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DISCUSSION
For optimal plant growth and yield, nutrients must be available in sufficient and balanced quantities. Soil contain natural reserves of plant nutrients, but these reserves are largely in forms unavailable to plants; and only a portion is released through biological activity and/or chemical processes. The rhizosphere is the zone of soil surrounding the root system which is majorly affected by these biological and/or chemical processes. The significance of the rhizosphere arises from the release of organic material from the root and the subsequent effect of increased microbial activity on nutrient cycling and plant growth. In the rhizosphere the quantities and the types of substrates are different from those in the bulk soil, and this leads to colonization by different microbial populations of bacteria, fungi, protozoa and nematodes. So, soil microorganisms play a significant role in regulating the dynamics of organic matter decomposition, and the availability of plant nutrients such as N, P, K and S. Over the past years, it is well-recognized that microbial inoculants constitute an important component of integrated nutrient management that leads to sustainable agriculture.
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As cellulose is the most abundant renewable natural polymer in the biosphere (Bakare 2005; Feng et al., 2007), cellulolytic microorganisms are fundamental for the transformation of cellulose into simple sugars that are essential nutrients for various organisms like plants and animals (Arifoglu and Ögel, 2000; Bhat and Bhat, 1997). Additionally, since the annual production of cellulose is estimated at $4.0 \times 10^7$ tons (Bakare et al., 2005), large quantities of industrial and agricultural cellulosic waste have accumulated due to inefficient use of natural microbial sources (Kim et al., 2003; Lee et al., 2008).

The primary objective of present study was to isolate potential cellulolytic bacterial species, through conducting a survey among cellulosic waste of diverse origin, like – kitchen wastes, agricultural waste, and industrial waste, etc, from different sampling sites of Nadia district, West Bengal, India; and their further feasible exploitation in modern day agriculture. Some of previous reports from Nevados Park Colombia, New Delhi in India, and other places also demonstrated similar types of studies on screening of cellulolytic bacteria from environmental sources (Gautam et al., 2012; Torres et al., 2014). Here, microorganisms with cellulolytic potential were selected
depending on qualitative cellulolytic activity by culturing microorganisms in media containing cellulose as the only carbon source, and using assays with congo red. A total of eight sample sites and sample types, designated as – S1 to S8, were selected (details in Chapter 4) from all over Nadia district, West Bengal, India.

According to obtained results, samples isolated from rotten tea leaves, jute rotting pond water, garden litter, and paper mill waste showed good amount microbes with positive cellulolytic activity (details in Chapter 5). The common characters among all the above mentioned sampling sites and types is – the waste materials collected from these sites are very rich in cellulose. The growth of these microorganisms on specialized culture media containing cellulose as a unique carbon source (CMC) varied among the various strains and species, depending on the efficacy of the organisms to grow. This finding is supported by previous reports that showed that small variations in carbon sources, macro- and micro-nutrients, and time of incubation induced differences in microorganism growth (Ahamed and Vermette, 2008; Arifoglu and Ögel, 2000; Grigorevski de Lima et al., 2005; Hanif et al., 2004; Ögel et al., 2001).
As per the primary screening, a total two extremely potent cellulolytic bacterial strains were isolated from all the sampling sites and sampling types. Though, the cellulolytic microbes in nature occur in mixed population of fungi, actinomycetes and bacteria. In present study, bacterial isolates were more abundant than fungi (see Table 5.2, in Chapter 5). Both these isolated bacterial strains showed a distinct growth curve under significant changes in pH and temperature. Some previous reports that showed that small variations in pH, temperature, and time of incubation induced differences in microorganism growth (Ahamed and Vermette, 2008; Hanif et al., 2004).

Finally based on the morphological, cultural and genetic characters (the sequence analyses of 16s rRNA); the most potent cellulolytic bacterial isolates were identified as - Bacillus altitudinis and Bacillus tequilensis. The bacteria isolated in present study have been reported by several other authors for their cellulolytic activity, e.g., Bacillus altitudinis (Shastry and Prasad, 2002; Sreeja et al., 2013) and Bacillus tequilensis (Khiannam et al., 2014). Most of the above isolates have been reported as cellulase producers, but with variable capabilities by several workers (Abhra et al., 1992; Siddiqui and Shankar, 2002;
Siddqui et al., 2006). Strom (1985) reported that a large majority of the total number of cellulolytic bacterial isolates were members of the genus Bacillus. Proom and Knight (1955) studied the bacteria required minimal nutritional requirement. Ezekiel et al. (2012) isolated 22 different cellulolytic fungi from different sites. Duncan et al. (2010) also isolated 72 fungi and screened for cellulase activity by using the carboxymethyl cellulose (CMC) Congo red plate technique.

Till date most of the published works on the screening and isolation of cellulolytic bacterial species primarily focused on their further industrial utilization. However, present study aims to exploit the plant growth promoting properties (PGPR) of both the isolates - *Bacillus altitudinis* and *Bacillus tequilensis*. From previous published reports it is evident that cellulolytic bacteria can degrade large cellolose molecules in to small simple sugars; and cellulose is the most abundant natural carbon polymer present in biosphere. So, if these cellulolytic bacteria can be utilized as biofertilizers; then, they will certainly help plants to grow and develop significantly well as compared to others (Ahamed and Vermette, 2008; Arifoglu and Ögel, 2000; Freire *et al.*, 1999; Grigorevski de Lima *et al.*, 2005; Hanif *et
Several bacteria associated with plant roots that exert beneficial effects on plant growth and development are referred to as plant growth–promoting rhizobacteria (PGPR) (Kloepper and Schroth, 1978; Kloepper et al., 2004). *Bacillus* and *Pseudomonas* spp. are predominant among the diverse bacterial genera that have been linked with PGPR activity (Podile and Kishore, 2006). Members of the *B. subtilis* group, including *B. subtilis*, *B. licheniformis*, *B. pumilus*, *B. amyloliquefaciens*, *B. atrophaeus*, *B. mojavensis*, *B. vallismortis*, *B. sonorensis*, and *B. tequilensis* have been identified as PGPR strains for their capacity to stimulate plant growth and suppress pathogens with in rhizosphere and phyllosphere (Kloepper et al., 2004; Hao et al., 2012; Kim et al., 2012). Strains of *B. amyloliquefaciens* are widely used for their positive effects on plant growth (Idriss et al., 2002). Reva et al. (2004) reported that seven *Bacillus* isolates from plants or soil are closely related yet distinct from *B. amyloliquefaciens* type strain DSM7T. In addition, these strains are more proficient for rhizosphere colonization than other members of the *B. subtilis* group. GB03 (Nakkeeran et al., 2005), INR7 (Kokalis–Burelle et al., 2002), and FZB42 (Chen et
al., 2007) are PGPR strains within the *Bacillus subtilis* group that have been widely used in different commercial formulations to promote plant growth.

Like most of the *Bacillus* sps., both the bacterial isolates, *i.e.* - *Bacillus altitudinis* and *Bacillus tequilensis*, also showed positive PGPR characters, like - phosphate solubilisation, IAA production, siderophore production, ammonia production and HCN production (see Table 5.8, and Figure 5.9, of Chapter 5). Now, the effects of PGPR on plant growth can be mediated by direct or indirect mechanisms (Glick, 1995). The direct effects have been most commonly attributed to produce or change the concentration of plant growth regulators like Indole acetic acid, Gibberellic acid, Cytokinins and Ethylene, Asymbiotic N$_2$ fixation (Boddey and Dobereiner, 2000). These PGPR also affect growth by indirect mechanisms such as antagonism against phytopathogenic microorganisms by production of Siderophores (Scher and Baker, 1982), Antibiotics and Cyanide, Solubilization of mineral phosphates and other nutrients (de Freitas et al., 1997).

As, soil always play a prime role in plants life, and cellulolytic bacteria can affect soil characters by degrading
large polymers and incorporating nutritional supplements. In present study, both the cellulolytic isolates, i.e. - *Bacillus altitudinis* and *Bacillus tequilensis*, were mixed with barren soil individually and in combination, to see whether the soil characters will improve. Obtained results showed significant improvement in major soil characters, like – pH, electrical conductivity, organic matter, organic carbon, available nitrogen, inorganic phosphorus, and potassium, with both, *Bacillus altitudinis* and *Bacillus tequilensis*, individual and combined amendments (see Figure 5.10, in Chapter 5). Previous reports also showed that, cellulolytic bacteria in forest soil provide carbon sources to improve the soil fertility and sustain the nutrient balance of the forest ecological system through the decomposition of cellulosic remains (Yang et al., 2014). These bacteria can also be utilized for the biological conversion of biomass into renewable biofuels. Investigations by other researchers showed that aerobic bacteria belonging to species of *Pseudomonas*, *Bacillus* and *Cellulomonas*, and anaerobe such as *Clostridium* have cellulolytic potential (Sindhu et al., 2001). *Serratia marcescens* EB 67 and *Pseudomonas* sp. CDB 35 have been shown to possess cellulolytic activity in the presence of crop residues (Hameeda et al., 2006). The residing microflora
maintains the soil health and affects the agronomic parameters of the crops planted on those plots. Thus, farming management trials along with the inoculants enhance the growth and yield of the crops (Dickey et al., 1994). Cellulolytic soil bacteria had been studied in various soils under different land use systems with respect to the effect of environmental conditions on the abundance and decomposing activity (Hiroki and Watanabe, 1996; Dilly et al., 2001).

Soil micro-organisms play a significant role in regulating the dynamics of organic matter decomposition and the availability of plant nutrients such as N, P and S. It is well-recognized that microbial inoculants constitute an important component of integrated nutrient management that leads to sustainable agriculture. In addition, microbial inoculants can be used as an economic input to increase crop productivity; fertilizer doses can be lowered and more nutrients can be harvested from the soil. Biofertilizer is defined as a substance which contains living micro-organisms and is known to help with expansion of the root system and better seed germination. A healthy plant usually has a healthy rhizosphere which should be dominated by beneficial microbes. Conversely, in unhealthy soil, dominated by pathogenic microbes, optimum plant growth
would not be possible. Biofertilizers differ from chemical and organic fertilizers in the sense that they do not directly supply any nutrients to crops and are cultures of special bacteria and fungi.

So, naturally every microbe which has some potential benefit for plant health and development can be utilized as biofertilizer. So, to check whether both the isolates, \textit{i.e.} \textit{- Bacillus altitudinis} and \textit{Bacillus tequilensis}, in present study have the ability or not; they have been treated with two important crop plants, \textit{i.e.} – rice (\textit{Oryza sativa} L.) and spinach (\textit{Spinacia oleracea} L.). Treatments with bacteria with PGPR characters increase germination percentage, seedling vigor, emergence, plant stand, root and shoot growth, total biomass of the plants, seed weight, early flowering, grains, fodder and fruit yields etc (Ramamoorthy et al., 2001). The enhancement of plant growth by PGPR indicates their potential as biofertilizers in the field of agriculture.

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To assess this hypothesis, the present investigation was undertaken to screen the bacterial isolates from the rhizospheric soil of Rice plant from Nadia, West Bengal, India based on Plant Growth Promoting Characters and the potential PGPR isolate was tried with rice plant and pot experiment was conducted to evaluate the efficacy of isolate to increase the germination (%), root length, shoot length and other growth related characters under in- vitro & in-vivo conditions. Rice is an important staple
food crop for more than 60 per cent of the world people. In 2008, more than 430 million metric tons of rice were consumed worldwide, according to the USDA. Rice straw is used as cattle feed, used for thatching roof and in cottage industry for preparation of hats, mats, ropes, sound absorbing, straw board and used as litter material. Rice husk is used as animal feed, for paper making and as fuel source. It was found that inoculation of rice seedlings with both - \textit{Bacillus altitudinis} and \textit{Bacillus tequilensis}, individually and in combination significantly increased the germination percentages, and number and length of root & shoots (see Figure 5.11, and 5.12, Chapter 5). In this study, an increase in the plant growth by seed bacterization has been demonstrated. It is a well-established fact that overall plant growth and root development influenced by improved phosphorous nutrition (Jones et al., 1994). A large number of evidence suggests that PGPR enhance the growth, seed emergence and crop yield (Herman et al., 2008). In this study, we investigated the effectiveness of PGPR isolates on seed germination percentage and growth of rice seedlings. To see the effect of PGPR isolates on rice seedlings, Seed germination was also increased when seeds were pretreated with \textit{Bacillus altitudinis} and \textit{Bacillus tequilensis}, which positively increase
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the germination of rice. The PGPR isolates significantly increased the root & shoot length of rice seedlings. Highest elongation (35.4 cm) was recorded when seeds were pre-treated with combination of both the bacterial isolates - *Bacillus altitudinis* and *Bacillus tequilensis*, in pot & 13 cm root length and 14.2 cm shoot length in germination petri-dishes. All statistical data are shown as mean ± SEM. These results suggest that Plant growth Promoting Rhizobacteria is able to induce the production of IAA, solubilization of phosphorus, production of siderophore & thereby improving growth of plants.

The other tested plant, spinach is an excellent source of vitamin K, vitamin A (in the form of carotenoids), manganese, folate, magnesium, iron, copper, vitamin B2, vitamin B6, vitamin E, calcium, potassium, and vitamin C. It is a very good source of dietary fiber, phosphorus, vitamin B1, zinc, protein, and choline. Several studies have already demonstrated improvement of growth and development of spinach plants under PGPR. Elkoca et al. (2010) determined an increase of P, K, Ca, B and Mg concentration in common bean with PGPR treatments. Furthermore, Cakmakci et al. (2009) found that PGPR treatments caused a similar or higher concentration of P, K, Ca and Mg than that of the control in spinach leaves. Adesemoye et. al.
(2008) also showed *Bacillus subtilis* enhanced the emergence of seedlings of African spinach. Presents results also supported the previous observations. The major growth and development characters of spinach plants, like – fresh weight, dry weight, total number of leaves, and total leaf area were significantly increased under individual and combined effects of both the isolates - *Bacillus altitudinis* and *Bacillus tequilensis* (see Figure 5.13 to 5.17, Chapter 5).

Now, the role of *Bacillus* sps. in plant growth promotion is widely known. Many species of *Bacillus* including *Bacillus subtilis*, *Bacillus amyloliquefaciens*, *Bacillus cereus*, *Bacillus pumilus* and *Bacillus polymyxa* are widely known for their biocontrol abilities as they produce several antibiotics and supports plant growth promotion by production of phytohormones, solubilization of phosphate, releasing ammonia from nitrogenous organic matter, etc. (Hayat et al. 2010). Lim and Kim (2009) reported the production of IAA and IBA by *B. subtilis* AH18 and *Bacillus licheniformis* K11. These bacteria also showed promotion in growth of red-pepper, spinach, tomato and radish. *Bacillus pumilus* and *B. licheniformis*, isolated from the rhizosphere of alder (*Alnus glutinosa* [L.] Gaertn.) has been
reported to produce C19-gibberellins which supports stem elongation in plants (Gutiérrez-Mañero et al. 2001).

However, this study is the first report of plant growth promoting properties of both cellulolytic isolates - *Bacillus altitudinis* and *Bacillus tequilensis*. Normally, the cellulolytic bacteria are available in animals gut (Hiroki and Watanabe, 1996; Dilly et al., 2001), for their importance as decomposer of cellolose in simple sugars. Now simple sugars are need by all the living organisms, staring from bacteria to plants and animals. So, if these potent bacteria can also be exploited as plant growth promoting agents, then that will make a good biofertilizer combination.