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RESEARCH ARTICLE

SCREENING OF POTENTIAL BACTERIA FOR POLYGALACTURONASE PRODUCTION FROM DIFFERENT COMMERCIAL CROP FIELD SOILS

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ABSTRACT

The studies on polygalacturonase producing bacteria form fertile soils are very meager. In the present study, bacteria were isolated from different commercial crop field soils of W.G.Dist. A.P. A total number of 12,325 bacterial colonies were isolated from 59 different fields in the cultivation of mango, maize, banana and lemon. A total of 69,371 hectares of land is under cultivation of these crops in the district. Among the isolated bacteria, 5935 were found to be polygalacturonase producers based on plate assay method. Of which1389 are from mango, 1741 are from maize, 1281 are from banana and 1503 are from lemon field soils. They were categorized as potential, good and poor producers of polygalacturonase based on the diameter of zone of clearance. The bacteria isolated from Maize fields were proved to be potential polygalacturonase producers with zone of 5cm.

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INTRODUCTION

The soil is such a diverse environment that the microbial populations differ tremendously from soil to soil; even within the same soil over the course of a season. Soil without microorganisms is a dead soil. The vast difference in the composition of soils, physical characteristics and the agricultural practices by which they are cultivated, result in corresponding large differences in the microbial population both in the numbers and kinds. Commercial crop fields of West Godavari District were selected to isolate polygalacturonase, producing bacteria which find applications in 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. Polygalacturonases/ Pectinases have found extensive application in fruit juice and beverage industry for clarification process. Pectinases from food and food bio product processed waste alone account to a total of one-third quaters of world's food enzyme production. Considering this in to account the present investigation was carried out to know the prevalence of pectinase producing bacteria from commercial crop field soils. West Godavari District the western delta of river Godavari has a richly cultivated land, divided into the Delta and the uplands. A substantial percentage of the district population over 70 per cent - depend on agriculture for the livelihood. A uniform pattern of land tenure was established throughout the district (and the State). Of the geographical area of 7,79,535 hectares the net cultivated area constitutes 58.1 per cent. West Godavari is basically an agrarian district with rich natural resources. The district has a distinct place in the State and it is popularly called as the 'Ricebowl' of Andhra Pradesh. Besides paddy the principal crops of cultivation in the district are jowar, maize, blackgram, chillies, turmeric, sugarcane, vegetables, groundnut, sesamum, tobacco, cashewnut, mango and banana. The Soils in the District are of Alluvial, Black Reger and Red Ferruginous besides a small belt of arenaceous sandy soils along coastal belt. It has a fertile land assured of irrigation facilities ties with less natural hazards. Soil microbes are immensely diverse and have numerous metabolic activities and products that could have industrial applications. This treasured reservoir is largely unexploited in many habitats. In this regard present investigation is taken up to screen potential polygalacturonase producing bacteria from field soils of commercial crops including mango, maize, banana and lemon from West Godavari district A.P.

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Selection of Habitat

Soil samples were collected from Mango, Maize, Banana, Lemon fields and also from the dump-yards of fruits and vegetables across West. Godavari. District, Andhra Pradesh, India.

Time of sample collection: Soil samples were collected from January to June2013

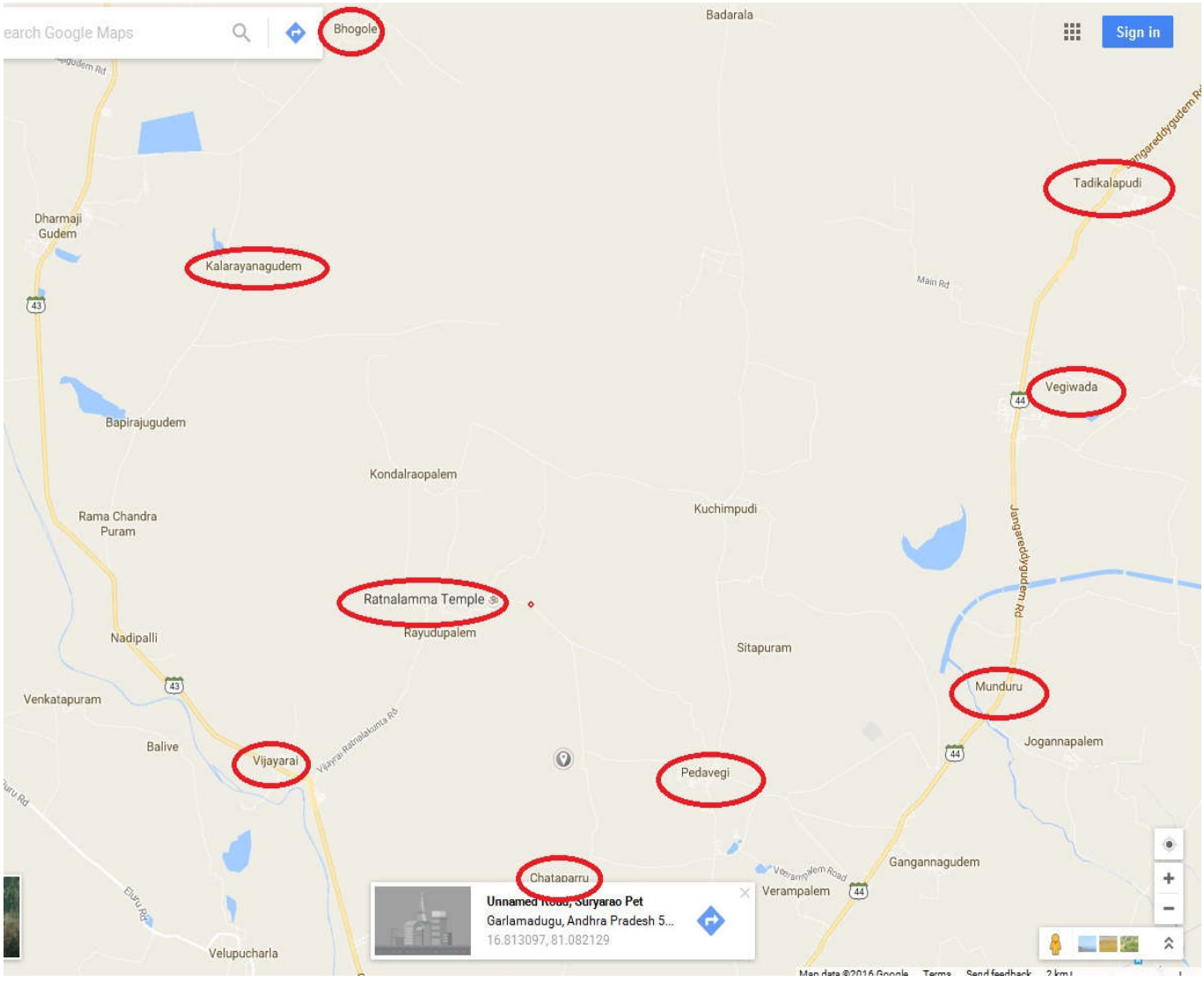
Collection of soil sample: Soil samples were collected by removing outer layer at a depth of 1cm from four corners and mixing it. The samples were collected in a polythene bag and bought to the laboratory for further processing. A total of 17 soil samples from mango, 16 from maize, 13 from banana and lemon fields were collected.

MATERIALS AND METHODS

Serial dilution: Series of sterile test tubes were taken with 10ml of sterile H_2O,1gm of soil sample was added to the test tube andmixed thoroughly which was labelled as 10^{-1} dilution. 1ml from 10^{-1}dilution was transferred to a second test tube containing 9ml of sterile H_2O(10^{-2} dilution) and mixed thoroughly before transferring1ml to next tube containing 9ml of H_2O (10^{-3} dilution). The dilutions were further carried until$10^{-7}$from which 1ml was discarded. The same was repeated with all the collected soil samples. This serial dilution ensures decrease in the number of organisms which allows less number of microbial colonies with clear morphology hence better isolation of pure microbial cultures.

Table 2. Commercial crop and the areas of sample collection

Commercial crop	Area/place of collection
Mango	Eluru, Rangapuram, Ayyapparajugudam, Bhogolu, Jangareddigudam, Dwaraka tirumala, Vegavaram, Kottapalli, Pedavegi, Gopannapalem, Ratnalakunta, Bhogolu.
Maize	Kalarayanagudem, Pragathipuram, Simhadripuram, Vemulapalli, Janampeta, Thadikilapudi, Rangapuram, Bhogolu, Monduru, Dwarakatirumala, Ammapalem, Koppaka, Ayyapparajugudam, Jangareddigudam
Banana	Chataparru, Eluru, Satrampadu, Kodelu, Ratnalakunta, Vijayarai, Venkatakrishnapuram, Pedavagi, Jangareddigudam, Dwarakatirumala.
Lemon	Vegiwada, Vegavaram, Kottapalli, Pedavegi, Gopannapalem, Ratnalakunta, Janampet, Vijayarai, Suryarampet, Dibbagudem, Gopannapalem, Bhogolu



Plating: Triplicates of 10^{-4} dilution from each sample were plated on nutrient agar medium by spread plate technique. All the plates were incubated at 37^0C for 24-48hrs.

Isolation of Bactiria: Different types of bacteria were identified based on morphological characteristics and number of colonies on each plate counted.

Screening of polygalacturonase producing (Ec.3.2.1.67) Bacteria: Each serially diluted (10^{-7}) soil samples were plated in triplicates on pectin agar medium to isolate pectinase producing bacteria. The plates were incubated at 37^0c for 24-48 hrs till the diameter of the colony reaches 3mm in size. Later the plates were screened for pectinase activity by adding1gm of iodine, 5gms of potassium iodide to 330 ml of water and observed for zone of clearance around the colonies. The diameter of zone of hydrolysis of specific colonies was measured and tabulated. Pectinase producing colonies show pale yellow colour while non-pectinase producers were indicated by browncolour. Total number of pectinase producing bacteria were counted and different types of pectinase producing colonies were categorized based on morphological characteristics. Pectin agar medium was prepared with the following composition 1% citrus pectin, 0.14% $(NH_4)_2SO_4$, 0.6%K_2HPO_4, 0.2%KH_2PO_4 and 0.01%Mgso47H_20, Agar agar 2%pH-6.0(Sanjay patel, 2015) Trace amounts of ferrous sulphate, mangonoussulphate and zinc sulphate were added and autoclaved at 121◦C. 15lbs pressure 15-20min.

Screening of potential polygalacturonase producing (Ec.3.2.1.67) bacteria

From the bacteria showing pectinase activity potential pectinase producing bacteria were screened based on the diameter of zone of hydrolysis. The colony showing maximum zone of hydrolysis was identified as potential pectinase producing bacteria. These were maintained for further study by sub-culturing on pectin agar slants.

Categorization of polygalacturonase producing (Ec.3.2.1.67) Bacteria

The plates were incubated till the colony reaches max size. After flooding plate with few ml ofiodine and potassium iodide reagent the dimeter of zone of clearance was measured and were categorized as potential (≥2cm), good (0.5-1.9cm) and poor polygalacturonase produces (0.2-0.4). Further screening of cultures was by measuring the diameter of zone of hydrolysis (i.e isolates with highest zone of hydrolysis). The cultures were individually plated on pectin agar medium and incubated at 37ºC. A 3 mm colony from the growing edge was transferred to1ml of sodium acetate buffer (0.1M; pH 5.0) and assayed for pectinase activity. The released sugars were estimated using DNS. Ten cultures were randomly selected based on the results of plate assay to compare the production of polygalacturonase. Four best isolates based on the polygalacturonase production were selected for further study.

RESULTS

Screening of bacterial isolates for polygalacturonase producing (Ec.3.2.1.67) bacteria

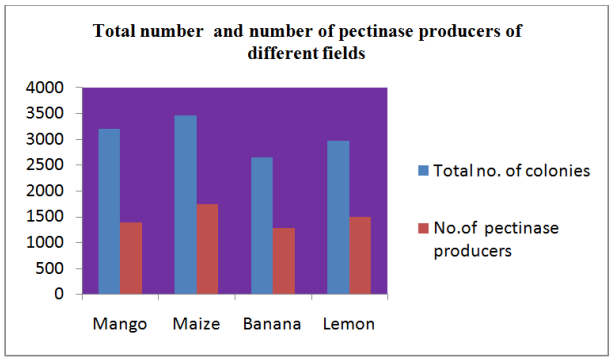
Bacterial enzyme production, in specific polygalacturonase, (E.C. 3.2.1.67) was confirmed by growing on specific media and observing zone formation. Rich bacterial diversity was observed in commercial crop field soils. A total number of 12,325 bacterial colonies were isolated from 59 soil samples. Out of these 5914 (48%) were found to be polygalacturonase producing bacteria. These were categorized as poor (3535), good (1710) and potential (669) producers based on diameter of zone of hydrolysis formed on pectin agar medium Table (I). A total number of 59 soil samples was collected from commercial crop fields which include 17 mango, 16 maize, 13 banana and 13 lemon. From mango fields a total number of 3213 bacteria were enumerated, out of these 1389 were polygalacturonase producers constituting 43.2% of total bacteria isolated. In a similar way from maize fields a total number of 3470 bacteria were enumerated, out of these 1741 were polygalacturonase producers constituting 50.1% of total bacteria isolated. Total number of bacteria from 13 banana and 13 lemon fields were 2657 and 2985 respectively, constituting 48% and 50.3 %. (Figure I, Table II). Polygalacturonase (E.C. 3.2.1.67) producing bacteria were more prevalent in maize fields followed by lemon, banana and mango. Based on the diameter of zone of hydrolysis the polygalacturonase (E.C. 3.2.1.67) producing bacteria were categorized as potential, good and poor producers with a zone diameter of >2, 0.5-1.9, 0.5cms respectively. Polygalacturonase producing bacteria showing >/ 2cm zone of clearance were considered as potential bacteria. Total number of potential producers was found to be11.31%.Mango 23%, maize 10.74%, banana 7.4%, lemon 4.4%. (Figure-II, Table-III) Screening studies revealed more of the isolates from mango fields were potential producers followed by maize, banana and lemon. More number of good polygalacturonase producers were isolated from maize and mango fields. When the percentage of good polygalacturonase producing bacteria of different commercial crop fields was determined mango constitutes 25.7% maize 48.47%, banana 14.75% and lemon 21.29% (Figure –III, Table-IV).

Table I. Total number of poor, good and potential pectinase producers

S.No.	No. of fields collected	Total no. of colonies	No. of polygalacturonase producers	No. of poor polygalacturonaseproducers	No. of good polygalacturonaseproducers	No. of potential pectinaseproducers
1.	59	12325	5914	3535	1710	699

Table II. Polygalacturonase producers from different soil samples

S.No.	Name of the soil sample	No. of fields	Total no. of colonies	No. of polygalacturonase producers	Percentage of polygalacturonase producers
1.	Mango	17	3213	1389	43.2%
2.	Maize	16	3470	1741	50.1%
3	Banana	13	2657	1281	48%
4.	Lemon	13	2985	1503	50.3%



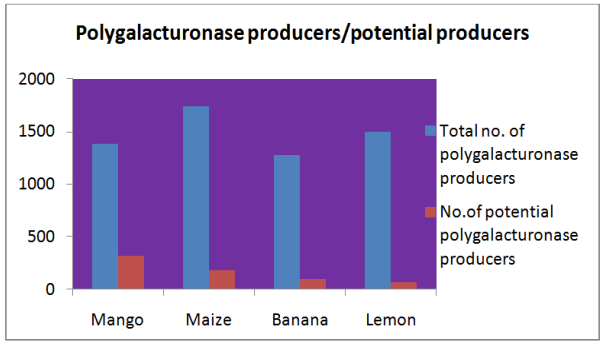


Table III. Potential polygalacturonase producers from different soil samples

S.No.	Name of the soil sample	No. of fields	Total no. of polygalacturonase producers	No.of potential polygalacturonase producers	Percentage of potential polygalacturonase producers
1.	Mango	17	1389	319	23%
2.	Maize	16	1741	187	10.74%
3	Banana	13	1281	96	7.4%
4.	Lemon	13	1503	67	4.4%

Table IV. Good polygalacturonase producers from different soil samples

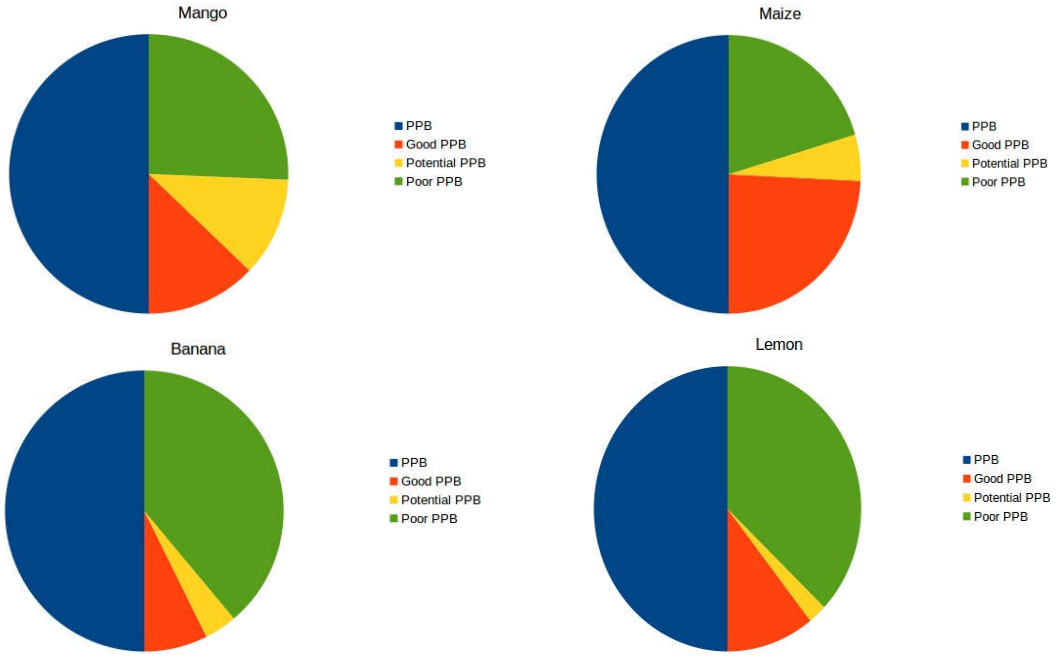
S.No.	Name of the soil sample	No. of fields	Total no. of polygalacturonaseproducers	No.of good polygalacturonaseproducers	Percentage of good polygalacturonase producers
1.	Mango	17	1389	357	25.7%
2.	Maize	16	1741	844	48.47%
3	Banana	13	1281	189	14.75%
4.	Lemon	13	1503	320	21.29%

Table V. Poor polygalacturonase producers

S.No.	Name of the soil sample	No. of fields	Total no. of polygalacturonase producers	No.of poor polygalacturonase producers	Percentage of poor polygalacturonase producers
1.	Mango	17	1389	713	51.3%
2.	Maize	16	1741	710	40.7%
3	Banana	13	1281	996	77.7%
4.	Lemon	13	1503	1116	74.2%

Table VI. Categorizatioon of polygalacturonase producers from different soil samples

S.No.	Name of soil sample	No. of fields	Total no. of colonies	Total no. of pectinase colonies	No. of good pectinaseproducers	No. of potential pectinase producers
1.	Mango	17	3213	1389	357	319
2.	Maize	16	3470	1741	844	187
3.	Banana	13	2657	1281	189	96
4.	Lemon	13	2985	1503	320	67



This shows the prevalence of good polygalacturonase producing bacteria in maize fields. Based on the above results it can be concluded that more of potential polygalacturonase producers were from mango and good producers were from maize fields. It reflects the fertility and favourable environmental conditions of the habitats. To sum up the screened bacteria were categorized as potential, good and poor producers (Figure-IV, Table-V)

Potential polygalacturonase producing bacteria were selected choosing one from each field i.e M1703 from mango, MZ501 from maize, B1303 from banana, L1303 from lemon, by measuring zone of hydrolysis as the zone corresponds to enzyme activity. (Table-VII)

Table VII. Isolates - Diameter of zone of clearance - selected from each field

S.No.	Name of soil sample	Ppotential pectinase producers	Persentage of potential pectinase producers	DIameter of zone of hydrolysis
1.	Mango	M1703	23%	5cm
2.	Maize	MZ501	10.74%	5.5cm
3.	Banana	B1303	7.4%	3.0cm
4.	Lemon	L1303	4.4%	4.0cm

From 17 mango field soil samples the selected potential isolate was from site Eluru, Similarly from 16maize field soil samples the potential isolate was from site Ammapalem, from 13 banana soil samples the potential isolate was from site Vijayarai, from 13 lemon soil samples the potential isolate was from site Ratnalakunta. The isolates screened from different places of west Godavari district, which is basically an agrarian city with good natural resources, fertile soil are the righteous/ merited characteristics in selecting the district as study area.

DISCUSSION

Screening of potential bacteria for polygalacturonase production from different commercial crop field soils was carried out in W.G.Dt the western delta of river Godavari W.G.Dist has a richly cultivated land divided in to Delta and the uplands. In the delta, coconut, lemon, rice farming and aquaculture are practiced. In the uplands, oil, palm, tobacco, cotton seed, corn, cashew, mango, banana and other fruit farming is practiced. Presence of fertile soil and rich cultivated area of the district are the reasons behind choosing the district as study area. The major types of soils in the district have been divided into Red sandy loams, clay loams, alluvial sandy and deltaic alluvial. The best isolate screened from mango fields was from sanivarapupet, one of the surroundings of Eluru having clay loamy soil, which is known for its organic content. Ammapalem, another surrounding area of Eluru also has clay loams which are highly fertile and good for all the crops. It is from this surrounding that the potential isolate from maize fields was selected. Vijayarai and Ratnalakunta, where the best isolate from banana and lemon were selected, are having red sandy loams. Red, yellow or brown colours are usually due to oxidation, hydration and diffusion of iron oxides in the soil, these oxides, are deep and well drained with a good nutrient supply and H_2O holding capacity and have all of the other physical and chemical characteristics required for intensive production of flora and fauna. Polygalacturonases producing Bacteria were isolated from commercial crop field soils, are rich sources of pectin. Nature of the soil and its physicochemical characteristics reflect the diversity of microorganisms. So as in the present study polygalacturonase producing bacteria were inhabitants of different soil types. Thus soils stand out to be the rich sources for diversified flora and fauna. Till date there were no reports on screening of polygalacturonase producing bacteria from commercial crops. Also these fields, with the cultivation of crops like mango, maize, banana and lemon, receive more amount of pectin in the form of fallen fruits and seeds. So fairly good number of polygalacturonase producing bacteria could be isolated from these crop field soils.

More number of these bacteria was isolated from soils of maize fields. Soils where maize is grown is enriched with pectin as it receives cobs with or without seeds during harvesting, after separation of seeds from the cobs and due to addition of left over corn again to the soil, making them as potent source of pectin. Next highest numbers of bacteria were isolated from lemon, followed by banana and mango. These fields also receive pectin in the form of fruits and leaves, also left over wastage after harvesting. There were reports in literature on isolation of polygalacturonase producing bacteria and fungi where in the percentage of isolates showed a great deal of variation, Soares *et al.* 1999 reported (60.7%), Alves *et al.* 2002, 96 %, Aminzadeh *et al* (2007) reported 71.4%, Rohban *et al* 12.1 %, Namasivayam *et al.* 2011, 35.29%, Kusuma and Rami Reddy, 2014, reported 12%. Soares et.al 1999, Jayani *et al*, 2005; Varalakshmi *et al* 2007 demonstrated the ability of microorganisms in the production of enzymes and gave an evidence by saying that Pectinase producing microorganisms hydrolyse pectin which can be detected by observing halo/clear zone formation on pectin agar medium. From the literature it is evident that the studies on zone of hydrolysis by polygalacturonase producing bacteria were meager, hence references on fungi were reviewed for discussion. Earlier Arotupin *et al* (2008) had reported the hydrolysis of pectin in the culture medium of *Aspergillusrepens*. The production of polygalacturonase by *Aspergillus, Fusarium,* Penicillium*, Thermoascus* sps on various substances during solid state and submerged fermentation (Favela-Torres *et al*., 2006) are strong evidences of the hydrolysis of pectin and pectin containing materials for the growth of fungi. Studies on *Trichodermaviridae* by Arotupin *et al* 2011 on polygalacturonase production by zone formation was another proof. The above studies affirmate that the screening of polygalacturonase production was mainly by observing zone formation and Zone diameter corresponds to enzyme activity.

Categorization of polygalacturonase producing bacteria

Polygalacturonase producers were classified as potential, good and poor producers based on the diameter of zone of hydrolysis in the present study. Although not comparable there were studies in the literature regarding categorization of polygalacturonase producing bacteria. Marcia *et al* 1999, Aminzadeh *et al* 2007 categorized as very good, good, weak and poor producers. Studies by Alves *et al* 2002 were supportive to the above methods; where they isolated the most productive strain by measuring clear zones formed around colonies, also evaluated the level of enzyme production by measuring the halo diameter. The isolates M170, M2501, B1303 and L1303 had a zone diameter of 5.0cm, 5.5cm, 3.0cm, and 4.0cm respectively. There were studies supportive to this classification but a little variation with regard to zone

diameter. There was variation in zone size from species to species. Alves *et al* 2002reported a zone diameter of ≥ 8cm for *Mucorcircinelloides*, ≥ 7cm for *Mucorgenevensis* and these fungi were isolated from herbivores dung. This is a manifestation of the bacterial (isolates M170, M2501, B1303 and L1303) bounteous ability to degrade the substrate. The polygalacturonase producing bacteria are visualized by flooding the plate with few ml of a solution containing 1gm of Iodine and 5gms of potassium Iodide dissolved in 330ml of H_2o as suggested by Marcia *et al* 1999. There were different ways of visualization of polygalacturonase producing microorganisms apart from the above mentioned one in the literature. For example, Beg *et al* 2000 used 1% cetrimide solution, Aminzadeh *et al* 2007, Nagapadma 2015- ruthenium red, Namasivayam *et al* 2011-3.3% CIAB, etc. Thus whatever may be the indicator used the confirmation is based on zone of clearance and the diameter of halo corresponds to enzyme activity. Thus the soils of commercial crop field's standout to be the suitable habitats for polygalacturonase producing bacteria. Also, proved that Habitats are inhabitants of diversified microorganisms.

Conclusion

The study is unique in that West Godavari district has not featured in any agrarian reports on bacterial polygalacturonase (EC. 3.2.1.67) production. It has thus been proved that the soils of the district are fertile and rich enough to yield ample bacteria capable of enzyme production. Thus potentially right area has been chosen for conducting the study fruitfully.

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