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Screening of cellulase and pectinase by using *Pseudomonas fluorescence* and *Bacillus subtilis*

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ABSTRACT

A study was conducted to determine the Production of cellulase and pectinase enzyme by using Plant growth promoting rhizobacteria like *Pseudomonas fluorescence* and *Bacillus subtilis*. These to micro organism are isolated by serial dilution method. One gram of soil sample was diluted in to 10 ml of sterile distilled water and 1 ml of sample solution was serially diluted in 9ml of sterile water up to 10 dilution. Each sample from dilution 10^{-5} and 10^{-6} were taken and streaked in to KB and NA medium and incubate at 24 hrs. After 24 hrs *Pseudomonas fluorescence* and *Bacillus subtilis* was observed in the medium of KB and NA medium. Both the culture was sub cultured and maintain in the same for the further work. CMCase medium was prepared and sterilized by autoclave for 121 ºC for 15 minutes after sterilization these medium contain petriplate was streaked by bacteria and incubates for 48h after incubation period a clear halo zone was produced by these bacteria among these bacteria *Pseudomonas fluorescence* are able to produce high amount of cellulose compare to *Bacillus subtilis*. Pectin agar medium was prepared and sterilized by autoclave for 121 ºC for 15 minutes after sterilization these medium contain petriplate was streaked by bacteria incubates for 48h after incubation period a clear halo zone was produced by these bacteria, among these bacteria *Pseudomonas fluorescence* are able to produce high amount of Pectinase compare to *Bacillus subtilis*. Plant growth promoting rhizobacteria (PGPR) are beneficial bacteria that colonize plant roots and enhance plant growth by a wide variety of mechanisms.

***Keywords*:** *Pseudomonas fluorescence*; *Bacillus subtilis* PGPR cellulase and pectinase

1. INTRODUCTION

The rhizosphere, representing the thin layer of soil surrounding plant roots and the soil occupied by the roots, supports large active groups of bacteria (Villacieros et al., 2003) known as plant growth promoting rhizobacteria (PGPR) (Kloepper et al., 1980). Plant growth promoting rhizobacteria are known to rapidly colonize the rhizosphere and suppress soil borne pathogens at the root surface (Rangajaran et al., 2003). These organisms can also be beneficial to the plant by stimulating growth (Bloemberg and Lugtenberg, 2001; Moeinzadeh et al., 2010). Among these organisms, Fluorescent *Pseudomonads* are considered to be the most promising group of plant growth promoting rhizobacteria involved in biocontrol of plant diseases (Gardner et al., 1984; Moeinzadeh et al., 2010). Application of some PGPR strains to seeds or seedlings has also been found to lead to a state of induced systemic resistance in the treated plant. In addition to improvement of plant growth, PGPR are directly involved in

increased uptake of nitrogen, synthesis of phytohormones, solubilization of minerals such as phosphorus, and production of siderophores that chelate iron and make it available to the plant root (Gyaneshwar et al., 1998). It has also been reported that PGPR is able to solubilize inorganic and organic phosphates in soil. It has also been reported that PGPR is able to solubilize inorganic and organic phosphates in soil. Diverse array of *Bacillus* as PGPR in crop ecosystem 47 of microorganisms contribute toward the biological control of plant pathogens, most research has utilized species of *Bacillus*, *Trichoderma* and *Pseudomonas* (McSpadden and Driks, 2004). Pseudomonades are gram-negative, straight or slightly curved rods with polar flagella; they are chemo-organotrophs with a respiratory, non-fermentative type of metabolism and are usually catalase and oxidase-positive. *Ps*eudomon*as* spp. is considered one of the most important biocontrol agents (Daniel and Fergal, 1992); they inhibit microorganisms by several mechanisms such as antibiotic production (Howie and Suslow, 1991), production of secondary metabolites (Dunne et al., 1996) or siderophore production (Meyer, 2007). They have been reported to play a role in controlling many plant diseases caused by bacteria, fungi and nematodes (Kavitha and Umesha, 2007). *Bacillus* species are well known for their ability to control plant diseases through various mechanisms, including the production of secondary metabolites. *Bacillus* spp., especially *Bacillus subtilis*, *Bacillus cereus* and *Bacillus amyloliquefaciens* are effective for the control of plant diseases caused by soil borne, foliar, and postharvest fungal pathogens (Abeysinghe, 2009).

2. MATERIALS AND METHODS

2. 1. General method

All glass ware first soaked in cromic acid cleaning solution containing potassium dichromate in 25 % sulphuric acid for 3 hrs and washed thoroughly in tap water. After a second wash in detergent solution. They were again washed thoroughly in tap water and finally rinsed in distilled water and dried in an oven

2. 2. Sterilization

Glass ware and culture media were sterilized in an autoclave at 121 ºC for 20 minutes at 15 psi.

2. 3. Chemicals

By using the following chemicals for isolation of PGPR bacteria and production of the enzyme like pectinase and cellulose.

King's B medium (Kings et al., 1954)

Peptone	: 20.0 g
Potassium dihydrogen phosphate	: 1.5 g
Magnesium sulphate	: 1.5 g
Glycerol	: 10.0 ml
Agar	: 15.0 g
Distilled water	: 1000 ml
pH	: 7.2

A. Nutrient agar (Anonymous, 1957)

Peptone	: 5.0 gm
Beef extract	: 3.0 gm
Sodium chloride	: 5.0 gm
Distilled water	: 1000 ml
pH	: 6.8-7.2
Agar	: 18.00 gm
L-Tryptophan stock solution (1%)	: 100 µl

B. CMC agar:

Peptone	: 5 gm
Nacl	: 5 gm
Yeast	: 3 gm
Agar	: 20 gm
Distilled water	: 1000 ml
carboxy methyl cellulose	: 5 gm

2. 4. Isolation of *Pseudomonas fluorescens* and *Bacillus subtilis*

Pseudomonas fluorescens and *Bacillus subtilis* were isolated from the rhizosphere soil of Botanical garden, Department of Botany, Annamalai university one gram of soil sample was diluted in to 10 ml of sterile distilled water and 1 ml of sample solution was serially diluted in 9 ml of sterile water up to 10 dilution Each 1 ml of sample from dilution 10^{-5} and 10^{-6}. Were taken and poured in to KB and NA medium and incubate at 48 hrs. Both the culture was sub cultured and maintain in the same for the further work.

2. 5. Detection and production of Cellulase

First we have to prepare the nutrient agar medium quadrate strength that means 1000 ml/4 and add carboxy methyl cellulose 0.5 % and autoclave at 121 ºC for 15 minutes and poured in to the petriplate after the medium solidification. Then streak the selected bacterium and incubate 48h.

2. 6. Detection and production of Pectinasa

First we have to prepare the nutrient agar medium quadrate strength that means 1000 ml/4 and add Pectine 0.5 % and autoclave at 121 ºC for 15 minutes and poured in to the petriplate after the medium solidification. Then streak the selected bacterium and incubate 48 h.

3. RESULT AND DISCUSSION

3. 1. Isolation of PGPR bacteria

Plant growth promoting rhizobacteria *Pseudomonas fluorescens* and *Bacillus subtilis* were isolated from the rhizosphere soil of Botanical garden, Department of Botany, Annamalai university. Pseudomonas fluorescens were isolated by using kings B medium, Bacillus subtilis were isolated by using NA medium. bacterial isolates were selected for further biochemical characteristics like Cellulase and Pectinase production.

3. 2. Enzyme Activities

The enzymes which were investigated in present study consisted of CMCase, pectinase. Present study, CMCase medium was prepared and sterilized by autoclave for 121 ºC for 15 minutes after sterilization these medium was poured in to petriplate and incubates for 48h after incubation peried a clear halo zone was produced by these bacteria among these bacteria *Pseudomonas fluorescens are able to produce high amount of cellulase compare to Bacillus subtilis.* Pectinase medium was prepared and sterilized by autoclave for 121 ºC for 15 minutes after sterilization these medium was poured in to petriplate and incubates for 48 h after incubation period a clear halo zone was produced by these bacteria, among these bacteria *Pseudomonas fluorescens are able to produce high amound of Pectinase compare to Bacillus subtilis.*

Pseudomonas fluorescens and *Bacillus subtilis* have the ability to produce the cellulase enzyme, these enzyme have the ability to degrade the fungal cell wall this is an important mechanism of fungal inhibition. Pectinase is a group of enzyme that known to catalyse the pectic substance through depolymerisation and deexrification reaction. These enzyme have the role in preventing plant from infection caused by pathogen. The improved plant growth by PGPR is due to its ability to produce phytohormones such as indole acetic acid (IAA) (Pattern and Glick, 2002), gibberellic acid (Mahmoud et al. 1984), cytokinin (Tien et al., 1979) and its ability to produce ACC-deaminase to reduce the ethylene in the roots of the developing plants thereby increasing the root length and growth (Penrose and Glick, 2001). The asymbiotic nitrogen fixation by PGPR also adds to its growth-promoting traits (Figueiredo et al., 2008).

Solubilization of mineral phosphates and mobilization of other essential nutrients by PGPR also helps in growth improvement of plants (Taurian et al., 2010). The cell wall of plant cells are mainly composed of cellulose, which is embedded in an amorphous polysaccharide matrix of hemicelluloses, pectin and some glycols and proteins (Myers, M.L. and D.H. Hubbell, 1987.) Also oxidized inositols serve as non cellulosic cell wall components (Loewus, F.A. and M.W. Loewus, 1983). Yield increase of groundnut by *Pseudomonas* isolates from rhizosphere positively correlated with the ability of these strains to increase available soil phosphorus (Dey et al., 2004 Since pectin is a major constituent of the primary cell wall and middle lamellae and low levels of pectinolytic and cellulolytic activities have been detected in *Azospirillum* cultures, the bacteria may eventually enter the root cortex intercellular spaces via enzymatic degradation of host cell wall middle lamellae (Okon, Y. and Y. Kapulnik, 1986). (Khammas et al 1989*)*. proposed that, except for *A. irakense*, none of the *Azospirillum species* is able to grow on pectin as sole C-source, but (Elbeltagy et al. 2001). showed *Azospirillum lipoferum isolated* of rice had pectinase. There are two general types of wall based on the relative amounts of pectic polysaccharides and the structure and amounts of hemicellulosic polysaccharides.

Type I walls (Gibeaut, D.M. and N.C. Carpita, 1993), which typically contain xyloglucan and/or glucomannan and 20-35 % pectin, are found in all dicotyledons, the non-graminaceous monocotyledons (e.g. Liliidae) and gymnosperms (e.g. Douglas fir). Type II walls are present in the Poaceae (e.g. rice and barley) and are rich in arabinoxylan, but contain < 10 % pectin (Gibeaut, D.M. and N.C. Carpita, 1993).

Table 1. Evaluation of the ability of microorganisms to exhibit Cellulase and pectinase activity invitro condition.

Isolates	Cellulase	Pectinase
Pseudomonas fluorescence	+++	+++
Bacillus subtilis	++	++

++ = medium producer; +++ = high producer.

4. CONCLUSIONS

From this present study, it can be concluded that rhizobacteria showed variation in their biocontrol characteristics. *Pseudomonas fluorescens* and *Bacillus subtilis* have the ability to produce the cellulase enzyme, these enzyme have the ability to degrade the fungal cell wall this is an important mechanism of fungal inhibition. Pectinase is a group of enzyme that known to catalyse the pectic substance through depolymerisation and deexrification reaction. These enzyme have the role in preventing plant from infection caused by pathogen. The experimental results also showed that cellulose and pectinase producing microbes are involved in plant growth promotion by preventing plant from fungal infection.

References

[1] Abeysinghe, *Plant Pathol. J.* 8 (2009) 9-16.

[2] Bloemberg G. V., Lugtenberg B. J., *Current Opinion Plant Biology* 4 (2001) 343-350.

[3] Daniel J. O., O. Fergal, *Microbiol. Rev.* 56 (1992) 662-676.

[4] Dey R., K. K. Pal, D. M. Bhatt, S. M. Chauhan, *Microbiol*. *Res*. 159 (2004) 371-394.

[5] Dunne C., I. Delany A. Fenton F. O. Gara, *Agronomie* 16 (1996) 721-729.

[6] Elbeltagy A., K. Nishioka, T. Sato, H. Suzuki, B. Ye, T. Hamada, T. Isawa, H. Mitsuia, K. Minamisawa, *Appl. Environ. Microbiol.* 67 (2001) 5285-5293.

[7] Figueiredo M. V. B., C. R. Martinez H. A. Burity, C. P. Chanway, *World J. Micro. Biotechnol.* 24 (2008) 1187-1193.

[8] Gardner J. M., Chandler L., Feldman A. W., *Plant Soil* 77 (1984) 103-113.

[9] Gibeaut D. M., N. C. Carpita, *Plant. J.* 3 (1993) 1-30.

[10] Gyaneshwar P., Naresh Kumar G., Parekh L. J., *World J. Microbial. Biotechnol.* (1998) 669-673.

[11] Howie W. J., T. V. Suslow, *Mol. Plant Microbe Interact.* 4 (1991) 393-399.

[12] Kavitha R., S. Umesha, *Crop Prot.* 26 (2007) 991-997.

[13] Khammas K. M., E. Ageron, P. A. D. Grimont, P. Kaiser, *Res. Microbiol.* 140 (1989) 679-693.

[14] Kloepper J. W., Leong J., Teintze M., Schroth M. N., *Nature* 268 (1980) 885-886.

[15] Loewus F. A., M. W. Loewus, *Annu. Rev. Plant Physiol.* 34 (1983) 137-161.

[16] Mahmoud S. A. Z., E. M. Ramadan, F. M. Thabet, T. Khater, *Zbl. Mikrobiol*. 139 (1984) 227-232.

[17] McSpadden Gardener B. B., A. Driks, *Phytopathol.* 94 (2004) 1244.

[18] Meyer J. M. (2007). Siderotyping and bacterial taxonomy: a siderophore bank for a rapid identification at the species level of fluorescent and non-fluorescent *Pseudomonas*. In: Varma, S., Chincholkar, S. (Eds.), Microbial Siderophores. Springer, New York, pp. 43-61.

[19] Moeinzadeh A., Sharif-Zadeh F., Ahmadzadeh M., Heidari Tajabadi F., *Australian Journal of Crop Science* 4 (2010) 564-570.

[20] Okon Y., Y. Kapulnik, *Plant. Soil* 90 (1986) 3-16.

[21] Pattern C. L., B. R. Glick, *Appl. and Environ. Micro.* 68 (2002) 3795-3801.

[22] Penrose D. M., B. R. Glick, *Canadian J. Microbiol.* 47 (2001) 368-372.

[23] Rangajaran S., Saleena L. M., Vasudevan P., Nair S., *Plant Soil* 251 (2003) 73-82.

[24] Taurian T., M. S. Anzuay, J. Angelini, M. L. Tonelli, L. Luduena, A. Fabra, *Plant Soil* 329 (2010) 1016-1024.

[25] Tien T. M., M. H. Gaskins, D. H. Hubbel, *Appl. Environ. Microbiol.* 37 (1979) 1016-1024.

[26] Villacieros M. et al., *Plant Soil* 251 (2003) 47-54.

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