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Population diversity of bacterial endophytes from jute (*Corchorus olitorius*) and evaluation of their potential role as bioinoculants

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ABSTRACT

Endophytes are bacterial or fungal organisms associated with plants in an obligate or facultative manner. In order to maintain a stable symbiosis, many of the endophytes produce compounds that promote plant growth and help them adapt better to the environment. This study was conducted to explore the potential of jute bacterial endophytes for their growth promotion ability in direct and indirect ways. A total of 27 different bacterial species were identified from different varieties of a jute plant (*Corchorus olitorius*) and different parts of the plant (leaf, root, seed, and seedling) based on 16S rRNA gene sequence. Two of the isolates showed ACC deaminase activity with *Staphylococcus pasteuri* strain MBL_B3 and *Ralstonia solanacearum* strain MBL_B6 producing 18.1 and 8.08 $\mu M\, mg^{-1}\, h^{-1}$ α-ketobutyrate respectively while eighteen had the ACC deaminase gene (*acdS*). Fourteen were positive for siderophore activity while *Kocuria* sp. strain MBL_B19 (133.36 μg/ml) and *Bacillus* sp. strain MBL_B17 (124.72 μg/ml) showed high IAA production ability. Seven bacterial strains were able to fix nitrogen with only one testing positive for *nifH* gene. Five isolates exhibited phosphorus utilization ability with *Bacillus* sp. strain MBL_B17 producing 218.47 μg P/ml. Three bacteria were able to inhibit the growth of a phytopathogen, *Macrophomina phaseolina* and among them *Bacillus subtilis* strain MBL_B4 was found to be the most effective, having 82% and 53% of relative inhibition ratio (RIR) and percent growth inhibition (PGI) values respectively. Nine bacteria were tested for their *in vivo* growth promotion ability and most of these isolates increased seed germination potential and vigour index significantly. *Bacillus subtilis* strain MBL_B13 showed 26.8% more vigour index than the control in which no bacterial inoculum was used. All inoculants were found to increase the dry weight of jute seedlings in comparison to the control plants and the most increase in fresh weight was found for *Staphylococcus saprophyticus* strain MBL_B9. *Staphylococcus pasteuri* strain MBL_B3 exhibited diverse *in vitro* growth promotion activity and significant growth promoting effect in *in vivo* pot experiments. These bacterial strains with plant growth enhancing abilities have the potential to be used as bioinoculants.

1. Introduction

Jute, a cash crop of Bangladesh and South-east Asia is one of the major sources of natural fiber. Initially, it was used only as a packaging material however with time a number of other uses of jute have reinforced the importance of this crop. *Corchorus olitorius* and *Corchorus capsularis* are two popular jute species cultivated mostly for their fiber quality. The main challenges for jute production are the availability of soil nutrients and adverse conditions arising from different biotic and abiotic stresses. Another hurdle farmers have to overcome in jute production is infection by a phytopathogen, *Macrophomina phaseolina* which drastically reduces its productivity. Endophytes are an interesting area studied mainly to understand plant-microbial interactions that enhance plant growth and protect plants from different stressors. However, not much work has been carried out to understand jute endophytes. So it is important to explore these communities of endophytes which form the jute microbiome and understand their growth promoting and biocontrol effects on jute.

Endophytes are plant associated microbes which grow symptomless within plants as an integral part of host metabolism and function. Commensalism and mutualism epitomize balanced stages of plant–-microbe communications (Hardoim et al., 2015). These organisms form either a commensal relationship where the endophyte is benefited by enabling itself a harmless existence and nutrient supply without

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affecting the host plants or a mutually beneficial relationship with their host plants through long term evolutionary processes (Kogel et al., 2006). Therefore, these bacterial communities are mostly advantageous for plant survival, providing protection from environmental stressors and microbial competitions (Rajendran et al., 2008). This symbiotic mutualism of endophytes and their host plants can be species and environment specific. Recent studies highlighted the potential of endophytes for the synthesis of bioactive compounds, promotion of plant growth and enhanced resistance to various pathogens (Rajendran et al., 2007; Sharma et al., 2016; Patel and Archana, 2017; Kandel et al., 2017). Plant growth promoting bacteria stimulate plant health and growth through three different mechanisms: phytostimulation, biofertilization, and biocontrol. Phytostimulators enhance plant growth in a direct way, usually by the production of phytohormones (auxins, cytokinins, gibberellins). The production of plant growth hormone, indole-3-acetic acid (IAA) is classified as "direct" growth promotion. Microbial IAA together with plant endogenous IAA work synergistically on the proliferation of plant cells and also stimulates the transcription of ACC (1-aminocyclopropane- 1-carboxylate) synthase that leads to ACC formation, the immediate precursor of ethylene production (Penrose et al., 2001). The ACC is taken up by the plant associated bacteria and cleaved by ACC deaminase to α-keto butyrate and ammonia (Penrose et al., 2001).

Nitrogen is the second major limiting factor for agricultural crop production (Dawe, 2000). Rhizobial bacteria are well studied for their biological nitrogen fixing activity (BNF). A wide range of diazotrophic bacteria having nitrogen fixing capacity have been isolated which include *Klebsiella, Enterobacter, Alcaligenes, Azospirillum* from rice (Baldani and Döbereiner, 1980) and *Herbospirrilium* from maize, sorghum, sugarcane (Olivares et al., 1996). Nitrogen fixation is catalyzed by nitrogenase, an enzyme that consists of a heterotetrameric core encoded by *nifD, nifK* and homodimeric dinitrogenase reductase encoded by *nifH* gene. *nifH* is the most commonly used biomarker for the detection of nitrogen fixing bacteria (Raymond et al., 2004). Knoth et al. (2014) have shown that when Salicaceae endophytes are inoculated in hybrid poplar plants they contribute to almost 65% of the total leaf N and help in the increase of plant biomass through BNF. This has recently been corroborated by Kandel et al. (2017).

One of the beneficial features of plant endophytes is solubilization of insoluble phosphate from the soil. Rhizobial bacteria are well known for their ability to solubilize inorganic phosphate and making it available for plants and in return, they use organic acids, sugar and root borne carbon compounds for their growth (Khan et al., 2010). Bacteria also liberate organic phosphate by the production of carbon-phosphorus lyases, phytases, phosphonatases (Oteino et al., 2015). Different studies have shown that inoculation of phosphate solubilizing bacteria reduces the use of phosphate fertilizer (without reducing crop yield) by 50% (Jilani et al., 2007; Yazdani et al., 2009).

Iron chelating siderophore production by bacterial endophytes has considerable influence on plant growth by making iron available to plants. Siderophores are the major sources of iron for plant growth and endophytes have been found to significantly increase root iron content in plants through the production of the same (Masalha et al., 2000).

In the last one decade, endophytic bacteria have also attracted researchers as novel resources in the biocontrol of plant diseases (Lin et al., 2013). Their advantages as biocontrol agents depend on their ability to live inside plants where they can provide reliable suppression of vascular diseases (Misaghi and Donndelinger, 1990). It is also well known that production of antibiotics by these endophytic bacteria is possibly a factor that contributes to the reduction of a pathogen's effects (Whipps, 2001).

Jute is prone to extensive infection by a phytopathogen, *Macrophomina phaseolina* which causes a stem rot disease that drastically reduces the production of jute. It attacks more than 500 plants and its host range comprises maize, sorghum, common bean, cotton, soybean and many more (Sarkar et al., 2014) There is an urgent need to find cost and environment friendly measures against this devastating phytopathogen. Strains of *Pseudomonas*, *Stenotrophomonas,* and *Bacillus* have been successfully used in attempts to control many plant pathogens (Bais et al., 2004). Recently endophytic bacteria from chickpea have been shown to alleviate stress of the host plant under saline conditions in addition to exhibiting biocontrol activity against *F. solani* (Egamberdieva et al., 2017)

Plant growth promotion by endophytes is a combined effect of different beneficial mechanisms (Koumoutsi et al., 2004). The effectiveness of microorganisms in influencing nutrient mobilization and assimilation by crop plants can only be gauged by studying the changes in plant properties such as plant height, length, plant biomass *etc.* and these give a comprehensive view of the impact of an inoculant on the functioning ecosystem (Caravaca et al., 2003; Sharma et al., 2011).

Twenty seven bacterial isolates belonging to seven different genera were isolated from jute and the predominant genera were found to be *Bacillus, Staphylococcus and Micrococcus. Bacillus and Micrococcus* are common endophytes while *Staphylococcus* have only recently been identified as beneficial plant associated microbes. The latter was been reported as endophytic bacteria in maize kernels (Liu et al., 2012), grapevine (*Vitis* sp.), and hybrid spruce (Collins et al., 2004). Their existence was also found in poplar trees, which are known to have phytoremediation ability (Moore et al., 2006). Adaptation with evolutionary changes have allowed some pathogenic strains like *Staphylococcus* to become endophytes (Chaudhry and Patil, 2016).

The objective of our study was to identify growth promoting activity of the jute endophytes by detecting their abilities to produce indole-3-acetic acid and iron chelating siderophores, solubilize phosphate and fix atmospheric nitrogen. Besides, we also screened their antagonistic activity against the devastating phytopathogen, *M. phaseolina* and studied the effect of bacterial inoculants on growth of jute (*Corchorus olitorius*). These beneficial entities can be potent candidates as bio-fertilizers or biocontrol agents capable of enhancing the yield of not only jute but other crops as well.

2. Materials and methods

2.1. Collection of plant materials and Macrophomina phaseolina

Fresh seeds and adult plants of different jute varieties (*Corchorus olitorius* var. O-9897, O-4, O-72 and accession 2015) were collected from the Bangladesh Jute Research Institute (BJRI), Dhaka. Fresh plant materials were used for endophyte isolation in order to reduce chances of contamination. The phytopathogen *M. phaseolina* was obtained from BJRI.

2.2. Isolation, morphological characterization and preservation of bacterial endophytes

For isolation of bacteria, surface sterilization techniques were used as described by Coombs and Franco (2003) with some modifications. Samples were washed under running tap water, soaked in ethanol for 1 min and then treated with 3% sodium hypochlorite for 3 min, 30 s wash in 70% ethanol. Finally, samples were rinsed with autoclaved dH_2O twice and dried on sterile tissue paper.

After surface disinfection, seeds, leaves, stems, roots and seedlings were ground with a sterile mortar pestle and dissolved in sterile phosphate buffer saline (PBS, 100 ml containing Na_2HPO_4 0.144 g; KH_2PO_4 0.24 g; KCl 0.02 g; NaCl 0.8 g; pH 7.4). Serial dilutions were prepared from the ground plant parts, and 100 µl aliquots from each dilution of 1×10^{-2}, 1×10^{-3}, and $1 \times 10^{-6,}$ 1×10^{-9} were spread on solid growth media (TSB, LB and MRS agar) and incubated at 37 °C for 24–72 h. Control plates were carefully prepared by spreading water (the same that was used in the final step of surface sterilization) and PBS buffer only.

Distinct morphological appearances such as color, shape and growth