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Cellulolytic bacteria with plant growth promoting properties as an efficient microbial strategy for composting

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Aims: The aim of this study was to screen plant growth promoting cellulolytic bacteria.
Methodology and results: Cellulolytic bacteria were isolated from soil, filter cake and distillery slop samples. Isolates were evaluated for their capacity to promote plant growth, harness soil fertility. Two isolates of bacteria screened from distillery slop (BDS 31) and filter cake (BFC8) decomposed carboxymethyl cellulose on the agar plate and showed the highest hydrolysis capacity 3.25-3.5. These isolates also fixed atmospheric nitrogen and produced Indole-3-acetic acid (IAA) in broth cultures. Bacterium isolate BFC8 solubilized Tri-calcium phosphate in Pikovskaya agar and bacterium isolate BDS31 inhibited the growth of rice blast fungus (*Pyricularia oryzae*).
Conclusion, significance and impact study: The results from this experiment suggested that bacteria isolate BFC8 and BDS31 had the properties of plant growth promoting. Moreover, these bacteria had high efficiency of degradation of carboxymethyl cellulose. Therefore, it is an alternative for using as microbial inoculums for composting.

Keywords: Cellulolytic bacteria, plant growth promoting bacteria, rice blast fungus

INTRODUCTION

Cellulose is a component of plant on earth and is a linear polysaccharide of glucose residues with β-1, 4 glycosidic linkages. Cellulolytic microorganisms are the group of microbes that are able to degrade cellulose. A variety of microbes (fungi, bacteria and actinomycetes) are capable of breaking down cellulose in the environment. Fungi such as *Aspergillus* and *Trichoderma* genera and actinomycetes such as *Streptomyces* etc. play a major role in degradation of cellulose. On the other hand, bacteria rather seem to help further decomposition of degraded cellulose under humid condition (Betrabet *et al*., 1968). Composting is a way to convert agricultural waste to a valuable product. Mesophilic and thermophilic cellulolytic microorganisms play a significant role in the composting process. Cellulolytic microbes are often added to compost to enhance decomposition of the organic materials.

Several bacteria and mold are known to decompose plant litter. A leaf litter with C/N ratio of 19.56 that was inoculated with bacteria and molds showed a faster process than untreated litter (Hidayanti *et al*., 2013). Study conducted on sandy soil and sugar beet haulms compost indicated that *Trichodema viride* NRC6 or *Streptomyces aureofaciens* NRC22 are effective decomposer of cellulose. (Badr EL-Din *et al*., 2000). Furthermore, compost treated with these microorganisms and arbuscular mycorrhizal fungi increased plant protection by 80%, 75%, and 73% (respectively) as compared to control that was treated only by the NPK chemical fertilizer (Badr EL-Din *et al*., 2000).

Plant growth promoting microorganisms may have direct or indirect role in promoting plant growth and development. They play vital role in solubilizing phosphate, fixing nitrogen, producing IAA and inhibiting plant pathogens etc. Identification and isolation of cellulolytic microorganisms that also enhance plant growth has agricultural significance value and increase importance of composts. Therefore, the aim of this study was to screen plant growth promoting cellulolytic bacteria.

MATERIALS AND METHODS

Screening of cellulolytic bacteria

Microorganisms isolated from soil compost and filter cake samples were obtained from Microbial fertilizer KKU Lab group, Department of Microbiology, Faculty of Science, Khon Kaen University, Thailand. Cellulose decomposition test was performed on carboxymethyl cellulose agar (CMC agar) with pH 7. The samples were incubated at 45 °C for 3 days. Isolates of bacteria that showed highest hydrolysis capacity value (HC) of halo zone in CMC agar after flooding with iodine solution for 5 min were selected for further study to check whether they have some properties of plant growth promoting microorganism. Their ability to fix nitrogen, solubilize phosphate, produce IAA was evaluated. The HC value was measured as the ratio

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of diameter of clearing zone and colony (Xu and Yang, 2010).

Study of some properties of cellulolytic bacteria as plant growth promoting microorganism

Nitrogen fixing cellulolytic bacteria

Preliminary screening for nitrogen fixing microorganisms was done as follow: cellulolytic microorganism was inoculated in Nitrogen free medium (Ashby's broth) and incubated at 45 °C for 5 days. The presence of turbidity in cultured broth was used as an indicator for positive nitrogen fixing microorganism.

Phosphate solubilizing cellulolytic bacteria (Patil *et al*., 2011)

Phosphate solubilization of cellulolytic microorganisms was tested on Pikovskaya agar containing tricalcium phosphate by spot inoculation method. The medium per liter consisted of 10 g of glucose, 0.5 g of $(NH_4)_2SO_4$, 0.2 g of KCl, 0.1 g of $MgSO_4 \cdot 7H_2O$, 0.5 g of yeast extract, 0.03 g of $FeSO_4 \cdot 7H_2O$ and 0.02 g $MnSO_4 \cdot H_2O$, 10 g of $Ca_3(PO_4)_2$ and 15 g agar. Plate was incubated at 45 °C for 3 days. Positive phosphate solubilization of microorganisms was shown by the presence of clearing zone around colony.

IAA producing cellulolytic bacteria (Husen, 2003)

Cellulolytic microorganism was inoculated in Nutrient broth (NB) which contained L-tryptophan as a precursor for 48 h on the shaker at room temperature. The production IAA was detected by adding 2 mL of Salkowski's reagent to broth culture and NB contained L-tryptophan (control) to development of a pink color indicates the presence of indole.

Anti- rice blast fungus activity assay

Two cellulolytic bacterial isolates were tested in their ability to inhibit rice blast fungus (*Pyricularia oryzae*) growth on PDA plates using the dual culture technique. Bacterial isolates were cultured on NA plate for 48 h whereas a rice blast fungus was cultured on PDA plate for 7 days. A plug of mycelium of a rice blast fungus was placed at the center of PDA plate. Cellulolytic bacteria was transferred to the same plate by streaking at the distance of 2 cm from the edge of the plate and incubated for 7 days at 30 °C. The diameter of inhibition zone (cm) of dual culture plate and the radial of mycelium growth of rice blast fungus in the control plate was measured and used for calculating of percent inhibition of radial growth (PIRG).

RESULTS AND DISCUSSION

Screening of cellulolytic bacteria

Twenty six of cellulolytic bacterial isolates were studied. The BDS31 and BFC8 isolates that were screened from distillery slop and filter cake sample, respectively, showed the highest hydrolysis capacity value (Figure 1). The HC values of two isolates were greater than 3.0. Two of the bacteria isolates were identified as effective decomposer of cellulolytic compound. Their hydrolysis capacity value was higher than the HC value from *Trichoderma* spp. (HC value 2-2.5) (Gochev and Krastanov, 2007).

Nitrogen fixing cellulolytic bacteria

Ashby's broth is used for isolation of a non-symbiotic nitrogen fixing bacteria which use mannitol as a carbon source and atmospheric nitrogen as nitrogen source. Dipotassium phosphate provides buffering to the medium. The medium contains essential ions required for promoting growth of the non-symbiotic nitrogen fixing bacteria, except nitrogen. Bacteria isolate BDS31 and BFC8 grew very well in nitrogen free medium. Change in turbidity of the medium indicates the presence of non symbiotic nitrogen fixing microorganism (Figure 2).

Phosphate solubilizing cellulolytic bacteria

Isolate BFC8 was able to solubilize a bound phosphate that was added to the medium in the form of calcium phosphate (Figure 3) Phosphate solubilization is indicated by clearance around the colony. This bacterium released acid to degrade phosphate and it helps neutralize the pH of saline soil. Therefore it can be used as bio-fertilizer in the saline soil (Patil *et al*., 2011).

IAA production of cellulolytic bacteria

Test tube inoculated with isolated BDS31 and isolate BFC8 showed pink color reaction when treated with Salkowski's reagent (Figure 4). No reaction was observed in the control test tube showing that those two cellulolytic bacteria are producing plant growth promoting hormone known as IAA. It enhances seed germination.

Antagonistic cellulolytic bacteria

The positive inhibition zone was revealed in the bacterium isolate BDS31 against *Pyricularia oryzae* (PIRG = 50%) after 7 days of incubation whereas bacterial isolate BFC8 was not able to inhibit the growth of rice blast fungus. This indicated that BDS31 was cellulolytic antagonistic bacterium (Figure 5). It suppresses the incidence of plant pathogens and thus, helps in the bio-control of diseases.

Plant requires a balanced supply of macro and micronutrient for normal growth and development. Microorganisms play vital role in the decomposition of organic matter and regulation of the flux of nutrient in the plant-soil-atmosphere continuum. Some microbes are

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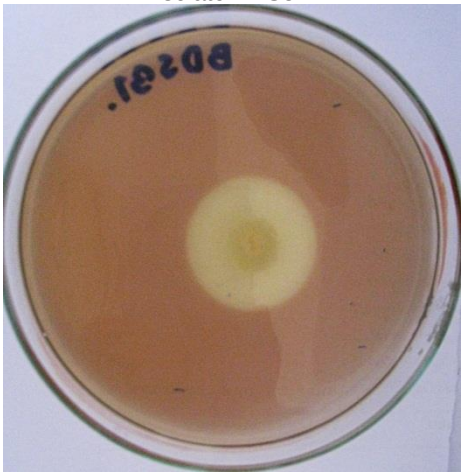
Isolate BDS31



Hydrolysis capacity value=3.50

Isolate BFC8

Hydrolysis capacity value=3.25

Figure 1: Cellulolytic bacteria showing the appearance of clear zone surrounding the colony indicated the decomposition of carboxymethyl cellulose in CMC agar.

Isolate BDS31

Ashby's broth + isolate BDS31 | Ashby's broth (Control)

Isolate BFC8

Ashby's broth (Control) | Ashby's broth + isolate BFC8

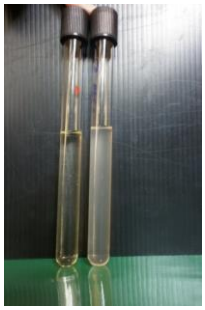
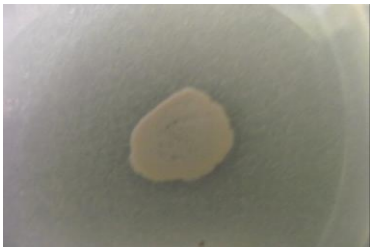


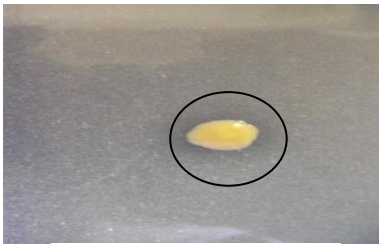
Figure 2: Nitrogen fixing where turbidity indicated growth of cellulolytic bacteria in nitrogen free medium.

Isolate BDS31



Solubilization: Negative

Isolate BFC8



Solubilization: Positive

Figure 3: Phosphate solubilizing cellulolytic bacterium. Clear zone indicated solubilization of Tri-calcium phosphate in Pikovskaya agar.

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Isolate BDS31

1, TSB (control)
2, TSB + reagent (control)
3, IAA production: Positive

Isolate BFC8

1, TSB (control)
2,TSB + reagent (control)
3, IAA production: Positive

Figure 4: IAA production of cellulolytic bacteria, isolate BDS31 and isolate BFC8. Development of a pink color indicated IAA production in NB + L-tryptophan cultured broth.

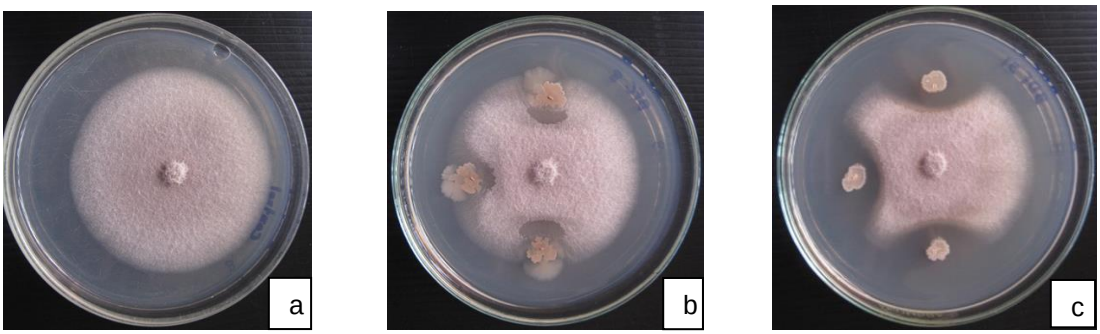

Figure 5: Dual culturing assay of cellulolytic antagonistic bacteria against rice blast fungus (*Pyricularia oryzae*). (a) Control plate: mycelium growth of rice blast fungus; (b) Negative inhibition zone of bacterium isolates BFC8 and (c) Positive inhibition zone of bacterium isolates BDS31.

specialized to fix atmospheric nitrogen and convert to a form that can be used by the plant; others solubilize nutrients and increase its availability, while few of them promote cell division and root growth to improve plants nutrient and water absorption. Identification of microbes that have two or more of these functions has great agricultural significance.

In this preliminary study we isolated cellulolytic bacteria that have a capability to fix nitrogen, solubilize phosphate and promote plant root growth. Two of the isolates (BFC8 and BDS31) appeared to be efficient decomposers of cellulose, have the ability to fix nitrogen non-symbiotically (without host plant) and produce plant growth promoting hormone (IAA). In addition, BFC8 bacterium has shown a promise to be used as phosphate solubilizing agent. Moreover, BSD31 was an antagonistic bacterium. In the previous study, bacteria identification by using full 16S rDNA sequence analysis and morphology study on the agar and under microscope found that isolate BFC8 was *Cronobacter sakazakii* and isolate BSD31 was *Bacillus licheniformis* (Riddech *et al*., 2013). In a separate study, by adding these bacteria to compost as inoculums, we have observed reduction in composting duration from 2-3 months to 1 month. We recommend further research to study large scale agricultural use of these two isolates under natural conditions and to find a way to tap their maximum potential as decomposer, nitrogen fixers, phosphate solubilizer and plant growth promoters.

CONCLUSION

Cellulolytic microbes are a group of microorganisms that can break down cellulose into simpler compounds. Cellulolytic bacteria decompose cellulose by enzymatic mechanisms. An enzyme called cellulase has a major role in cellulose decomposition process. In this experiment, we were able to screen two cellulolytic bacteria that can be used as non symbiotic nitrogen fixer, phosphate solubilizer and plant growth promoters. Two of the bacteria isolates were identified as effectively decomposers of cellulolytic compound.

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