

Analysis of phosphate solubilizing bacteria in soil



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1. INTRODUCTION

Cereals are the world's major source of food for human nutrition and rice (*Oryza sativa* L.) is one of the predominant cereal crop and represent staple diet for more than two fifth of the world population. To feed the ever increasing human population, the world's yearly rice manufacturing to be bound by an obligation act out of 560 million tonnes to 760 million tonnes by 2020. The future increase in rice production has to come from the same or even reduced land area and the productivity yield (per ha) must be greatly enhanced by providing additional nutrient input and through effective control of phytopathogens. Blast disease of rice caused by *Pyricularia oryzae* is one of the most destructive fungal diseases of rice causing loss up to 90% and has a ubiquitous occurrence in almost all the rice growing countries (Mehrotra, 1980).

Phosphorus has been called " the key of life" because it is directly involved in most of the life processes. Next to nitrogen it is invariably classified as one of the macronutrients and it is a key element in frequency of use as fertilizer. It serves as a primary energy source for microbial oxidation. It is a constituent substance in life processes. Soil cannot give high yields if it is deficient in phosphate.

Different mechanisms have been suggested for the solubilization of inorganic phosphorus by phosphate solubilize. It is usually accepted that the important mechanism of mineral phosphate solubilization is the movement of organic acids the combination of various thing into one whole by soil microorganisms

(Halder *et al.* , 1990). Manufacturing of organic acids results in acidification of the microbial cell and its lying on the outskirts.

Resultant, inorganic phosphate may be released from a mineral phosphate by proton addition for Calcium ion (Goldstein, 1994). The production of organic acids by PSB has been deep notified. Among them, gluconic acid seems to be the most repeated occurrence agent of mineral phosphate solubilization.

Being an in specified microorganism is known to be involved in the solubilization of insoluble phosphates (Alexander, 1977). These phosphate-solubilizing microorganisms render insoluble phosphate into soluble group formed completely the series of acidification and chelation of some reactions. This process not only compensates for higher cost of manufacturing fertilizers in industry but also mobilizes the fertilizers added to soil (Rodriguez and Reynaldo, 1999). Therefore, many researchers have tried to increase the plant-available phosphate fraction by means of Phosphate solubilizing microorganisms (PSMs) such as *Achromobacter* sp, *Agrobacterium* sp, *Alcaligenes* sp , *Bacillus cereus*, *B. polymyxa*, *B. megaterium*, *B. subtilis*, *Pseudomonas striata* and *Xanthomonas* sp and Fungi like *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *Penicillium* sp. and *Rhizopus* sp.

Several mechanisms of plant - microbe interaction may participate in the association and affect plant growth, including IAA, Siderophore production and biocontrol against *Pyricularia oryzae* . Thus, the Plant Growth Promoting Rhizobacteria (PGPR) affect the plant growth through direct promotion by

producing plant growth promoting substances and through indirect promotion by acting against plant pathogenic microorganisms (Kloepper *et al.*, 1989).

Plant growth promoting rhizobacteria (PGPR) are free - living, soil - borne bacteria, which enhance the growth of the plant either directly or indirectly (Kloepper *et al.*, 1980; Glick and Ibid, 1995). The direct mechanisms involve nitrogen fixation, phosphorus solubilization, HCN production, manufacturing of phytohormones such as auxins, cytokinins and gibberellins, and lowering of ethylene concentration (Glick and Ibid, 1995; Glick *et al.*, 1999). Bacteria belonging to the genera *Azospirillum*, *Pseudomonas*, *Xanthomonas*, and *Rhizobium* as well as *Alcaligenes faecalis*, *Enterobacter cloacae*, *Acetobacter diazotrophicus* and *B radyrhizobium japonicum* have been shown to that which is produced auxins which help in inducing plant growth increase (Patten and Glick, 2002).

There are many reports on plant growth promotion and yield enhancement by plant growth promoting rhizobacteria (PGPR) (Lugtenberg *et al.*, 2001). The mechanisms of plant growth increase the promotion by PGPR include: the ability to produce phytohormones, N₂ fixation, antagonism against phytopathogens and solubilization of insoluble phosphates (Lugtenberg and Kamilova, 2009). It was also suggested that the PGPR can also prevent the deleterious effects of stresses from the environment (Paul and Nair, 2008).

Composting is a biotechnological process by which different microbial communities convert organic wastes into a stabilized form. During the process, temperatures arise because of the heat released due to biological

activity. These temperatures are responsible for pathogen inactivation.

Composting is an aerobic process that requires O_2 , optimal moisture and enough free air space and C: N ratio within certain limits. The treatment by composting leads to the development of microbial populations, which causes numerous physicochemical changes within mixture. These changes could influence the metal distribution through release of heavy metals during organic matter mineralization or the metal solubilization by the decrease of pH, metal biosorption by the microbial biomass or metal complexation with the newly formed humic substances or other factors (Rahul Kumar *et al.*, 2010).

One of the most effective means of recycling any organic wastes for agricultural use is by means of composting, an accepted practice in India and elsewhere. In many cases in India, it is valuable to add nutrients to compost to increase its fertilizer value. Although, sugar industry wastes are relatively high in nitrogen, calcium, magnesium and potassium, they are generally deficient in phosphorus, iron and zinc when compared to fertilizers commonly used in India. Further, the possibility of enriching organic wastes with micronutrients like Fe and Zn, which have become critical in crop production, have been studied and their effectiveness is increased appreciably through combined application of organics with $FeSO_4$ and $ZnSO_4$ in addition to N, P, K fertilizers (Deepa Devi, 1992; Sennimalai, 1994).

Therefore, it is appropriate to develop composting systems that are capable of converting these agroindustrial wastes into valuable organic fertilizers.

Among the microbes, bacteria are the most important one for decomposing waste. Bacteria use press mud for their metabolism and finally they produce

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some simple and useful compounds from them which are important for soil health, plant growth and over all to keep well balance of natural ecosystem (Zaved *et al.*, 2008). Moreover, efficiency of bacterial in bioconversion or organic compounds is well documented (Petre *et al.*, 1999; Suhaimi *et al.*, 2012). Unfortunately, knowledge on physico-chemical and microbial diversity of bacteria on bioconversion of sugarcane press mud is limited. Therefore, this study was conducted to monitor the chemical and biological changes during composting of sugarcane press mud with cattle manure in order to get high quality stabilized product within the goal of shortening the stabilization time.

The pressmud biocompost contains appreciable amount of plant nutrients *viz.*, organic carbon, nitrogen, phosphorus, potassium, calcium and magnesium along with traces of micronutrients *viz.*, Zn, Fe, Cu and Mn (Banulekha, 2007). The beneficial effect of the organic matter for enhancing the soil fertility and thereby improving the crop productivity is well established (Laird *et al.*, 2001).

Objectives of the present study

1. To collect the soil samples from ten different locations in Cuddalore district, Tamil Nadu, India.
2. To isolate and identify the Phosphate solubilizing bacteria (*Bacillus subtilis* , *Bacillus megaterium* , *Enterobacter asburiae*) from collected soil samples.
3. To screen the efficient Phosphate solubilizing bacterial isolates based on production of plant growth promoting substances.

4. Composting of sugar mill waste (pressmud) by Phosphobacterial isolates and analysis of nutrient status of compost mixtures.
5. To study the combined effect of efficient phosphobacterial isolates and compost mixture for the growth and yield of Paddy BPT-5804(*Oryza sativa* L.)

Cellulose, hemicellulose and lignin become or make less indicate in the present study during the bacterial consortium based composting process. Singh and Sharma (2002) reported rapid decomposition of wheat straw with a mixture of cellulolytic fungi, *Pleurotus sajor-caju* , *Trichoderma reesei* , *Aspergillus niger* along with nitrogen fixing bacteria *Azotobacter chroococcum* . The simulated activity of bacterial consortium present in the waste substrate ability to do have characterised by intensity cellulolysis and lignolysis as advised done by Loquet *et al* . (1984).

Hemicellulosic residue of pressmud contains a large quantity of xylans. Xylanase are enzymes that are capable of degrading xylan units yielding large quantities of monomeric xylose units. The xylanases activities during composting. The xylanase activity in all the treatments was found to be on the 30th day and thereafter a sharp decline was noticed. During 90th days maximum xylanase activity recorded in CM - 8 (Pressmud + *Bacillus subtilis* + *Bacillus megaterium* + *Enterobacter asburiae*) (5. 91 U ml⁻¹ of protein) followed by CM -5 (4. 93 U ml⁻¹ of protein), CM - 6 (3. 91 U ml⁻¹ of protein), CM - 3 (3. 89 U ml⁻¹ of protein), CM - 2 (3. 00 U ml⁻¹ of protein), CM - 4 (2. 92 U ml⁻¹ of protein) and CM - 6 (2. 00 U ml⁻¹ of protein). The minimum

amount of xylanase activity recorded in CM - 1 (pressmud alone) (1. 82 U ml⁻¹ of protein).

The survival and microbial activity during the entire composting process was studied by estimating dehydrogenase activity. During 90th day, maximum dehydrogenase activity recorded in CM - 8 (Pressmud + *Bacillus subtilis* + *Bacillus megaterium* + *Enterobacter asburiae*) (3. 09 mg formazon formed h⁻¹ g⁻¹) followed by CM - 7 (2. 95 mg formazon formed h⁻¹ g⁻¹), CM - 6 (2. 39 mg formazon formed h⁻¹ g⁻¹), CM - 5 (2. 30 mg formazon formed h⁻¹ g⁻¹), CM - 4 (1. 71 mg formazon formed h⁻¹ g⁻¹), CM - 3 (1. 70 mg formazon formed h⁻¹ g⁻¹) and CM - 2 (1. 68 mg formazon formed h⁻¹ g⁻¹). Lower amount of dehydrogenase activity recorded in CM - 1 (Pressmud alone) (1. 00 mg formazon formed h⁻¹ g⁻¹).

In general, percentage cellulose reduction increased thereafter decrease in the period of decomposition in press mud up to end of 90days. The initial cellulose content of pressmud was 15. 75 % explained. During 90th day, maximum cellulose reduction recorded in CM - 8 (Pressmud + *Bacillus subtilis* + *Bacillus megaterium* + *Enterobacter asburiae*) (3. 81%) followed by CM - 5 (3. 88%), CM - 6 (3. 99%), CM - 7 (4. 09%), CM - 3 (4. 2%), CM - 2 (4. 62%) and CM - 4 (4. 65%). The minimum cellulose reduction recorded in CM - 1 (pressmud alone) (5. 62%).

Changes in reduction of hemicellulose content during composting of pressmud are studied. From the results, it was noticed that the reduction of hemicellulose content increased thereafter decreased in all treatments.

During 90th day, reduction in hemicellulose content recorded in CM - 8 (Pressmud + *Bacillus subtilis* + *Bacillus megaterium* + *Enterobacter asburiae*) (7.76%) followed by CM - 6 (7.85%), CM - 5 (7.90%), CM - 7 (7.92%), CM - 3 (7.95%), CM - 2 (9.22%) and CM - 4 (9.37%). Least amount of reduction in hemicellulose content recorded in CM - 1 (Pressmud alone) (13.00%).

Inoculation levels were found to have significant effect on reduction in lignin content. During 90th day, reduction of lignin content in triple inoculants compost mixture recorded in CM - 8 (Pressmud + *Bacillus subtilis* + *Bacillus megaterium* + *Enterobacter asburiae*) (6.10%), followed by CM - 5 (6.31%), CM - 6 (6.64%), CM - 7 (6.69%), CM - 2 (6.81%), CM - 2 (7.60%) and CM - 4 (7.70%). The lowest reduction in lignin content recorded in CM - 1 (Pressmud alone) (8.24%).

Faryal *et al.* (2006) have suggested the effects of inoculation with three thermophilic species of *Bacillus* on the composting activity of drainage water that passes through sewers sludge amended with black combustible mineral fly the grey soft remains as the causer or source.

The bacteria population present in the compost mixtures were estimated quantitatively during the 90th day. In all the treatments the bacterial population was more in CM - 8 (29.32×10^6 cfu g⁻¹) and less in CM-1 (15.00×10^6 cfu g⁻¹).

The effect of Compost mixture (Pressmud + *Bacillus subtilis* + *Bacillus megaterium* + *Enterobacter asburiae*) and Phosphate solubilizing bacteria on plant height of paddy var BPT - 5804 (*Oryza sativa* L.) was measured.

Among the various treatments tested, the highest plant height was recorded in the treatment T₈ (75% NPK + Compost Mixture - 8 + BS + BM + EA) (121 . 98 cm). The least plant height was recorded in T₉ (Control) (89. 30 cm).

The effect of Compost mixture (Pressmud + *Bacillus subtilis* + *Bacillus megaterium* + *Enterobacter asburiae*) and Phosphate solubilizing bacteria on Dry matter production of paddy var BPT - 5804 (*Oryza sativa* L.) was investigated. Among the nine treatments tested, the dry matter production was maximum in treatment T₈ (75% NPK + CM+BS+BM+EA) (8. 12 t ha⁻¹). The lowest chlorophyll content was recorded in T₉ (Control) (4. 00 t ha⁻¹).

The effect of Compost mixture (Pressmud + *Bacillus subtilis* + *Bacillus megaterium* + *Enterobacter asburiae*) and Phosphate solubilizing bacteria on Leaf area index at flowering of paddy var BPT - 5804 (*Oryza sativa* L.) was investigated. Among the nine treatments tested, the Leaf area index at flowering was maximum in treatment T₈ (75% NPK + CM+BS+BM+EA) (6. 42 cm). The lowest grain yield was recorded in T₉ (Control) (3. 98cm).

The Compost mixture (Pressmud + *Bacillus subtilis* + *Bacillus megaterium* + *Enterobacter asburiae*) and Phosphate solubilizing bacteria on chlorophyll content of paddy var BPT - 5804 (*Oryza sativa* L.) was investigated. Among the nine treatments tested, the chlorophyll content was maximum in the treatment T₈ (75% NPK + CM+BS+BM+EA) (3. 09 mg/g of leaf). The lowest chlorophyll content was recorded in T₉ (Control) (3. 00 mg/g of leaf).

The effect of Compost mixture (Pressmud + *Bacillus subtilis* + *Bacillus megaterium* + *Enterobacter asburiae*) and Phosphate solubilizing bacteria on number of tillers plant⁻¹ of paddy var BPT - 5804 (*Oryza sativa* L.) was investigated. Among the nine treatments tested, the number of tillers plant⁻¹ was maximum in treatment T₈ (75% NPK + CM+BS+BM+EA) (18.86). The treatment T₁ (100% NPK) (18.52) was on par with the treatment T₈. The lowest tillers plant⁻¹ was recorded in T₉ (Control) (10.56).

The effect of Compost mixture (Pressmud + *Bacillus subtilis* + *Bacillus megaterium* + *Enterobacter asburiae*) and Phosphate solubilizing bacteria on Number of tillers panicles hill⁻¹ of paddy var BPT - 5804 (*Oryza sativa* L.) was investigated. Among the nine treatments tested, the Number of tillers panicles hill⁻¹ was maximum in treatment T₈ (75% NPK + CM+BS+BM+EA) (6.37). The treatment T₁ (100% NPK) (6.01) was on par with the treatment T₈. The minimum tillers panicles hill⁻¹ was recorded in T₉ (Control) (3.00).

The effect of Compost mixture (Pressmud + *Bacillus subtilis* + *Bacillus megaterium* + *Enterobacter asburiae*) and Phosphate solubilizing bacteria on Number of filled grains panicles⁻¹ of paddy var BPT - 5804 (*Oryza sativa* L.) was investigated. Among the nine treatments tested, the Number of filled grains panicles⁻¹ was maximum in treatment T₈ (75% NPK + CM+BS+BM+EA) (73.03). The treatment T₁ (100% NPK) (72.78) was on par with the treatment T₈. The lowest filled grains panicles⁻¹ was recorded in T₉ (Control) (57.24).

The effect of Compost mixture (Pressmud + *Bacillus subtilis* + *Bacillus megaterium* + *Enterobacter asburiae*) and Phosphate solubilizing bacteria on Number of thousand grain weight of paddy var BPT - 5804 (*Oryza sativa* L.) was investigated. Among the nine treatments tested, the No of thousand grain weight was maximum in treatment T₈ (75% NPK + CM + BS + BM + EA) (21. 56). The treatment T₁ (100% NPK) (21. 01) was on par with T₈. The minimum grain weight was recorded in T₉ (Control) (14. 23).

The effect of Compost mixture (Pressmud + *Bacillus subtilis* + *Bacillus megaterium* + *Enterobacter asburiae*) and Phosphate solubilizing bacteria on grain yield and straw yield of paddy var BPT - 5804 (*Oryza sativa* L.) was investigated. Among the nine treatments tested, the grain yield and straw yield content was maximum in treatment T₈ (75% NPK + CM + BS + BM + EA) (47. 36), (63. 76) and the treatment T₁ (100% NPK) (46. 88), (63. 24) was on par with the treatment T₈. The lowest grain yield was recorded in T₉ (Control) (25. 23), (49. 76).

The effect of Compost mixture and Phosphate solubilizing bacteria on nutrients uptake (N, P & K) was determined. Among the treatments tested, maximum NPK uptake was recorded in the treatment T₈ (75% NPK + BS + BM + EA) (121. 56 kg ha⁻¹), (22. 86 kg ha⁻¹), (118. 73 kg ha⁻¹). The treatment T₁ (100% NPK) (121. 02 kg ha⁻¹), (22. 41 kg ha⁻¹) and (118. 31 kg ha⁻¹) was on par with the treatment T₈. The lowest NPK was recorded in T₉ (Control) (92. 85 kg ha⁻¹), (11. 77 kg ha⁻¹) and (89. 82 kg ha⁻¹).

The effect of Compost mixture and Phosphate solubilizing bacteria on nutrients uptake (N, P & K) was determined. Among the treatments tested, maximum NPK uptake was recorded in the treatment T₈ (75% NPK + BS + BM + EA) (72.85 kg ha⁻¹) (18.73 kg ha⁻¹) and (81.67 kg ha⁻¹). The treatment T₁ (100% NPK) (72.43 kg ha⁻¹), (18.51 kg ha⁻¹) and (81.47 kg ha⁻¹) was on par with T₈. The minimum NPK was recorded in T₉ (Control) (46.21 kg ha⁻¹), (08.05 kg ha⁻¹) and (56.12 kg ha⁻¹).

The effect of Compost mixture and Phosphate solubilizing bacteria on bacterial population was studied. Highest bacterial population was recorded in the treatment T₈ (Bacteria - 36.66×10^6 cfu g⁻¹) and the lowest bacterial population (19.27×10^6 cfu g⁻¹).