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RESEARCH ARTICLE

EXPLOITATION OF PLANT GROWTH PROMOTER ACTIVITY OF *BACILLUS MEGATERIUM* UNDER *IN VIVO* CONDITIONS

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ABSTRACT

Plant growth promoting ability of *Bacillus megaterium*, isolated from mango fruit compost samples collected from Vinsari and Varsha fruit processing industries around Tirupati, Chittoor district, Andhra Pradesh, was tested on groundnut variety JL24 obtained from S.V. Agricultural University in potted condition. Bacillus megaterium promoted growth in terms of shoot length, root length, no. of leaves, total dry mass and it had improved the percentage of germination over uninoculated controls was recorded during 15 to 45 days in potted conditions. In the present study, four treatments were set up with different variables to evaluate the effect of inoculation with seed treatment. A significant increase was recorded in the shoot length, root length, 100% seed germination, no. of leaves, dry mass weight in grams per plant and mean daily germination were observed in treated seeds when compared to control. Among all treatments, Treatment4 (T4), in which soil was amended with mango peel pectin medium kept for fermentation showed maximum growth of the plant. The present study also suggested that mango fruit peel found to be the best substrate for pectinase production by *Bacillus megaterium* among the various agricultural wastes. *Bacillus megaterium* has been found to be a good plant growth promoter with the ability to increase growth of ground nut plants.

INTRODUCTION

Biodegradation of agricultural waste by microorganisms play a vital role in carbon recycling and to maintain soil fertility by its conversion into humus which favours the growth of the plant. In India there is an immediate need for ensuring sustainability in all facets of development, including the environment. In this line the area of renewable energy plays an important role in sustaining resources and combating environmental degradation while utilizing the organic matter. The biological treatment of these wastes appears to be most cost effective and carry a less negative environmental impact (Coker, 2006). The use of various agricultural waste and agro-industrial by-products, in the present study suggested that mango fruit peel found to be the best substrate for polygalacturonase production by *B. megaterium*. The similar studies were also carried out by Silva *et al.,* (2002) where orange bagasse and wheat bran gave higher yields of PG by the culture *P. viridicatum* RFC3.

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For the commercial usage of enzyme from the isolates, it should have desirable biochemical, physio-chemical characteristics and low cost of production. Mango peel is very cheap, abundantly available and could be easily stored after sun drying. This waste is generated after the extraction of juice and available in high quantity from fruit processing industries, but has a limitation of availability in only particular season. Its dumping in nature causes pollution problems; hence its eco-friendly utilization is essential which tempted to use agro waste for pectinase production by solid state fermentation (Afifi and Foaad *et al.,* 2002). Different natural substrates used as pectin instead of commercial pectin. Natural subtracts like Rice bran (Farooqui, 2012), Wheat bran (Naderi *et al.,* 2012), Fruit and vegetable waste (Soares *et al.,* 1999), cassava waste (Mukesh Kumar, *et al.,* 2012) etc, used for pectin substrate. Conventional means of utilization of waste has been via biotransformation of Kinnow waste (mainly Kinnow peels and Kinnow Pomace) into humus. This is the 2nd important fruit crop after mango. Many useful products like pectinases, pectin, peel oil and dietary fibres can be obtained from exploitation of such waste. Of these, pectin and pectinase have appreciable global importance. Pectin's are extracted by heating the plant materials in water (60-90^{0}C) and at an acidic pH (2.5). The pectin are precipitated with ethanol and removed

by centrifugation. (www.lifeviv.edu). Many plants have intimate relationships with soil microbes, which improve the plant's growth and fitness through a variety of mechanisms. *Bacillus* spp. isolates are natural root-associated bacteria, isolated from *Nicotiana attenuate* plant roots growing in native soils shown a significant improvement (Meldau *et al*., 2012). *Bacillus megaterium* isolated from tea rhizosphere and tested for its ability to promote growth and cause disease reduction in tea plants. *In vivo* studies revealed the ability of this bacterium to promote growth of tea plants very significantly. The present study was undertaken to determine how *Bacillus megaterium* isolated from compost of mango processing industries influences the growth of ground nut plats and development of some successful bacterial degradation of the mango fruit waste with the help of their enzymes in less span of time under natural conditions without producing any foul odour.

MATERIALS AND METHODS

Isolation of Pectinolytic bacteria

Collection of soil samples: The soil samples were collected from the mango fruit processing industries around Tirupati, Chittoor district of Andhra Pradesh, India (Subbarao, 1999). The soil samples were transported to the laboratory in sterile polythene bags, air-dried and mixed thoroughly to make a composite sample. All the soil samples were serially diluted and are plated according to the method of Aneja, 2003.

Screening of pectinolytic bacteria from fruit waste by using Citrus Pectin agar medium (g/L) preparation: Isolates were streaked on 1% citrus pectin Agar with 67% of metoxilation, 0.14% $(NH_4)_2$ SO_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 the isolated morphological colonies were purified by repeated streaknig.

Plate assay of depolymerised 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°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 (Fernandes-Salomão *et al.,* 1996).

Identification and characterization of efficient pectinolytic bacterial isolates from the soil sample: Morphological, cultural and biochemical characteristics were used for the identification of the bacterial isolates from the sample. It was done according to the Bergey's Manual of Systemic Bacteriology, and with assistance of IMTECH, Chandigarh.

Composition of production media

The composition of mango peel (g/100g dry residue): moisture 68.5%, total sugar 48.1%, reducing sugar 40.8, starch 291, pectin 12.85%, protein 3.9%, fibre 8.4% and tannins 2.3% were collected from the local mango fruit processing industries of Tirupati. The mango peel is subjected for oven at 45^0C for 5 days and powered after shade drying. The powder was refrigerated at 4^0C till use. It can be used for suitable substrate (instead of commercial citrus pectin) for pectinase production in a cost effective production (Mukesh kumar, 2012).

Mango pectin agar medium: A modified yeast extract pectin (YEP) medium (Kashyap *et al.,* 2003) was used for the production of pectinases. Yeast extract 10g, mango pectin 4g, citric acid 1g, agar-agar 20gm, distilled water 100 ml. The medium was autoclaved at 121^0C (15 psi) for 15 minutes. Commercial pectin producin potent bacterial isolate was tested for the degradation of mango pectin.

Preparation of production medium: The same above media was used for production medium without agar. 5 ml of overnight grown *Bacillus megaterium* culture was added to the flask containing 250 ml of production medium and incubated at 37^0C for 3 days under agitation (120 rpm) and optimum conditions were studied for further investigations.

Effect of *B. megaterium* on growth of ground nut variety JL-24

***In vivo* pot assay studies:** *Bacillus megaterium* having efficient exo PG producing was selected to study the effect of exo-PG on growth of ground nut variety JL-24. In vivo studies by pot assays based on the ability to solubilise pectin into available form.

Inoculum preparation and Application: A loopful of 24 hours old culture of efficient exo PG producing *B. megaterium* was added to 100 ml of sterile citrus pectin broth. The flasks were incubated at 37°C for 3 days on rotary shaker. The bacterial count of the isolates was 36x10-6/ml (Zou *et al.,* 2010). Groundnut JL-24 seeds were subjected for surface sterilizion with 0.01% $HgCl_2$ for 2 minutes and washed several times with distilled water. The liquid culture of *B. megaterium* of 4 days old is inoculated to seed by soaking for 2 hours and dried in shade and sowed immediately (within an hour) (Sudhansu and Pal, 1998). Per kg of soil the pectin amendment was 5 grams in case of citrus pectin (commercial) and 20 grams in case of Mango peel pectin. The inoculums were diluted and added 5 ml/kg with dilution factor 36x10-6. The soil selected was from groundnut growing fields from Chittoor district.

T0 Control: Sterile soil not amended with any pectin and without bacterial inoculum was control.

T1 (Test 1): In T1, the sterile soil was amended with commercial citrus pectin and without bacterial inoculum.

T2 (Test 2): In T2, soil was amended with commercial citrus pectin (5 grams/ kg soil) and bacterial inoculum.

T3 (Test 3): In T3, soil was amended with Mango peel pectin (20 gms/kg) and bacterial inoculum inoculums.

T4 (Test 4): In T4, soil was amended with mango peel pectin medium kept for fermentation.

Pot assays studies include setting up of with triplicates for all five treatments and the average for the following of all five treatments and the average for the following variables was calculated on 15,30 and 45 days. Percentage of seed germination, height (root length and shoot length) and weight of the plant were calculated. Seedling Vigour Index (SVI) (Agarwal, 1980) was calculated by the formula described by Abdul Baki and Anderson (1973).

Seedling Vigour Index = (Root length + shoot length) x Seed germination

RESULTS

Isolation of soil sample from fruit processing industries: Mango fruit compost samples were collected from the mango fruit processing industries around Tirupati, Chittoor district, Andhra Pradesh. Samples of required quantity were taken according to the standard methods. Bacteria were isolated from the compost sample collected from mango fruit processing industrial area by serial dilution and plating technique as mentioned earlier). Sample was inoculated into Nutrient Agar medium and incubated for 48 hours at 37^{o}C. All the isolates were subjected for their pectinolytic property by puncturing the Citrus pectin agar (CPA) medium which is a quantitative test for pectin degrading bacteria.

Screening of the isolates for pectin degrading bacteria: Efficient bacterial isolates were 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). Good pectinolytic activity exhibiting was isolated and numbered as 'mango pectinolytic bacteria' mpb2 (Fig. 1). Based on the morphological, biochemical and physiological tests performed, both the isolates were initially identified as *Bacillus sp.* These were characterized according to the guidelines of Bergey's Manual of Systemic Bacteriology (Volume- III) (Sneath *et al*., 1986) and Manual of Medical Microbiology (Mackie and MacCartney, 2008).

Identification and Characterization 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. The physiological conditions for growth are temperature 37^{o}C, pH 7 and 1% NaCl concentration. It can tolerate even upto 12% of NaCl concentration. 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). The identity of the bacterium was confirmed at species level as *Bacillus megaterium* MTCC 10773 (mpb2) by the characterization with the assistance of IMTECH, Chandigarh, India. The screened *Bacillus megaterium* cultures are further cultured by using mango peel pectin a cost effective substrate in production medium.

Production of pectinase by using Mango peel pectin as a pectin substrate: The use of various agricultural waste and agro-industrial by-products, in the present study suggested that mango peel waste found to be the best substrate for pectinase production by *Bacillus megaterium*. A clear zone was observed by *B. megaterium* with mango peel pectin using as a substrate. Further investigations were made to use the fermented medium for evaluating the plant growth promotion activities. Application of enzyme alone or in combination with other enzymes like cellulase, hemicellulase and amylase may increase its utilization in many other applications.

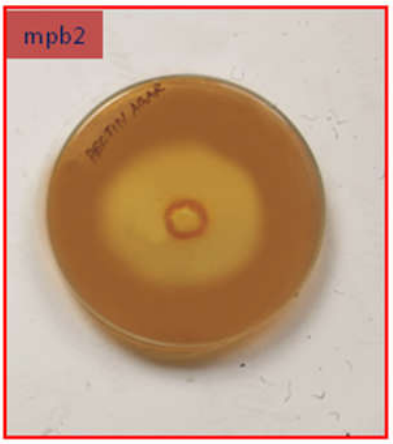

Fig. 1. Screening for pectinolytic activity of mango pectinolytic bacteria (mpb2) using Citrus Pectin Agar

Fig. 2. Fruit peel as pectin substrate

Fig. 3. Pectinase production medium

Plant growth promotion of *B. megaterium* on groundnut variety JL24: Plant growth promotion as influenced by inoculation with *B. megaterium* was measured for groundnut plants. The growth in terms of shoot length, root length, no. of leaves and total dry mass over uninoculated controls was recorded during 15 to 45 days. The following treatments were set up with different variables to evaluate the effect of inoculation with seed treatment.

T1 (Test 1): In T1, the sterile soil was amended with commercial citrus pectin and without bacterial inoculum.

T2 (Test 2): In T2, soil was amended with commercial citrus pectin (5 grams/ kg soil) and bacterial inoculum.

T3 (Test 3): In T3, soil was amended with Mango peel pectin (20 gms/kg) and bacterial inoculum inoculums.

T4 (Test 4): In T4, soil was amended with mango peel pectin medium kept for fermentation.

Table 1. Effect of *B. megaterium* on plant growth promotion of groundnut JL-24 on 15th day

S. No.	Seed Treatment	% Seed germination	No. of Leaves	Root length (cm)	Shoot length (cm)	Height +Root +Shoot (cm)	Dry mass weight (gms)	Seedling Vigour Index
1	T0	70	8	10	22	32	5.20	2240
2	T1	80	13	15	25	40	6.50	3200
3	T2	90	16	17	24	41	6.90	3690
4	T3	100	15	16	24	40	6.80	4000
5	T4	100	18	16	26	42	7.25	4200

Fig. 4. Effect of *B. megaterium* on plant growth promotion of groundnut JL-24 on 15th day

Table 2. Effect of *B. megaterium* on plant growth promotion of groundnut JL-24 on 30th day

S. No.	Seed Treatment	% Seed germination	No. of Leaves	Root length (cm)	Shoot length (cm)	Height +Root +Shoot (cm)	Dry mass weight (gms)	Seedling Vigour Index
1	T0	70	16	11	23	34	5.90	2380
2	T1	80	25	14	28	42	6.50	3360
3	T2	90	28	18	30	48	8.97	4320
4	T3	100	32	18	32	50	10.00	5000
5	T4	100	30	19	33	52	10.5	5200

Fig. 5. Effect of B. megaterium on plant growth promotion of groundnut JL-24 on 30th day

Table 3. Effect of *B. megaterium* on plant growth promotion of groundnut JL-24 on 45th day

S. No.	Seed Treatment	% Seed germination	No. of Leaves	Root length (cm)	Shoot length (cm)	Height +Root +Shoot (cm)	Dry mass weight (gms)	Seedling Vigour Index
1	T0	70	16	12	27	39	9.10	2730
2	T1	80	26	18	29	47	11.25	3760
3	T2	90	36	21	36	57	13.78	5130
4	T3	100	35	20	37	57	13.30	5700
5	T4	100	38	20	36	56	17.28	5600

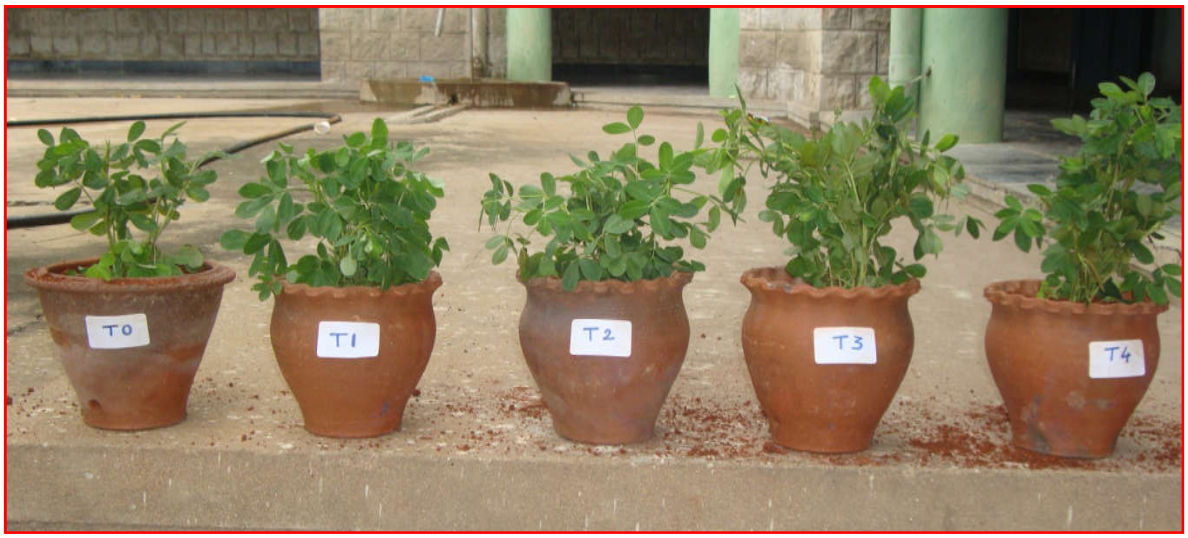

Fig. 6. Effect of B. megaterium on plant growth promotion of groundnut JL-24 on 45th day

The purpose of the pot assay study was to assess the ability of *B. megaterium* to solubulize mango peel pectin in green house conditions at different days of plant growth (Table 1, 2 and 3). A significant increase was recorded in the shoot length (26, 33, 36); root length (16, 19, 20); seed germination 100%, no. of leaves (18, 30, 38) and (7.25, 10.05, 17.28) of dry mass weight in gms per plant. Total height of root+shoot is (42, 52, and 56) and with seedling vigour index of 4200, 5200 and 5600 respectively during 15th, 30th and 45th day. The result of soil experiments in the pot assay had shown the efficiency of this mango pectin utilization by *B. megaterium* in direct and fermented. The above observations concluded that by using citrus pectin with bacterial inoculums (T2), mango pectin with inoculum and pectin degraded already in the fermented mango peel medium were almost gave similar results when compared to control. The result of Soil experiments in the pot assay study shown the efficiency of this isolate to make available nutrients from mango peel pectin substrate with bacterial inoculation (T4) 4200, 5200, 5600 had good growth when compared to controls 2240, 2380, 2730 (T0); 3200, 3360, 3760 (T1); 3690, 5000, 5130 (T2) and 4000, 5000, 5700 (T3) respectively during 15th, 30th and 45th day.

DISCUSSION

In the present study, the *Bacillus megaterium* (MTCC 10773) was isolated, screened and characterized with the assistance of IMTECH, Chandigarh. The bacterium *B. megaterium* produced significant amount of pectinase after 72 hours of incubation in fermentation medium at 37^0C and pH 7. In the present study suggested that mango peel waste found to be the best substrate for pectinase production by *B. megaterium*. The study illustrate that the usage of mango wastes as a substrate for pectinase production.

The impact of *B. megaterium* on seed germination and plant growth was detected. Treatment of Groundnut seeds (JL-24) obtained from S.V. Agricultural University was treated with inoculum (seed bacterization) and it had improved the percentage of germination and overall height / weight of the plants. Germination up to 80-100% was recorded with maximum Seedling Vigor Index (4200, 5200 and 5600); peak value (at T3 and T4), germination value (100%) and mean daily germination were observed in treated seeds when compared to control (Table 22, 23 and 24). Similar reports were made by Bijender Singh and Satyanarayana, (2011); Zou *et al.,* (2010).

Summary and conclusion

The present study is an attempt to isolate potential pectinase producing strains from the potent sites. Among all isolates, strain *Bacillus megaterium* showed highest activity and predominance of exo PG enzyme. Furthermore, *Bacillus megaterium* was found to utilize agricultural waste and by-products for enzyme production. Among the tested substrates mango peel pectin was found to be the best substrate for PG production. This study has potential of utilizing agricultural waste provides cost effective and eco-friendly method for pectinase production on large scale. Peel is abundantly available in high quantity at particular season. 141 tons of pectin can be produced annually in India. The industrial utilization of mango peels for manufacturing pectin would not only solve the problem of waste disposal but also save valuable foreign exchange by reducing the pectin and pectinase imports. With the increase in the price of world pectin a country like India, with free access to abundant supplies of quality raw material may also find it feasible in setting up pectin factories more favourable than before in order

to meet the internal demand. Substitution of commercial pectin by mango waste could not only reduce the cost of the enzyme but open an avenue for successful waste utilization. This was the first study reported that *B. megaterium* as efficient exo PG producer by utilizing mango peel as pectin substrate. It was also evident that higher enzyme activity was measured with fruit peel which is a cost-effective method for the development.

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Author contribution

- **Dr. K. Sridevi:** Carried out research work, data collection and paper writing.
- **Mr. G.Venkatesh:** Assisted in carrying research work and paper writing.
- **Mr. M. Sumanth:** Encouragement during the work, assisted in carrying research work and paper writing.
- **Dr. K. Vijayalakshmi:** 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.

Research Guide: DR. K. Vijayalakshmi, Dept. of microbiology, Sri Padmavathi Mahila Viswa Vidyalayam, Tirupati, Andhra Pradesh.

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Conflicts of interest: None declared.

REFERENCES

Abdul Baki AA and Anderson JD. 1973. Vigor determination of Soybean seed by multiple bacteria. *Crop Science*, 13:630-633.

Afifi AF and Foaad MA. 2002. Purification and characterisation of pectin lyase produced by *Curvularia inaequalis* NRRL 13884 on orange peels waste, solid state cultivation. *Annal Microbiol*, 52, 287-297.

Agarwal RL. 1980. Seed Technology. Oxford and IBH Publishing Co., New Delhi, pp.685.

Aneja KR. 2003. Experiments in Microbiology, *Plant Pathology and Biotechnology revised fourth edition*, New Age International Pvt. Ltd..

Bijender Singh and Satyanarayana T. 2011. Microbial phytases in phosphorus acquisition and plant growth promotion. *Physiology and Molecular Biology of Plants*, 17(2):93-103.

Coker C. 2006. Environmental remediation by composting. *Biocycle*., 47: 18-23.

Farooqui MJH. 2012. Cost effective production and process optimization of pectinase under submerged fermentation. *Asiatic Journal of Biotechnology resources*. 03(10):1419-1423.

Fernandes-Salomao TM, Amorim ACR, Chaves-Alves VM, Coelho JLC, Silva DO, Araújo EF. 1996. Isolation of pectinase hyperproducing mutants of *Penicillium expansum*. Rev. Microbiol., 27: 15-18.

Kashyap DR, Soni SK and Tiwari R. 2003. Enhanced production of pectinase by *Bacillus* sp. DT7 using solid *satefermentaion, Bioresource Technology*, 88(3):251-254.

Mackie and McCartney, 2008. Practical Medical Microbiology. (College G, Dugid, JP Fraser, AG and Marmion BP Eds.) 16th Edition. Churcchill Livingstone, Edinburgh, London, Melbourne and New York 2:43-160.

Meldau DG, Hoang LH, and Baldwin IT. 2012. A native plant growth promoting bacterium, *Bacillus megaterium* B55, rescues growth performance of an ethylene insensitive plant genotype in nature. *Frontiers in Plant Sci*., 3: 1-13.

Mukesh kumar DJ, Saranya GM, Suresh K, Andal Priyadharshini D, Rajakumar R and Kalaichelvan PT. 2012. Production and optimization of pectinase from *Bacillus sp.* MFW7 using Cassawa Wate. *Asian Journal of Plant Science and Research*, 2(3): 369-375.

Mukesh kumar DJ, Saranya GM, Suresh K, Andal Priyadharshini D, Rajakumar R and Kalaichelvan PT. 2012. Production and optimization of pectinase from *Bacillus sp.* MFW7 using Cassawa Wate. *Asian Journal of Plant Science and Research,* 2(3): 369-375.

Naderi S, Naghavi N.S. and Shahanipoor K. 2012. Pectinolytic Activity of *Aspergillus niger* on pectic agricultural substractes. The first international and the 4th National Congress on Recycling of Organic waste in agriculture in Isfahan, Iran.

Silva D, Silva ED, Silva RD and Gomes E. 2002. Pectinase production by *Penicillium viridicatum* RFC3 by solid state fermentation using agricultural wastes and agro industrial by-products. *Braz. J. Microbiol*., 33: 318-324.

Sneath PA, Mair NS, Sharpe ME and Holts JG. 1986. Bergey's Manual of Systematic Bacteriology Vol 2, Baltimore MD: Willam and Wilkins.

Soares MMCN, Da Silva R and Gomes E. 1999. Screening of bacterial strains for pectinolytic activity: characterisation of the polygalacturonase produced by *Bacillus* sp. Rev. Microbial, 30(4):299-303.

Subba Rao NS. 1999. Soil microorganisms and plant growth. Oxford and IBH Publishing Co., New Delhi, India.

Sudhansu S and Pal, 1998. Interaction of an acid tolerant strain of phosphate solubilizing bacteria with a few acid tolerant crops. *Plant and Soil*.,198: 169-177.

Zou C, Li Z and Yu D. 2010. *Bacillus megaterium* strain XTBG34 promotes plant growth by producing 2-pentylfuran. *J. Microbiol*., 48(4): 460-466.

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