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Effect of Various Factors (Temperature, pH, Nacl Conc., Carbon, Nitrogen and Amino Acids) on Growth of Pectinolytic Bacteria Isolated and Screened from Mango Fruit Yards

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ABSTRACT

Mango occupies a prominent place amongst the various fruits grown in India because of its great utility and health benefits and is acknowledged as the king of fruits. One of the agro-wastes currently causing pollution problems is the mango peel from fruit processing industries Mango peel is rich in carbohydrate, protein and pectin. Pectin is a major component peel of mango fruit. The degradation of mango peel by the bacterial populations is highly significant. The action of different extracellular enzymes on mango peel leads to the biodegradation of pectin. The type of enzymes is known as pectinolytic enzymes or pectinases which hydrolyses pectic substances readily. The isolates of B.megaterium were collected from different mango fruit processing industries around Tirupati, Chittoor district of Andhra Pradesh and it was isolated and screened on CPA medium under laboratory conditions. Effect of different pH levels, temperature, NaCl concentrations and effect of carbon, nitrogen and amino acids were tested against the growth of B.megaterium under in vitro. Results indicated that the growth of B. megaterium was maximum at pH range of 6.50-7.00 and temperature range of 35-40°C and NaCl concentration of 0.5 to 1%. And fructose and galactose as carbon sources, casein and peptone as nitrogen sources, phenyl alanine and histidine as the amino acids were observed as optimum for the growth of B. Megaterium respectively. Therefore, the main objective of the present study was to isolate, screen and to study the effect of various factors on pectinolytic activities of B. Megaterium from soils of decomposed fruit waste from mango processing industries.

Key words: *Pectin, Mango peel, Pectinolytic enzymes or Pectinases, Temperature, pH, NaCl concentration, Carbon sources, Nitrogen sources, Amino acid sources.*

INTRODUCTION

It is a matter of astonishment to many that mango (*Mangifera indica* L.), one of the most celebrated fruits of tropical part of India, is a member of the family Anacardiaceae, which is notorious for embracing a number of highly poisonous plants.

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It has rich luscious, aromatic flavor and a delicious taste in which sweetness and acidity is delightfully blended. Mango production has experienced continuous growth in the last decades of the twentieth century[2]. India with a production of 125.4 lakh tonnes of mango accounts for 40 per cent of total world production (312.5 lakh tonnes). Among the various states of India, Andhra Pradesh ranks first both in area and production and is followed by Uttar Pradesh. The productivity of mango in Andhra Pradesh is 8.1 tonnes per hectare and is higher than the country's average productivity of 6.1 tonnes per hectare. The waste produced from the mango processing industries is becoming one of the major agro-waste causing pollution. Mango peel is a major by-product during processing of mango fruit into pulp which is rich in carbohydrate, protein and pectin. Pectin, a major component peel of these fruit, is a polymer of galacturonic acid residues connected by α-1, 4 glycosidic linkages[12]. The degradation of organic wastes by the bacterial populations is highly significant. It reduces the time span of degradation and produces no foul odour[15]. Many microorganisms viz.., bacteria, yeast, fungi could produce pectinases[17]. Pectin is hydrolysed by pectinase enzymes produced extracellularly by micro flora available in our natural environment[3]. With the help of the pectinase enzyme, micro-organisms can convert fruit wastes into sugars which can be used for food and value added products[10]. These micro-organisms can also be exploited for production of pectinase which is an industrially important enzyme and have potential applications in fruit, paper, textile, coffee and tea fermentation industries. In order to isolate a potential pectinase producer the microbial populations have to be screened. Because of the ever growing cost for energy enzymatic technologies will stay in focus of science and technology, and its relevance will increase significantly in the future[9]. Hence, the present investigation was to study the pectinolytic activities and effect of various factors on the growth of *Bacillus megaterium* isolated from soils of decomposed fruit waste from mango processing industries in Chittoor district, Andhra Pradesh, India.

MATERIAL AND METHODS

Isolation of Pectinolytic bacteria:

Collection of soil samples from mango fruit processing industries:

Soil samples were collected from 12 different locations at a depth of 15cm from fruit waste dumping yards of Vinsari and Varsha mango fruit processing industries around Tirupati, Chittoor district of Andhra Pradesh[16]. The soil samples were transported to the laboratory in sterile polythene bags, air-dried and mixed thoroughly to make a composite sample[1].

Serial dilution and plating technique:

Soil sample was serially diluted and plated according to the method of Aneja[1]. One gm of soil sample was serially diluted and 0.1 ml of 10^{-1} to 10^{-8} dilutions and plated in duplications on to Nutrient agar (NA) medium plates and incubated at 37^{o}C for 24 hours. Replicates were maintained for each dilution. Potential isolates were achieved at mesophilic temperatures ranging from 30 ^{o}C to 37^{o}C.

Screening of pectinolytic bacteria from fruit waste:

Citrus Pectin agar medium (g/L) preparation:

Isolates were streaked on 1% citrus pectin Agar with 67% of metoxilation, 0.14% $(NH_4)_2SO_4$, 0.2% K_2HPO_4, 0.02% $MgSO_4.7H_2O$ and 0.10% nutrient solution (5mg /L $FeSO_4.7H_2O$, 1.6 mg/L $MnSO_4$, H_2O, 1.4 mg/L $ZnSO_4.7H_2O$, 2.0mg/L $CoCl_2$ with pH 6.0. All morphological colonies were purified by repeated streaking.

Plate assay of depolymerized pectin:

The medium was the same used for isolation of cultures, supplemented with 2% agar agar. Pure cultures were inoculated by making puncture in the medium and incubated for 48h at 30^{o}C. After the colonies reached around 3 mm, iodine –potassium iodide solution (1.0 gm Iodine, 5g of KI and 330 mL H_2O) was added to detect clearance zones[6].

Identification and characterization of efficient pectinolytic bacterial isolates:

Identification of the bacterial isolates was carried out by determining morphological,

cultural and biochemical characteristics. It was done according to the Bergey's Manual of Systemic Bacteriology[13,11] and with assistance of IMTECH, Chandigarh.

Morphological characterization:

Identification of the isolates was carried out by studying the morphological characters after incubating nutrient agar plates for 24-48 hours at 37°C. The colony morphology, pigment production and its diffusibility on the nutrient agar were observed and isolated colonies were selected for staining techniques like Gram's staining, hanging drop technique (for motility) and Spore staining were recorded[4,5].

Biochemical characterization:

Further characterization of the isolates was carried out by performing IMVIC tests adopting standard protocols as described in Mackie and McCartney[11].

Sugar utilization test:

The ability of the isolates to ferment different carbohydrates was tested. Different carbohydrates used in the test were monosaccharides like glucose, galactose, fructose and arabinose; disaccharides like lactose, sucrose and maltose; polysaccharides like soluble starch and glycogen. Peptone water was supplemented with 0.5% of each sugar Andrade's indicator (0.005%) was added to the medium and Durham tubes were inserted into the medium tubes. Medium was sterilized at 10 lbs pressure for 10 minutes. The tubes were inoculated with the isolates and incubated at 37°C for 24-48 hours and acid/gas production was recorded.

IMVIC tests:

Indole test:

The ability of the isolates to convert the amino acid Tryptophan to indole was tested by adding 0.5ml of Kovac's reagent to 48 hrs peptone broth tubes containing culture. The production of red color in the alcohol layer indicates a positive indole reaction.

Methyl Red (MR) test:

The ability of the isolates to ferment glucose and production of acid was determined using Glucose Phosphate Peptone (GPP) medium. 2-3 drops of methyl red indicator was added to 48 hrs culture tubes and results were recorded immediately. Appearance of bright red color in the medium indicated a positive reaction. *E.coli* and *K.pneumoniae* were used as positive and negative controls respectively for MR and VP tests.

Voges – Proskauer (VP) test:

The ability of the isolates to produce Acetoin as the end product was detected by adding 5% solution of α-napthol in ethanol and 0.2 ml of 40% KOH to 1 ml of GPP broth cultures of all the isolates incubated at 37°C for 48 hrs. The development of eosin pink color indicates a positive reaction.

Citrate utilization test:

The ability of the organism to utilize citrate as the sole carbon and energy source for growth and an ammonium salt as the sole source of nitrogen was determined by streaking isolates on Simmon's citrate agar slant (pH 6.8) and incubating for 24 hrs at 37°C. A positive test indicates change in the color of the medium from green to blue and appearance of a streak of growth. *K.pneumoniae* and *E.coli* were used as positive and negative controls.

Effect of various factors on the growth of isolate:

The effect of various factors on the growth of the isolates was carried by studying the growth at different temperatures, pH, salt concentrations, nitrogen, carbon sources and different amino acids sources.

Growth at different temperatures:

The effect of temperature on growth of the isolate was studied. Different temperatures maintained for the growth of isolate were 35°C, 45°C, 55°C, 65°C and 75°C. Nutrient broth tubes were inoculated with 0.1ml of overnight cultures of each isolate and incubated at different temperatures for 48 hours. The tubes were observed for visible turbidity after incubation and the optimum temperature for the growth of each isolate was recorded.

Growth at different pH:

The effect of pH on the growth of the isolate was studied as per the method followed by Grant and Tindall[7] using CPA medium. Different pH levels *viz.,* 2.0, 4.0, 6.0, 8.0, 10.0, and 12.0 were used. The pH levels of the

medium were adjusted in a digital pH meter using 0.1N Hydrochloric acid and 0.1N Sodium hydroxide. The media with different pH levels were sterilized, cooled and poured in the sterilized Petri plates in 20 ml quantities and allowed to solidify which are inoculated with the isolate and incubated as above and observed for the growth at different pH range and optimum pH of growth of bacterial isolates were recorded.

Growth at different salt concentration:

The ability of the bacterial isolates to grow at different salt concentrations between 1-12% was studied[8]. Nutrient broth tubes with respective salt concentrations were prepared and inoculated with 0.1 mL overnight culture of each of the isolates and incubated at 37^{o}C for 48 hours and observed for visible turbidity. The ability of organisms to grow under different salt concentration was recorded.

Different carbon sources:

The effect of nitrogen sources on growth of the isolate was studied. Production medium was supplemented with different carbon sources like glucose, sucrose, starch, lactose, maltose, galactose, fructose, cellulose and pectin were introduced into the pectin medium at an equimolecular amount located at 1% (W/V) glucose were prepared and inoculated with 0.1 mL overnight culture of each of the isolates and incubated at 37^{o}C for 48 hours and observed for visible turbidity. The ability of organisms to grow under different carbon sources was recorded.

Different nitrogen sources:

The effect of nitrogen sources on growth of the isolate was studied. Production medium was supplemented with different nitrogen sources at an equimolar of nitrogen that present in diammonium sulphate in pectin medium. Nutrient broth tubes with respective nitrogen sources like ammonium molybdate, ammonium chloride, ammonium oxalate, ammonium nitrate, diammonium hydrogen phosphate, potassium nitrate, gelatin, peptone, casein and urea were prepared and inoculated with 0.1 mL overnight culture of each of the isolates and incubated at 37^{o}C for 48 hours and observed for visible turbidity. The ability of organisms to grow under different nitrogen sources was recorded.

Different amino acid sources:

The effect of different amino acids on growth of the isolate was studied. Production medium was supplemented with different amino like glycine, lysine, cysteine, tryptophan, methionine, phenylalanine, histidine, alanine and aspartic acid etc. were prepared and inoculated with 0.1 mL overnight culture of each of the isolates and incubated at 37^{o}C for 48 hours and observed for visible turbidity. The ability of organisms to grow under different amino acid sources was recorded. The production medium for most potent isolates was prepared.

RESULTS

Collection of soil sample from the mango fruit processing industries:

Mango fruit compost samples were collected from Vinsari and Varsha fruit processing industries around Tirupati, Chittoor district, Andhra Pradesh. Samples of required quantity were taken according to the standard methods. The fruit waste was collected after peeling and crushing operations, only after which they lose their utility. Rotten fruits were also included in the collection. Before preparation of the composite sample, hand sorting was emphasized to segregate and to remove unwanted materials like straw, plastic covers, packaging materials etc.

Isolation of Bacteria from the soil sample of Mango Fruit processing Industries:

Bacteria were isolated from the compost sample collected from mango fruit processing industrial area by serial dilution and plating techniques. Sample was inoculated into Nutrient Agar medium and incubated for 48 hours at 37^{o}C. The pectinolytic property of all the 16 isolates was studied by puncturing the medium on Citrus pectin agar (CPA) medium, a quantitative test for pectin degrading bacteria.

Screening of the isolates for pectin degrading bacteria:

One of the efficient bacterial isolate is selected according to their highest pectinolytic activity on the basis of their growth and formation of clearing zones on Citrus Pectin Agar (CPA) medium by using iodine-potassium Iodide solution (I-KI solution). The 16 bacterial isolates were able to grown on medium containing citrus pectin as a source of carbon. The strain is tested for pectin hydrolysis by plate assay, at pH 6.0. The strain is classified as very good producer of pectin depolymerising enzymes when showed clear zones around colonies of 15 mm when the zones were at least 10 mm weak producers when the zones were at least 5 mm and poor producers when no pectinolytic activity and no clear zones were observed. Good pectinolytic activity was exhibited by the isolate. The isolate is designated and numbered as 'mango pectinolytic bacteria' as mpb2. Based on the morphological, biochemical and physiological tests, one of the isolate was initially identified as *Bacillus sp.* It was characterized according to the guidelines of Bergey's Manual of Systemic Bacteriology[13] and Manual of Medical Microbiology[11].

Identification of selected bacterial isolates:

At all stages of growth, the cells were found as Gram positive Bacilli. Carbon, Nitrogen utilization pattern, morphological and other biochemical tests were performed according to standard methods of the bacteria.

B.megaterium is Gram positive, motile, endospore producing, aerotolerant and non-lactose fermentor. It is a Catalase positive, Oxidase positive, Urease positive organism. It can ferment sugars like Dextrose, Fructose, Millibiose, Mannitol, Raffinose, Mannose, Trehalose and Ionositol. Biochemical tests like Indole negative, Methyl Red negative, Voges-proskauer test negative, Citrate positive, Nitrate Reductase positive, H_2S production negative. It can hydrolyze cellulose, starch, pectin, Gelatin, Arginine, Tween 60, casein and Urea (Fig below).

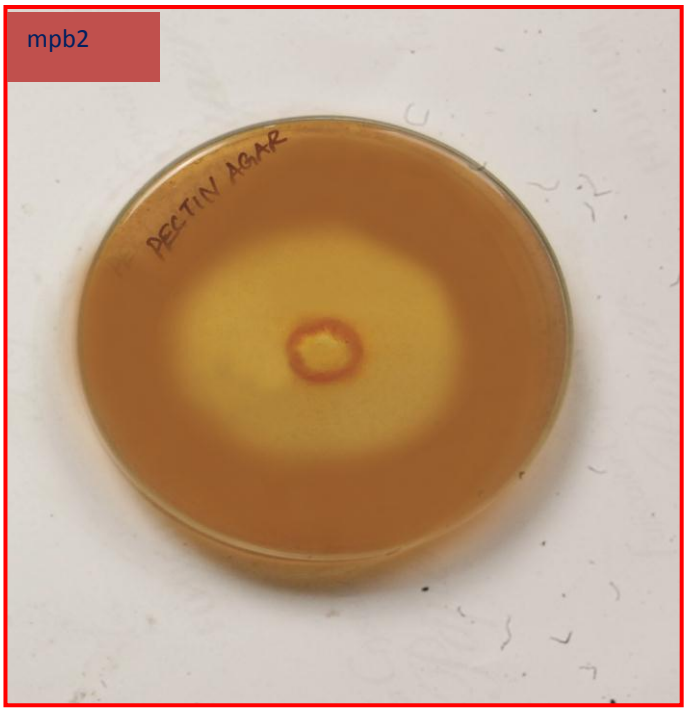


Fig. 1: Screening for pectinolytic activity of mango pectinolytic bacteria (mpb2) using Citrus Pectin Agar

Characterization of the isolates:

Based on the morphological, biochemical and physiological tests performed the mpb was initially identified in the laboratory as *Bacillus sp.*, which were further confirmed at species level as *Bacillus megaterium* MTCC 10773 (mpb2) by the characterization with the assistance of IMTECH, Chandigarh, India.

Table 1: Morphological, physiological and biochemical characteristics of and *Bacillus megaterium*

S.No.	Characteristics	*B.megaterium*
Morphological Characteristics -		
Colony morphology		
1.	Configuration	Irregular
2.	Margin	Undulate
3.	Elevation	Flat
4.	Surface	Smooth
5.	Colony color	Cream
6.	Opacity	Opaque
7.	Gram's reaction	+ve
8.	Cell shape	Long-rods
9.	Spore	+
10.	Endospore	+
11.	Position	Central
12.	Spore Shape	Oval
13.	Sporangia bulging	Non-bulging
14.	Motility	Motile
15.	Fluorescence	-
Biochemical Test - Degradation/Hydrolysis of		
1.	Casein	+
2.	Cellulose	+
3.	Starch	+
4.	Gelatin	+
5.	Tween 60	-
6.	Urea	+
7.	Esculin	+
Enzymatic activity - Enzyme		
1)	Catalase activity	+
2)	Oxidase activity	+
3)	Urease activity	+
Biochemical tests		
IMVIC Tests		
1.	Indole test	-
2.	Methyl Red test	-
3.	Voges-proskauer test	-
4.	Citrate Utilization	+
5.	Hydrogen sulphide Test	-
6.	Nitrate reduction	+
7.	Arginine dihydrolase	+

Identification of *Bacillus megaterium:*

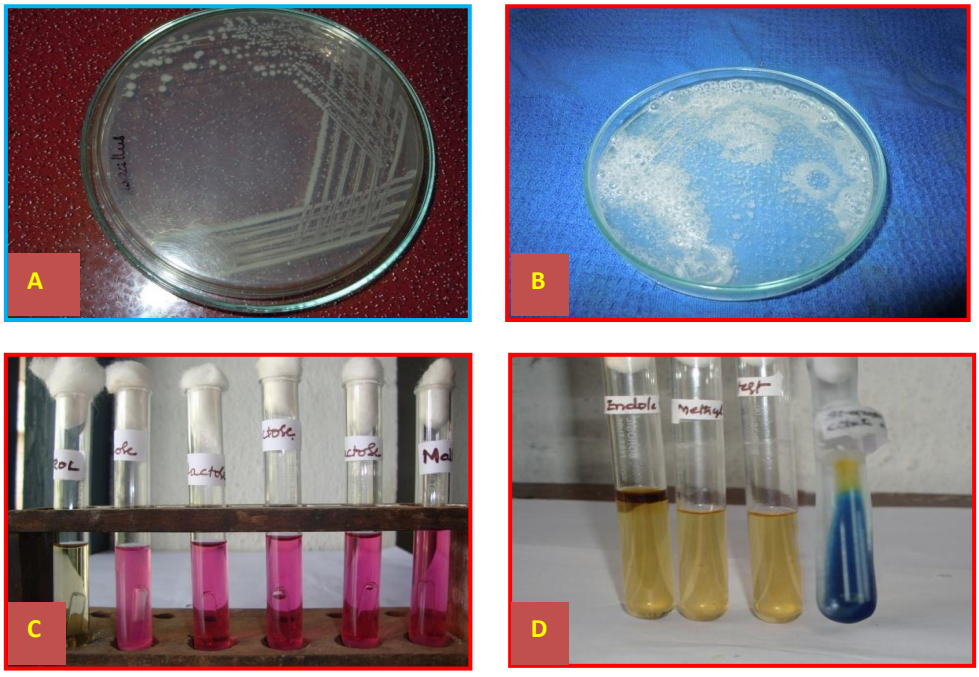


Fig. 2: A) Colony morphology; B) Catalase production; C) Sugar fermentation test D) Biochemical (IMVIC) tests

Effect of physiological conditions like NaCl, Temperature, pH, Carbon and Nitrogen sources influencing the growth of organism:

Effect of physiological conditions like Temperature, pH, NaCl concentration, carbon, nitrogen and amino acid sources was studied to determine the optimum growth conditions of the organism. A temperature of 35°C, pH 6.0, NaCl concentration of 0.5 to 1% and, fructose and galactose as carbon sources for *Bacillus megaterium.* Casein and peptone as nitrogen sources, phenyl alanine and histidine as the amino acids were observed as optimum for the growth of *B. megaterium* isolates. The growth was measured by turbidity by taking O.D values at 660 nm.

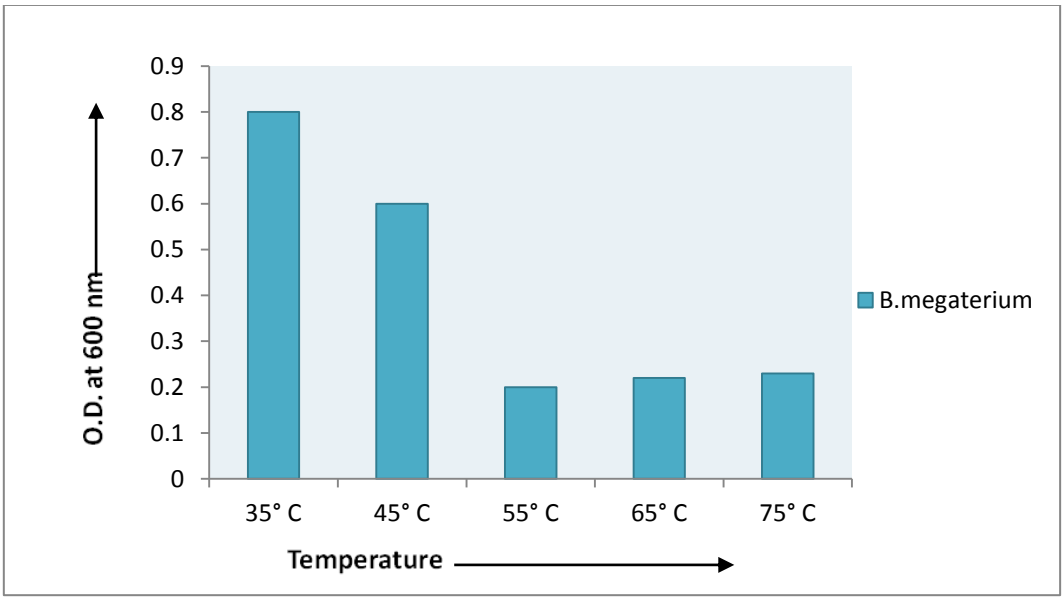
Effect of temperature on growth:

Effect of different temperatures on the growth of *B. megaterium* was studied. Incubation temperature had influenced the metabolic reactions through enzymatic activities which had effected the growth of organism *B. megaterium* produced maximum growth when incubated at 35°C and minimum growth at 75°C.

Table 2: Effect of temperature on growth of *Bacillus megaterium* Summary of One-way ANOVA and Duncan's Multiple Range Test

Organism	Temperature	N	Mean	Std. Deviation	F-value	p-value
B. megaterium	35° C	3	.8000 a	.02000	777.825**	0.000
	45° C	3	.6200 b	.02000		
	55° C	3	.2167 c	.01528		
	65° C	3	.2267 c	.02517		
	75° C	3	.2333 c	.01528		
	Total	**15**	**.4393**	**.29107**		

** Significant at 1% level – 0.01



Graph-1: Effect of temperature on growth of *Bacillus megaterium*

One way ANOVA was carried out to know whether temperature has any impact on the growth. Results are noted in Table 1. From the F-Value it can be observed that there is significant impact of temperature on growth of *B. megaterium*. The optimum temperature for the growth of *B. megaterium* is 35°C. Since the maximum growth of organism is noted highly as 0.92 at this temperature.

Further, Duncan's Multiple Range Test reveals that at 55 °C, 65 °C and 75 °C same level of growth was noted and differed from the growth at other temperatures.

Effect of pH on growth:

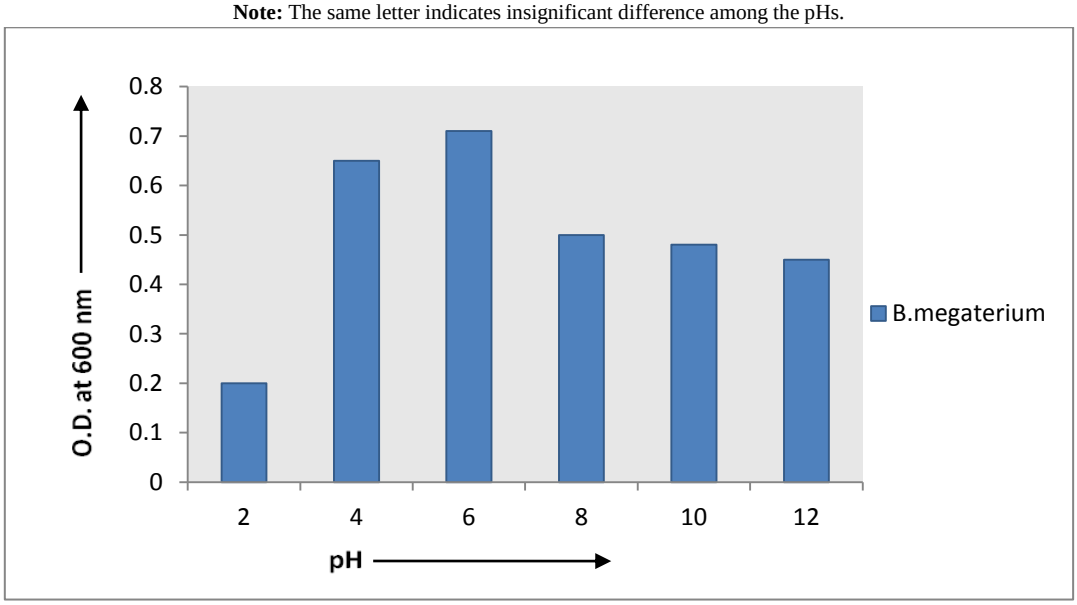
Effect of different pH concentrations on the growth of *B. megaterium* was studied. pH of the medium played an important role in the growth and metabolism of the organism *B. megaterium* produced maximum growth when incubated in pH 6.0 and minimum growth in pH 2.0.

Table 3: Effect of pH on growth of *Bacillus megaterium* Summary of One-way ANOVA and Duncan's Multiple Range Test

Organism	pH	N	Mean	Std. Deviation	F-value	p-value
B. megaterium	2	3	.197 a	.0153	350.241**	0.000
	4	3	.667 b	.0153		
	6	3	.737 c	.0153		
	8	3	.523 d	.0153		
	10	3	.503 d	.0208		
	12	3	.477 e	.0208		
	Total	**18**	**.517**	**.1764**		

** Significant at 1% level – 0.01

Note: The same letter indicates insignificant difference among the pHs.



Graph-2: Effect of pH on growth of *B.megaterium*

From the results of ANOVA it can be observed that pH has impact on the growth of B.megaterium and was showing significant difference at 1% level among the different pH (Table 3). Since P value 0.000 < 0.01 for the corresponding F- values were significant. pH 6 is the optimum pH for the growth of the organism *B. megaterium*

Further, Duncan's Multiple Range Test reveals that there is same level of growth was observed at pH 8 and 12 in *B. megaterium*.

Effect of NaCl Concentration on the growth:

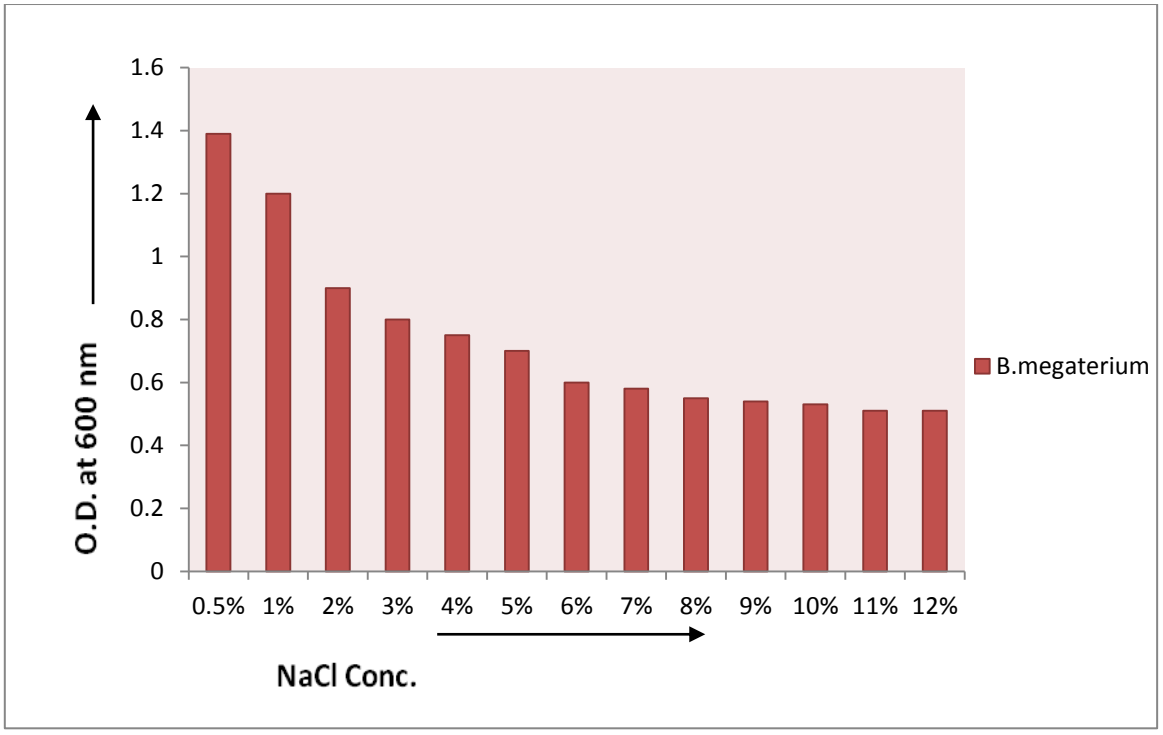
Growth of *B.megaterium* was studied in CP broth with a range of 0.5% to 12% NaCl concentrations. NaCl concentration of 0.5% to 1% was found to be the optimum for growth and a decrease in the growth was observed from 3 % NaCl onwards in the organism *B.megaterium* (Graph-2).

Table 4: Effect of NaCl Concentration on the growth of *B.megaterium* Summary of One-way ANOVA and Duncan's Multiple Range Test

Organism	NaCl	N	Mean	Std. Deviation	F-value	p-value
B. megaterium	0.5%	3	1.367 a	.1528	32.538**	0.000
	1%	3	1.167 b	.2082		
	2%	3	.933 c	.1528		
	3%	3	.807 d	.0643		
	4%	3	.777 d	.0153		
	5%	3	.737 d	.0153		
	6%	3	.637 e	.0153		
	7%	3	.580 e	.0100		
	8%	3	.547 e	.0321		
	9%	3	.533 e	.0231		
	10%	3	.500 e	.0100		
	11%	3	.437 f	.0153		
	12%	3	.433 f	.0252		
	Total	**39**	**.727**	**.2866**		

** Significant at 1% level

Note: The same letter indicates insignificant difference among the various NaCl concentrations.



Graph-3: Effect of NaCl concentration on growth of *B.megaterium*

The growth of *B.megaterium* was showing a significant difference at 1% level among the various NaCl concentrations since P value 0.000< 0.01 for the corresponding F- Value (32.538**).

Based on DMRT analysis the influence of different NaCl concentrations with respect to the growth of *B.megaterium* were significant. However *B. megaterium* has luxuriantly grown up to 12% NaCl concentration.

Effect of carbon sources on growth

Effect of different carbon sources on the growth of *B. megaterium* was studied. Nine different carbon sources, like – glucose, sucrose, starch, lactose, maltose, galactose, fructose, cellulose and pectin were amended in

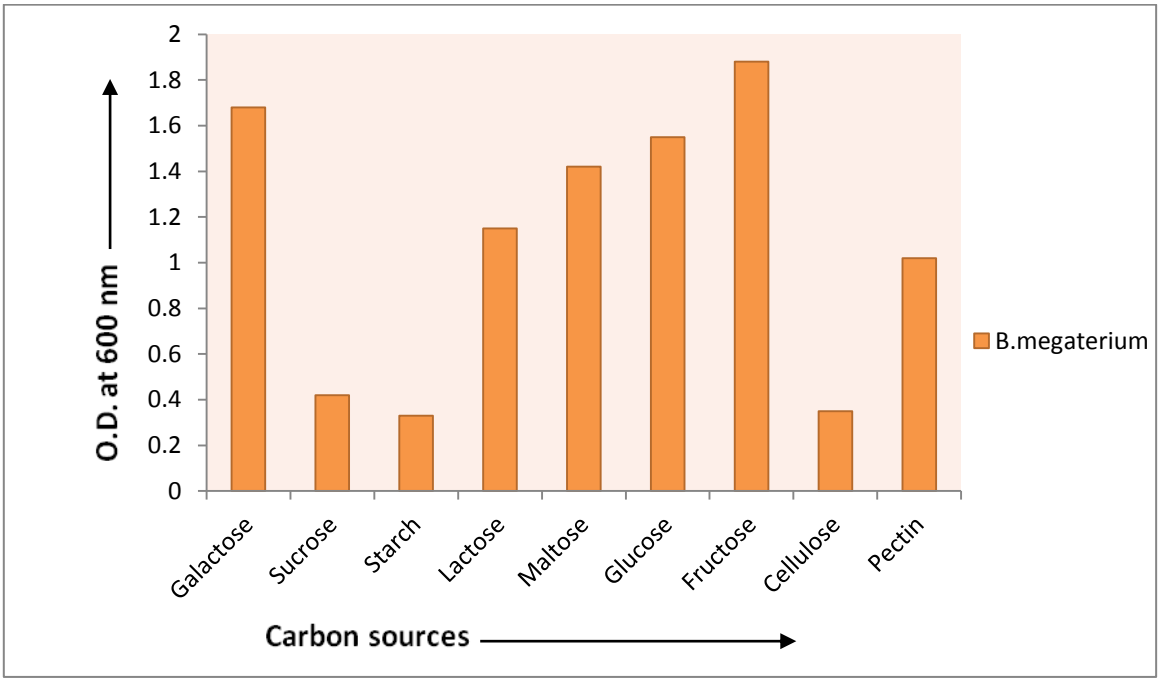
CP medium to find out suitable carbon source for optimum growth of the organism (Table 5). Fructose followed by galactose was found to be the best carbon sources for the maximum growth of *B.megaterium* when compared to the other carbon sources and minimum growth in starch (Graph 4).

Table 5: Effect of carbon sources on growth of *B.megaterium* Summary of One-way ANOVA and Duncan's Multiple Range Test

Organism	Carbon sources	N	Mean	Std. Deviation	F-value	P-value
B.megaterium	Galactose	3	1.6833 a	.02082	1294.256**	0.000
	Sucrose	3	0.4167 b	.02082		
	Starch	3	0.3300 c	.02000		
	Lactose	3	1.1500 d	.01000		
	Maltose	3	1.4233 e	.02517		
	Glucose	3	1.5533 f	.01528		
	Fructose	3	1.8833 g	.01528		
	Cellulose	3	0.3533 c	.02517		
	Pectin	3	1.0233 h	.06658		
	Total	**27**	**1.0907**	**.57785**		

** Significant at 1% level

Note: The same letter indicates insignificant difference among the various carbon sources



Graph-4: Effect of carbon sources on growth of *B.megaterium*

The growth of *B. megaterium* isolate was showing a significant difference at 1% level among the carbon sources since P value 0.000 < 0.01 for the corresponding F- Value (1294.256**).

Based on DMRT analysis the influence of different carbon sources with respect to the growth of *B.megaterium* was significant. The result reveals that the same alphabet (a, b, c, d) beside mean values suggests insignificant difference among the O.D values for respective carbon sources.

Effect of nitrogen sources on growth:

Effect of different nitrogen sources on the growth of *B. megaterium* was studied. Provision of utilizable nitrogen source to organisms was the basic requirement for the optimum growth; hence the CP medium was

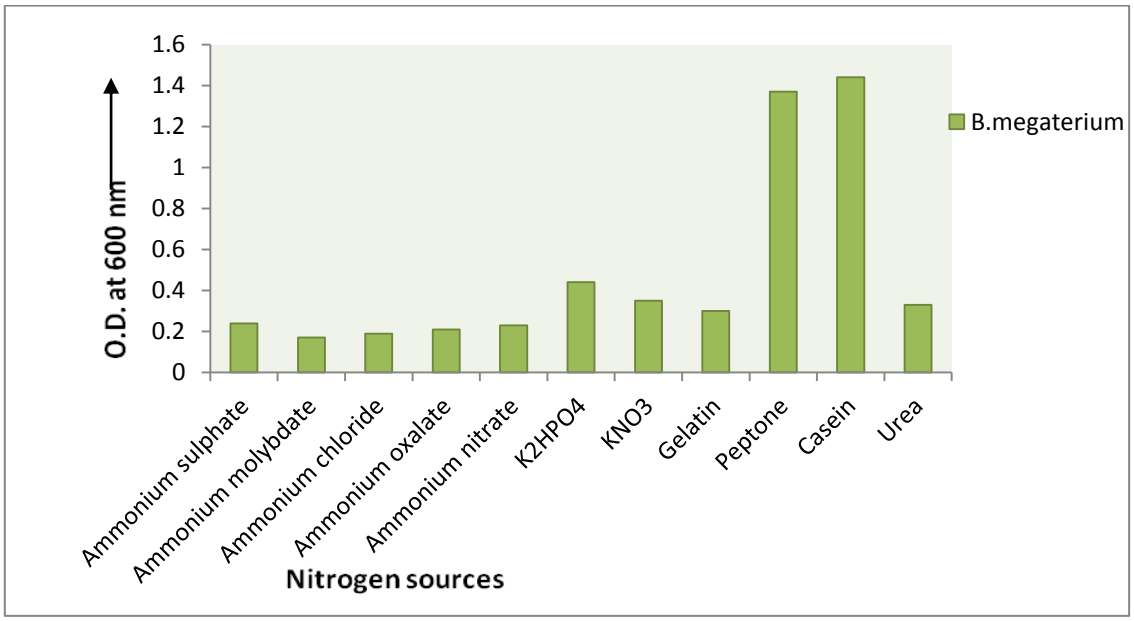
supplemented with eleven different nitrogen sources namely ammonium sulphate, ammonium molybdate, ammonium chloride, ammonium oxalate, ammonium nitrate, K_2HPO_4, KNO_3, gelatin, peptone, casein and urea. Among the nitrogen sources tested in the present study casein and peptone have shown high influence on the growth of *B.megaterium*.

Table 6 : Effect of nitrogen sources on growth of *B.megaterium* Summary of One-way ANOVA and Duncan's Multiple Range Test

	Nitrogen sources	N	Mean	Std. Deviation	F-value	P-value
B.megaterium	Ammonium sulphate	3	.2433 c	.02082	1499.894**	0.000
	Ammonium molybdate	3	.1733 a	.02082		
	Ammonium chloride	3	.1867 a	.01528		
	Ammonium oxalate	3	.2133 b	.01528		
	Ammonium nitrate	3	.2267 c	.02082		
	K_2HPO_4	3	.4400 f	.03000		
	KNO_3	3	.3467 e	.01528		
	Gelatin	3	.2967 d	.02517		
	Peptone	3	1.3733 g	.02082		
	Casein	3	1.4400 h	.02000		
	Urea	3	.3300 e	.02000		
	Total	**33**	**.4791**	**.45101**		

** Significant at 1% level

Note: The same letter indicates insignificant difference among the various Nitrogen sources.



Graph-5: Effect of nitrogen sources on growth of *B.megaterium*

The growth of *B.megaterium* were showing a significant difference at 1% level among the nitrogen sources since P value 0.000 < 0.01 for the corresponding F- Value (1499.894**). Casein and peptone showed maximum growth in both organisms (Fig 5).

Based on DMRT analysis the influence of different nitrogen sources with respect to the growth of the isolate was significant. The results were listed in Table 6. The result suggests that the same level of growth was observed in ammonium sulphate

and ammonium nitrate followed by ammonium molybdate and ammonium chloride in *B.megaterium*.

Effect of amino acids on growth:

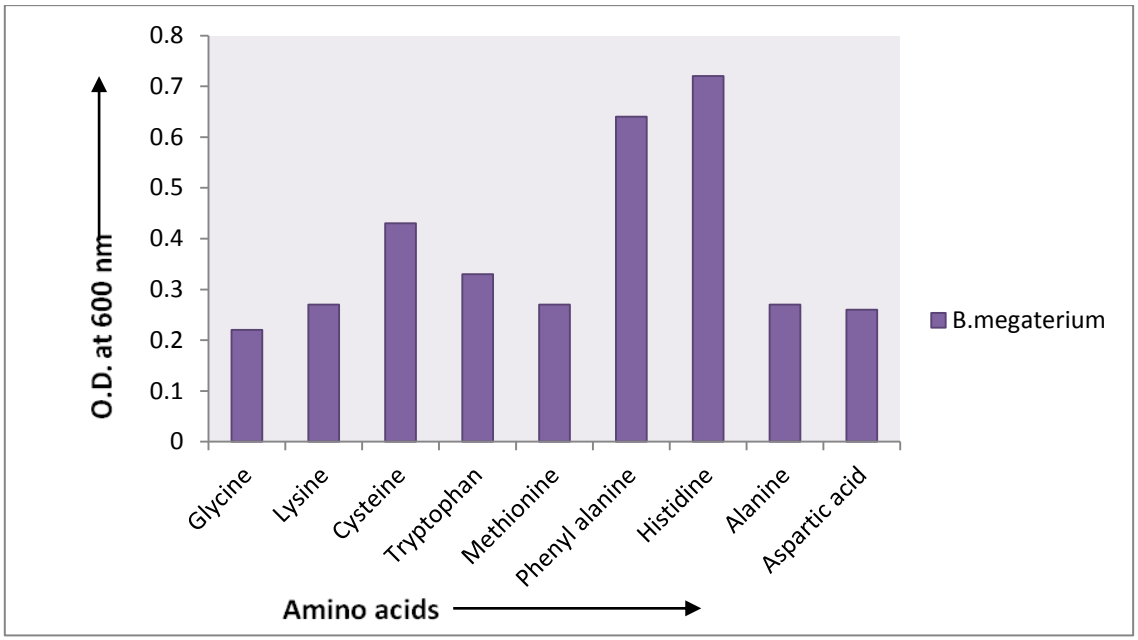
Effect of different amino acids on the growth of *B. megaterium* was studied. Nine Different amino acids were amended to find out suitable amino acid for the optimum growth of *Bacillus megaterium* isolates (Table 7). Among the different amino acids histidine and phenyl alanine have shown the best for the growth of two isolates.

Table 7: Effect of amino acids on growth of *B. megaterium* Summary of One-way ANOVA and Duncan's Multiple Range Test

	Amino acids	N	Mean	Std. Deviation	F-value	p-value
B.megaterium	Glycine	3	.217 a	.0208	312.593**	0.000
	Lysine	3	.267 b	.0153		
	Cysteine	3	.433 c	.0153		
	Tryptophan	3	.330 d	.0200		
	Methionine	3	.273 b	.0153		
	Phenyl alanine	3	.643 e	.0208		
	Histidine	3	.717 f	.0153		
	Alanine	3	.273 b	.0208		
	Aspartic acid	3	.257 b	.0153		
	Total	**27**	**.379**	**.1757**		

** Significant at 1% level

Note: The same letter indicates insignificant difference among the various amino acids



Graph-6: Effect of different Amino acids on growth of *B. megaterium*

The growth of *B.megaterium* was showing a significant difference at 1% level among the amino acids. Since P value 0.000 < 0.01 for the corresponding F- Value (312.593**). Phenyl alanine and histidine shows highest growth when compared to other amino acids (Graph-6).

Based on DMRT analysis the influence of different amino acids with respect to the growth of isolates was significant. The result suggests that the same alphabets (a,b,c,d….) beside mean values suggests no difference among the growth O.D values for respective amino acids.

DISCUSSION

Mangoes have been named the most widely consumed fruit in the world. Because their range of possible benefits. Mango is one of the important fruit crop of Andhra Pradesh cultivated in an area of 3.99 lakh hectares and producing 3.19 million tonnes (Government of India, 2005). Mango is one of the most delicious and widely cultivated fruits of the tropical world. It is processed extensively, thereby generating huge quantity of solid and liquid waste. Solid waste is comprised of mango peel, stones, stalk, trimmings, fibrous material and rotten fruits. This constitute about 40-50 % of total waste. One of the agro-wastes currently causing pollution problems is the mango peel from fruit processing industries, which poses considerable disposal problems and ultimately leads to environmental pollution. Utilization of waste is both a necessity and challenge. Mango fruit compost was collected from fruit processing industries. The efficient bacterial isolate was selected according to their highest pectinolytic activity on the basis of their growth and formation of clearing zones on Citrus Pectin Agar (CPA) medium by using iodine potassium iodide solution[14]. Among 16 bacteria strains screened on the specific medium, one of the bacterial strains showed clear zones and found as pectinase producers with the assistance of IMTECH, Chandigarh. The bacterial colony showing maximum zone diameter was selected as the best strain. The isolate were designated and numbered as 'mango pectinolytic bacteria' mpb2, the morphological examination, biochemical and physiological tests of screened isolate were performed and mpb2 isolate was initially identified as *Bacillus megaterium* (MTCC 10773). In studies on an optimum range of temperature, pH levels, NaCl concentrations for growth of *B. megaterium* were 35°C to 40oC, pH of 6.5 to 7.0, NaCl concentration of 0.5 to 1% and, fructose and galactose as carbon sources for *Bacillus megaterium.* Casein and peptone as nitrogen sources, phenyl alanine and histidine as the amino acids were observed as optimum for the growth of *B. Megaterium* respectively. However, *B. megaterium* has grown up to 12% NaCl concentration.

CONCLUSION

This study revealed bacterial isolates were found associated with compost, collected from fruit processing industries around Tirupati, Chittoor district, Andhra Pradesh. Based on the morphological examination, biochemical and physiological tests of the isolate resembles as *Bacillus megaterium* and is suitable for the production of pectinase enzyme useful for the pectin degradation. Finally, concluded that abiotic factors such as temperature, pH, saline concentration and nutritional sources like carbon, nitrogen and amino acids are also influenced the growth of *Bacillus megaterium.*

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Author contribution:

1) DR. K. Sridevi: Carried out research work, data collection and paper writing.
2) Mr. G.Venkatesh: Assisted in carrying research work and paper writing.
3) Mr. M. Sumanth: Encouragement during the work, assisted in carrying research work and paper writing.
4) Dr. P. Sivaraagini: Assisted in carrying research work and paper writing.
5) DR. K. Vijalakshmi: Research guide, assisted in carrying research work and paper writing.

Ethical Approval:

This article does not contain any studies with human participants or animals performed by any of the authors.

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