[16].
2. **Enzyme isolation:**
   After 24, 96 and 168 hours of growth, the production media was filtered through 4 layers of muslin cloth and centrifuged at 8000 rpm for 10 minutes at 4^0C in a refrigerated centrifuge. The supernatant taken as enzyme extract was used to assay the enzyme activity[17].
3. **Assay of Pectinase:**
   Pectinase enzyme was assayed by measuring the reducing sugars released from the action of pectinase on pectin using 3, 5-dinitrosalicylic acid reagent. The reaction mixture consists of 0.8ml of pectin (1% w/v) and 0.2 ml of enzyme in 2ml of sodium acetate buffer (0.1 M, pH 5). The reaction mixture was incubated at 40^0C for 10 min followed by the addition of 1 ml of NaOH and 1 ml of DNS to the tubes to stop the reaction and the tubes were boiled in water bath until colour change is observed. Then the solution is made up to 10 ml using distilled water and the absorbance was read at 540 nm using visible spectrometer and its concentration was determined using galacturonic acid standard curve[18]. The reducing sugars released by enzymatic hydrolysis were determined. A separate blank was set up to correct the non-enzymatic release of sugars.

One unit of enzyme activity is defined as the amount of enzymes required to liberate 1μ mole of reducing sugars per minute with galacturonic acid as standard under the assay conditions.

From graph, the concentration of the reducing sugar liberated by the action of enzyme is determined and the enzyme activity is expressed.

Enzyme activity = μ moles of the product liberated per mole of enzyme per ml minute of Glucose liberated.

$$\text{IU/ml/min enzyme} = \frac{(\mu\text{mol of glucose equivalent released})\ (10)}{(0.2)\ (10)\ (2)}$$

10 = Total volume (in millilitres) of assay [present in numerator]

10 = Time of assay (in minute) as per the Unit Definition [present in denominator]

0.2 = Volume of enzyme (in millilitre) used
2 = Volume (in millilitres) used in Colorimetric Determination[18].

B. Effect of Incubation Time, Temperature and pH:

The isolated bacteria were subjected to different culture condition to derive the optimum conditions for pectinase production. Growth and pectinase production were estimated at time intervals (24, 96 and 168 hours), selected temperatures (30, 37 and 40^{0}C), different substrates (wheat bran, Gram husk and pigeon peas husk) and different pH (4, 6 and 8)[19].

Result and Discussion:

The 4 soil samples were collected from the fruit dumping area of city and this soil was screened for isolation of pectinase producing bacteria.

Isolation of *Bacillus spp.* from soil sample:

On HiCrome Bacillus Agar plates after incubation of 24 hours green colour, light blue (large flat), yellow (mucoid colonies) and pink colour colonies were obtained. The colonies were streaked on HiCrome Bacillus Agar slants and identified on the basis of morphological, biochemical and cultural characteristics. Total 4 types of organisms i.e. *Bacillus spp*.(S1, to S14) were obtained from 4 soil samples (1 to 4).

Identification of *Bacillus spp.:*

A. MORPHOLOGICAL CHARACTERISTICS:

- **Gram staining:** All isolates of *Bacillus species* were gram positive because they showed short purple colour rods in Gram staining.
- **Motility:** Isolates were motile in nature.

Table No. 1: Morphological characteristics of *Bacillus sp.*

Soil Samples	Isolated Bacteria	*Bacillus spp.*	Gram staining	Colour of colonies	Motility
SS1	S1, S3, S7, S9	BS1	Gram positive (rod shaped)	Green colour colonies	Motile
SS2	S2, S5, S8	BS2	Gram positive (rod shaped)	Light blue, large, flat	Motile
SS3	S4, S6, S10, S13	BS3	Gram positive (rod shaped)	Yellow mucoid colonies	Motile
SS4	S11, S12, S14	BS4	Gram positive (rod shaped)	Pink colour colonies	Motile

SS – Soil Sample; S – Isolated Bacteria; BS – *Bacillus spp.*

BIOCHEMICAL CHARACTERISTICS:

The results of biochemical characteristics of the *Bacillus spp*. were given in the table:

Table No. 2 : Biochemical characteristics of *Bacillus sp.*

Soil Samples	Isolated Bacteria	*Bacillus spp.*	Indole	MR	VP	Citrate	Urease	Catalase	TSI
SS1	S1, S3, S7, S9	BS1	-ve	-ve	-ve	-ve	+ve	+ve	-ve
SS2	S2, S5, S8	BS2	-ve	+ve	-ve	+ve	+ve	+ve	-ve
SS3	S4, S6, S10, S13	BS3	-ve	-ve	-ve	-ve	+ve	+ve	-ve
SS4	S11, S12, S14	BS4	-ve	+ve	-ve	+ve	+ve	+ve	-ve

SS – Soil Sample; S – Isolated Bacteria; BS – *Bacillus spp.*

Table No. 3 : Sugar fermentation characteristics of *Bacillus sp.*

Soil Samples	Isolated Bacteria	*Bacillus spp.*	Glucose	Maltose	Lactose	Mannitol	Sucrose
SS1	S1, S3, S7, S9	BS1	A +ve G +ve	A –ve, G –ve	A +ve G+ve	A +ve G +ve	A –ve G –ve
SS2	S2, S5, S8	BS2	A –ve G –ve	A +ve G –ve	A –ve G –ve	A –ve G –ve	A –ve G –ve
SS3	S4, S6, S10, S13	BS3	A +ve G –ve	A +ve G –ve	A –ve G –ve	A +ve G –ve	A –ve G –ve

SS4	S11, S12, S14	BS4	A +ve G –ve	A –ve G –ve	A –ve G –ve	A –ve G –ve	A –ve G –ve

SS – Soil Sample; S – Isolated Bacteria; BS – *Bacillus spp.*

(A= acid, G= gas, +ve = presence, -ve = absence)

B. CULTURL CHARACTERISTICS:

- **HiCrome Bacillus Agar:** Green coloured colonies of BS1, light blue, large and flat colonies of BS2, yellow mucoid colonies of BS3 and pink coloured colonies of BS4 were obtained on HiCrome Bacillus Agar medium.

  Cultural characteristics confirmed that the isolated organisms i.e. BS1, BS2, BS3 and BS4 were *Bacillus subtilis, Bacillus cereus, Bacillus megaterium* and *Bacillus thuringiensis* respectively.

C. Screening for Pectinolytic bacteria:

- **Pectin Agar media:** *Bacillus subtilis, Bacillus cereus, Bacillus megaterium* and *Bacillus thuringiensis* were observed for the zone formation.

  Bacillus cereus and *Bacillus thuringiensis* both the bacterial culture recorded significant quantities of pectinolytic enzyme and formed zone.

D. Test for the potent isolates for cellulolytic, proteolytic and amylolytic activity:

- **Cellulolytic activity:** After adding 1% congo red and 1M NaCl, zone was observed in case of *Bacillus subtilis*. Others *B. cereus, B. megaterium* and *B. thuringiensis* gave negative results for cellulolytic activity.

A Similar kind of results were found in the study of Lori M. Robson[20]. In their study, isolated a group I Bacillus strain, DLG, was isolated and characterized as being most closely related to *Bacillus subtilis*. When grown on any of a variety of sugars, the culture supernatant of this isolate was found to possess cellulolytic activity, as demonstrated by degradation of trinitrophenyl-carboxymethyl cellulose. Growth in medium containing cellobiose or glucose resulted in the greatest production of cellulolytic activity. The cellulolytic activity was not produced until the stationary phase of growth, and the addition of glucose or cellobiose to a culture in this phase had no apparent effect on enzyme production[20].

- **Proteolytic activity:** After 48 hours plates of Casein Agar media was observed for zone formation. Zone were formed by *B.subtilies* and *B.cereus* showing proteolytic activity. *B.megaterium* and *B.thuringiensis* showed negative results.

A I. Aronson *et.al.,* worked on Twenty-nine mutants of *Bacillus cereus* T were selected on casein agar for their inability to produce large amounts of extracellular protease. They all formed spores, and 27 were also auxotrophs for purines or pyrimidines. Upon reversion to prototrophy, a large fraction regained the capacity to produce protease. Conversely, reversion to normal protease production resulted in loss of the purine or pyrimidine requirement in a large fraction of the revertants. One spontaneous low protease- producing pyrimidine auxotroph studied in detail grew as well as the wild type and produced spores which were identical to those produced by the wild type on the basis of heat resistance, dipicolinic acid content, density, and appearance in the electron microscope. The rate of protein turnover in the mutant was the same as the wild type. The mutant did grow poorly, however, when casein was the principal carbon source[21].

- **Amylolytic activity:** After incubation of 24 hours Starch Agar media plates were observed for zone formation. *Bacillus cereus* showed positive result by forming zone. *B.subtilis*, *B.megaterium* and *B.thuringiensis* gave negative results for amylolytic activity.

A. PRODUCTION OF PECTINASE BY USING PECTINOLYTIC BACTERIA:

Two pectinolytic bacteria were used i.e. *B. cereus* and *B. thuringiensis.* Three types of substrate were used (Wheat husk, Gram husk and Pigeon peas husk) for production of pectinase enzyme. *B. cereus* and *B. thuringiensis* were treated with three different substrates i.e. wheat husk, gram husk and pigeon peas husk respectively to check the maximum production of pectinase enzyme. After extraction and pectinase assay absorbance were recorded at 540 nm.

Table No. 4 : Observation table of Glucose as standard.

Sr. No.	Test tube	Absorbance (nm)	Concentration of Glucose (µg/ml)
1.	Blank	0.00	0.00
2.	1	0.05	1000
3.	2	0.1	2000
4.	3	0.16	3000

5.	4	0.20	4000
6.	5	0.21	5000

Table No. 5 : Pectinase activity produced by *B. cereus* using different substrate at different incubation time.

Sr. No.	Substrate	O.D. of 1st day	O.D of 4nd day	O.D of 7th day
1.	Wheat bran (E_1)	0.17	0.21	0.39
2.	Gram husk (E_2)	0.13	0.36	0.38
3.	Pigeon peas husk (E_3)	0.15	0.36	0.40

Table No. 6 : Pectinase activity produced by *B. thuringiensis* using different substrate at different incubation time.

Sr. No.	Substrate	O.D of 1st day	O.D of 4nd day	O.D of 7th day
1.	Wheat bran (E_1)	0.19	0.39	0.41
2.	Gram husk (E_2)	0.15	0.36	0.37
3.	Pigeon peas husk ($E_{3)}$	0.11	0.34	0.36

From above result, *B. cereus* and *B. thuringiensis* recorded maximum pectinolytic activity with Wheat bran as a substrate. Pectinase produced by *B. cereus* and *B. thuringiensis* showed an increase in activity with increase in incubation time. The results confirmed the finding of Nithisha K. T. *et al*.,[18]

Table No. 7 : Pectinase activity produced by *B. cereus* and *B. thuringiensis* using wheat bran as a substrate at different temperature.

Sr. No	Substrate	30^{0}C	37^{0}C	40^{0}C
1.	Wheat bran (E_1)	0.11	0.23	0.13

The maximum pectinase activity had shown by *B.cereus* and *B. thuringiensis* at temperature 37^{0}C.
The result of the pectinase activity at temperature 37^{0}C is in the confirmation with the finding of K. Sridevi *et al*., [16].

Table No. 7 : Pectinase activity produced by *B. thuringiensis* using wheat bran as a substrate at different pH.

Sr. No.	Substrate	4	6	8
1.	Wheat bran (E_1)	0.30	0.36	0.35

Pectinase produced by *B. thuringiensis* had an optimum pH of 6. The activity exhibited a steady increase as the pH increase from 4 to 6. Further increase in pH led to decrease in the activity and it remained constant. This shows that the pectinase enzyme can be produced by maintaining the pH of 6.

Table No. 8 : Pectinase activity produced by *B.cereus* and *B. thuringiensis* using three substrates

Sr. No.	Bacteria	Substrates	Enzyme Concentration (in µg/ml)
1.	***B.cereus***	Wheat bran (E_1)	108.32
		Gram husk (E_2)	105.00
		Pigeon peas husk (E_3)	111.10
2.	***B. thuringiensis***	Wheat bran (E_1)	113.87
		Gram husk (E_2)	102.77
		Pigeon peas husk (E_3)	100.00

Out of three substrate, all three substrates were useful for pectinase production. The four bacteria were isolated from soil samples were *Bacillus subtilis, Bacillus cereus, Bacillus megaterium* and *Bacillus thuringiensis.* From these four bacteria, *Bacillus cereus* and *Bacillus thuringiensis* showed highest potential to produce pectinase enzyme. *Bacillus cereus* produced highest pectinase enzyme 111.10 µg/ml from pigeon peas husk. *Bacillus megaterium* produced highest pectinase enzyme 113.87 µg/ml from Wheat bran. The best substrate for pectinase enzyme was Wheat bran. From this result it was concluded that *Bacillus megaterium* produced highest pectinase enzyme from Wheat bran.

The result of the effects of pH on pectinase activity confirmed the finding of Nithisha K. T. *et al*.,[18] studied on isolation of bacteria from soil sample and screened for the production and characterization of pectinase. The bacterial isolate was identified as *Bacillus sp.* The optimum temperature and pH for enzyme activity was found to be 35^{0}C, pH-6.0 respectively.

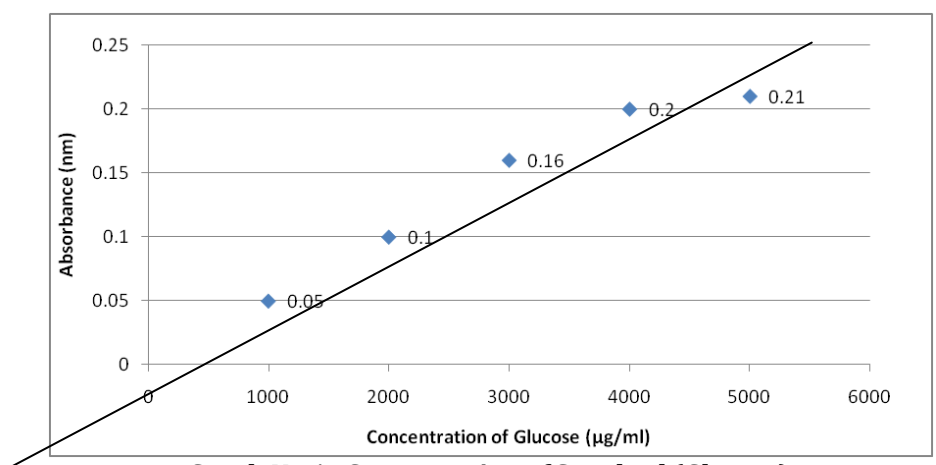
The result of the temperature in the present study is also in the confirmation with the findings of B. Lalitha Kumari *et. al*. When *Bacillus subtilis* was seeded into flasks containing both dry orange and banana peel powder as a substrate with optimum temperature 37^{0}C and pH 7 constant, pectinase activity was increased from 0.5% to 1.5%[22].

The results of the isolates for temperature and pH confirmed the findings of Menka Gauthwal *et. al.,* worked on Pectinolytic enzymes play an important role in fruit processing industries by degrading fruit pulp pectin and thus increasing yield and production of juice. A pectinase has been produced from newly isolated strain *Bacillus cereus* under submerged fermentation at pH6 and temperature 37^{0}C[23].

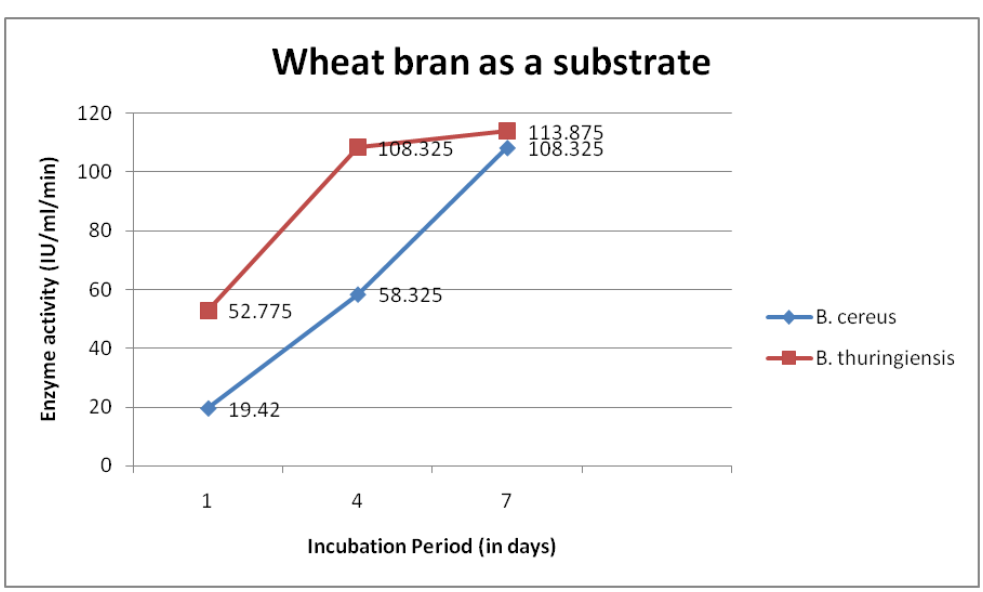
A. Rasheedha Banu *et. al.,* worked on ten moulds isolated from municipal waste soil were screened for pectinolytic enzyme production when grown on pectin containing (YPSS) solid media. *Penicillium chrysogenum* was selected based on clearance zones. Enzyme production was higher at temperature of 35^{0}C but pH was 6.5[24].

Conclusion :

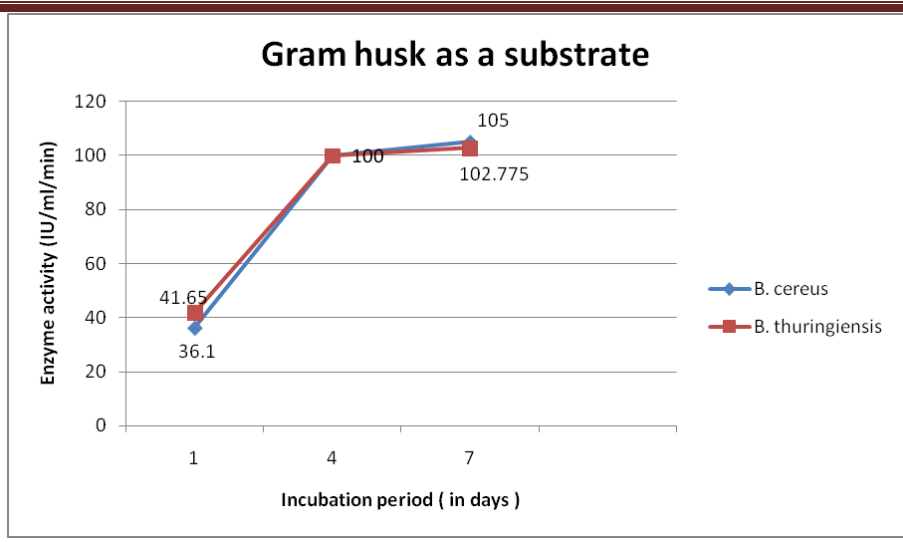
In conclusion, the soil of fruit waste dumping was found to be rich source for the isolation of pectinase producers. Screening of pectinase producing microbial strain from farm soil samples of Nagpur region may help to supplement the increasing requirement of pectinase enzyme by the industries. The identification shows that the more number of isolates were belong to *Bacillus* spp. Further studies on molecular characterization of four maximum pectinase producing bacterial isolates from the study may be helpful for the production of industrially important enzyme in near future.



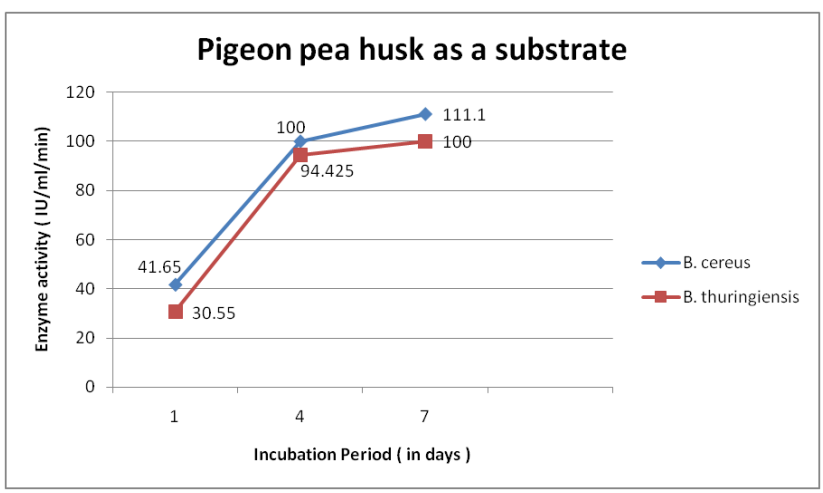
Graph No.1: Concentration of Standard (Glucose)



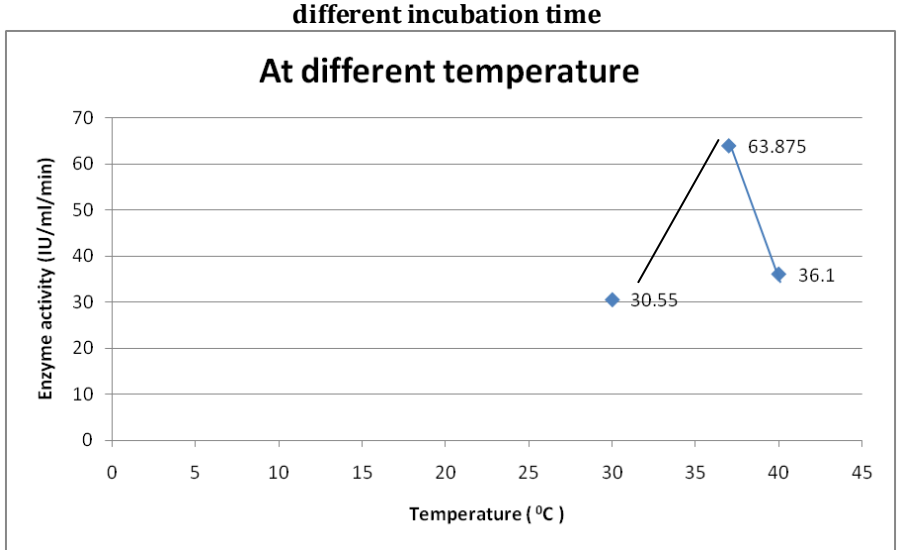
Graph No.2: Enzyme activity of *B. cereus* and *B. thuringiensis* using Wheat bran as a substrate at different incubation period.



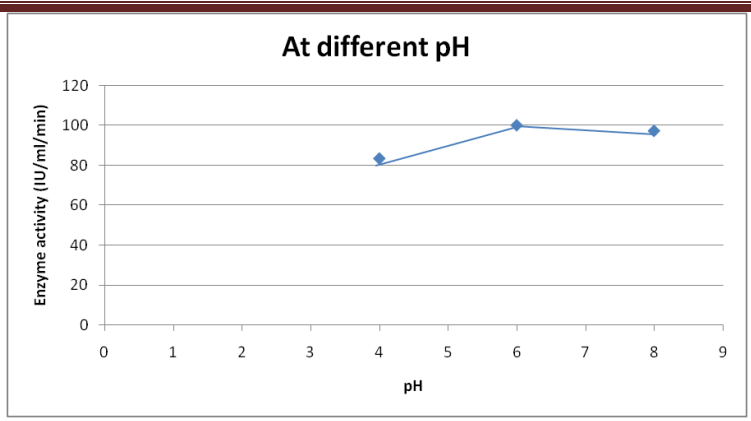
Graph No.3: Enzyme activity of *B. cereus* and *B. thuringiensis* using Gram husk as a substrate at different incubation period.



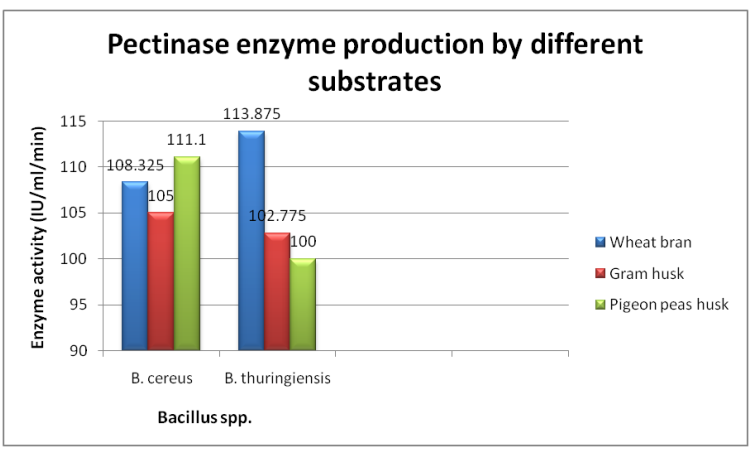
Graph No.4: Enzyme activity of *B. cereus* and *B. thuringiensis* using Pigeon pea husk as a substrate at different incubation time



Graph No.5: Enzyme activity by *B. thuringiensis* using wheat bran as a substrate at different temperature.



Graph No.6: Enzyme activity by *B. thuringiensis* using wheat bran as a substrate at different pH.



Graph No.7: Pectinase production by *B. cereus* and *B. thuringiensis* using 1) Wheat bran, 2) Gram husk, and 3) Pigeon peas husk.

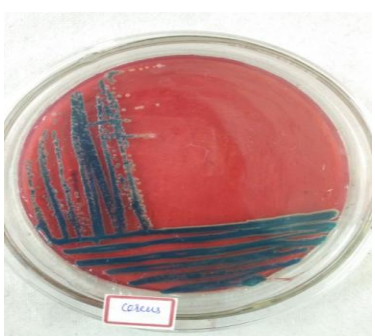


Figure No.1 : Cultural characteristics of *B. cereus* on HBA

Figure No.2: Cultural characteristics of *B. megaterium* on HBA

Figure No.22: Pectinolytic activity of *B. cereus* media

Figure No.23. Pectinolytic activity of on Pectin *B. thuringiensis* on Pectin media

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