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Colonization Potential of *Ochrobactrum intermedium*, *Bacillus cereus* and *Brevibacterium* sp. on *Triticum aestivum* and *Helianthus annuus* Roots*

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Abstract: The colonization potential of plant growth promoting bacterial strains (*Ochrobactrum intermedium*, *Bacillus cereus* and *Brevibacterium* sp.) on *Triticum aestivum* and *Helianthus annuus* roots. The roots of *Triticum aestivum* were heavy colonized with *Ochrobactrum intermedium* and *Bacillus cereus* as compared to *Brevibacterium*. *Ochrobactrum intermedium* mainly colonized the rhizoplane while *Bacillus cereus* was present both on the rhizoplane and near the root zone. *Ochrobactrum intermedium* and *Bacillus cereus* were found to be present both on the rhizoplane as well as near root zones of both crops used (*Triticum aestivum* and *Helianthus annuus*) while *Brevibacterium* was found in the form of groups in the rhizosphere of *Helianthus annuus* roots. From the results of both crops it was observed that the number of *Ochrobactrum intermedium* cells were more where some root exudates seen.

Key words: Colonization, PGPR, rhizosphere, *Triticum aestivum*, *Helianthus annuus*, *Ochrobactrum*, *Bacillus*, *Brevibacterium*

Introduction

Plant growth-promoting Rhizobacteria (PGPR) are beneficial native soil bacteria that result in increased plant growth (Faisal and Hasnain, 2005). The rhizospheric zone is rich in nutrients when compared with the bulk soil, due to the accumulation of a variety of organic compounds released from roots by exudation, secretion, and deposition (Curl and Truelove, 1986). Because these organic compounds can be used as carbon and energy sources by microorganisms, microbial growth and activity is particularly intense in the rhizosphere. Plant-associated bacteria that are able to colonize roots are called rhizobacteria and can be classified into beneficial (Kalaptur *et al*., 2004), deleterious (Berggren *et al*., 2004) and neutral groups on the basis of their effects on plant growth. Bacteria may colonize the plant root both externally (Schmidt *et al*., 2004) as well as internally (Sturz *et al*., 2000). For several non-pathogenic bacteria, colonization of the root exterior/interior of various crops has been described (Bressan and Borges, 2004; Kalaptur *et al*., 2004). The present study deals with the colonization potential of the *Ochrobactrum intermedium*, *Brevibacterium* and *Bacillus cereus* on the *Triticum aestivum* and *Helianthus annuus* roots.

Materials and Methods

Bacterial Strains

Three bacterial strains *Ochrobactrum intermedium*, *Brevibacterium* and *Bacillus cereus* (Fig. 2) used in this study were isolated from wastewater of tannery, ICI Chemicals and chromium polluted

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soil, Lahore, Pakistan (Faisal and Hasnain, 2004). They are gram negative motile rods and showed very high level resistance to K_2CrO_4 both on the nutrient agar (up to 40 mg mL^{-1}) as well as in acetate-minimal medium (up to 10 mg mL^{-1}). Strains were routinely grown on nutrient agar (grams per litres; peptone 5, beef extract 3, NaCl, 3, agar 10) plates supplemented with 1 mg mL^{-1} of K_2CrO_4.

Experimental Setup

Present study was conducted in the year 2004-2005 at Microbiology and Molecular Genetics Research Lab, Department of Botany, University of the Punjab, Lahore, Pakistan. *Triticum aestivum* var Inqlab-97 and *Helianthus annuus* var SF-187 seeds were obtained from National Agriculture Research Centre, Islamabad, Pakistan. Seeds were surface sterilized in 5% sodium hypochlorite solution for 5 min and then thoroughly washed with sterilized glass-distilled water three times. For seeds inoculation freshly prepared overnight cultures were suspended in a 10 mL sterilized glass distilled water and were adjusted to an absorbance of 1.0 at 600 nm for all the strains to ensure equal number of bacteria for each inoculation. Un-inoculated seeds were used as a control treatment. Both inoculated and un-inoculated (control) seeds (10 seeds per plate) were spread uniformly on the filter papers in petri dishes. Seeds were kept in dark for germination and after that seedlings were provided with nutrient solution (Hewitt, 1963) and were shifted to light with a 12 h photoperiod. After 10 days, seedlings were harvested and different observations were made under microscope.

Results

Triticum Aestivum

In *Triticum aestivum* roots, thick population of *Ochrobactrum intermedium* were observed on the rhizoplane (Fig.1a) as compared to non-inoculated control seedlings. The mode of colonization of *Brevibacterium* strain on wheat roots was different as compared to *Ochrobactrum intermedium* and less population of bacteria were observed and majority of them colonized near the root zone (Fig.1b). *Bacillus cereus* was found to be highly efficient root colonizer of wheat root externally. This strain frequently present in the form of thick mass on the rhizoplane of wheat roots (Fig.1c).

Helianthus Annuus

In case of *Helianthus annuus* root, *Ochrobactrum intermedium* was present on the rhizoplane, but high density of cells were observed on the tip of root hairs where some root exudates were also observed (Fig.1d). *Brevibacterium* was present in the form of groups near the root curvature (Fig. 1e). In *Helianthus annuus*, *Bacillus cereus* was observed in high population around and upon the roots (Fig. 1f) as observed in *Triticum aestivum*. The population of *Bacillus cereus* increases rapidly where some root exudates released by the inoculated sunflower seedlings.

Ochrobactrum intermedium and *Bacillus cereus* were found to be present both on the rhizoplane as well as near root zones of both crops (*Triticum aestivum* and *Helianthus annuus*) while *Brevibacterium* was located in the form of groups in the rhizosphere of *Helianthus annuus*.

Discussion

All the three strains promote plant growth both in the laboratory as well as in the pots experiments (Faisal and Hasnian, 2004). Roots of *Triticum aestivum* were heavy colonized with *Ochrobactrum intermedium* and *Bacillus cereus* as compared to *Brevibacterium*. Ochrobactrum

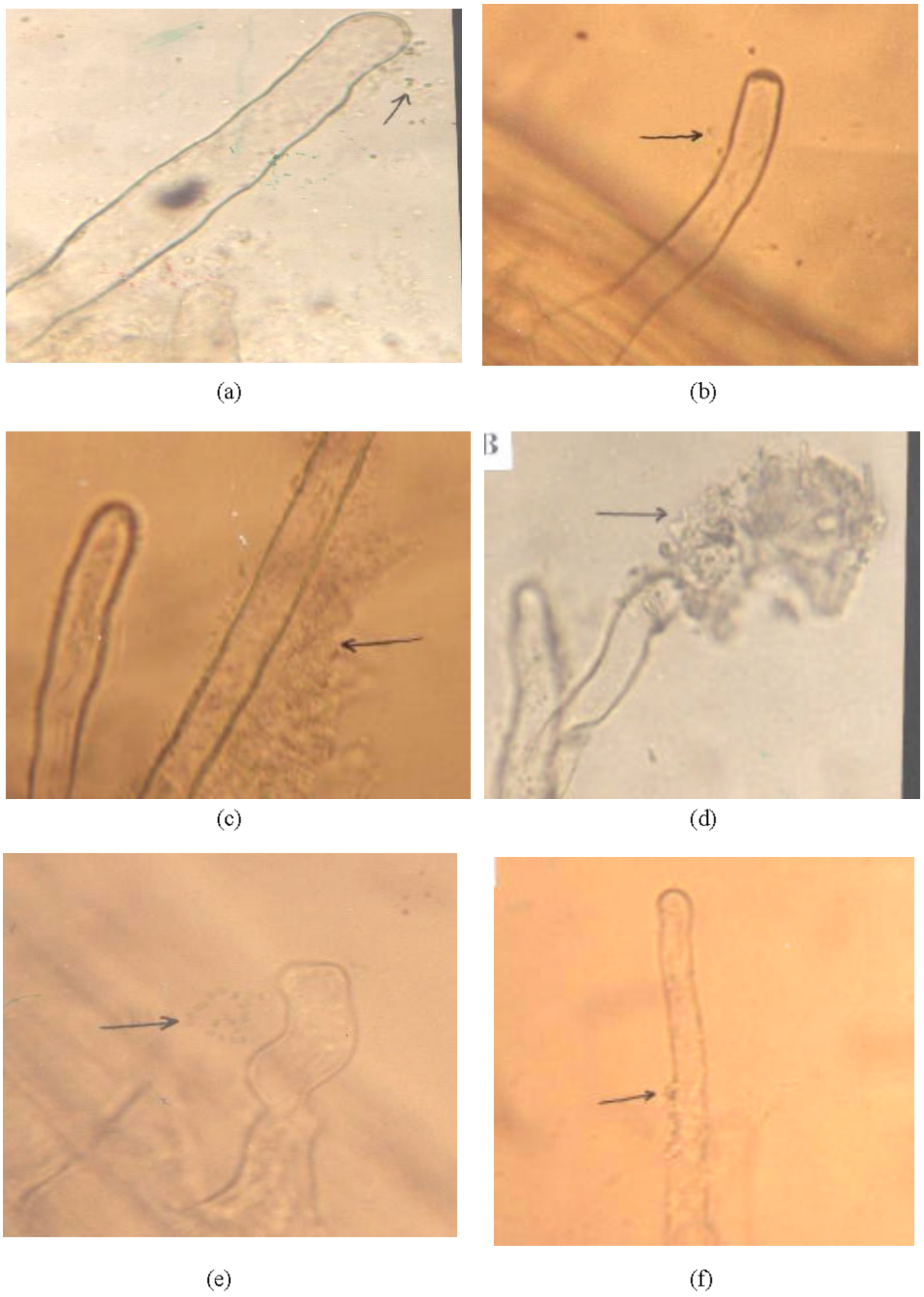

Fig. 1: Colonization of *Ochrobactrum intermedium*, *Bacillus cereus* and *Brevibacterium* sp. with *Triticum aestivum* (a, b, c) and *Helianthus annuus* (d, e, f) roots. Arrow shows the point of bacterial attachment/presence in the rhizosphere or on rhizoplane. bar = 10 μm

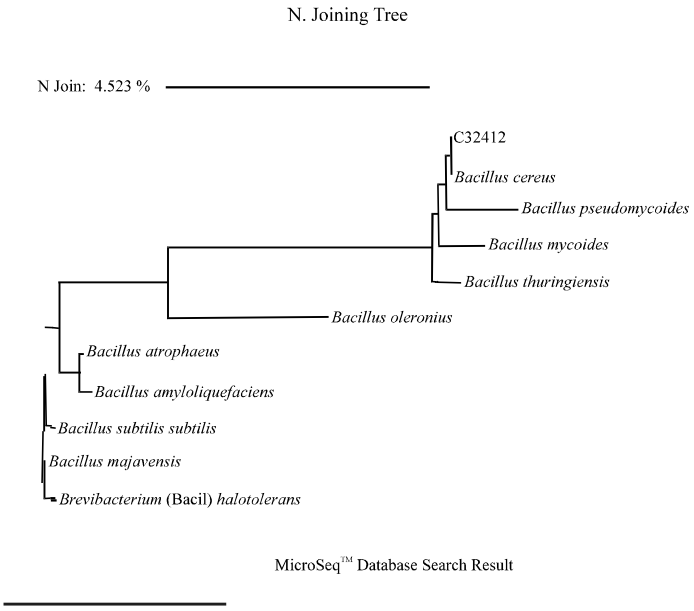

Fig. 2: Phylogenetic tree of *Bacillus cereus* and other *Bacillus* species based on 16S rRNA gene sequence analysis. The bar indicates 4.52% of estimated sequence divergence. The percentage differences between *Bacillus cereus* and other members of genus *Bacillus* are given at the top of the tree

intermedium mainly colonizes the rhizoplane while *Bacillus cereus* was present both in the rhizoplane and near the root zone. The population of *Brevibacterium* was less and mainly exists as single cells near the root zone of wheat. It was also observed that rhizospheric and phyllospheric colonization of *Pantoea agglomerans* was more extensive in *Triticum aestivum* as compared to barley and rye. It means that colonization behavior of each strain vary from crop to crop. The cells of *Brevibacterim* were present singly as compared to *Ochrobactrum intermedium* and *Bacillus cereus* that are present in groups and thick mass around the wheat root. It was earlier observed that cells of strain *Azospirillum bresilence* demonstrate a characteristic pattern of wheat colonization, which was present in rhizoplane of root hair (Assmus *et al*., 1995) and root tip invasion (Schloter and Hartmann, 1998).

In *Helianthus annuus*, *Ochrobactrum intermedium* invades the root tip region in the form of thick mass. Probably here some root exudates were also observed which favors the growth of this strain. Colonization potential and plant growth promoting effect of *Phyllobacterium myrsinum*, *Phylollobacterium rubiacearum* and *Pseudomonas fluorescence* strains in the rhizosphere and rhizoplane of *Triticum aestivum* and related crops has been reported (Bertrand *et al*., 2001; Lucas-Garcia *et al*., 2003). There are certain genetics elements in the bacteria or in the crops that can affect the colonization behavior. In soybean the colonization pattern of *Azospirillum lipoferum* 137 genes and *Pseudomonas fluorescence* 21377 genes on root tips was different in both strains (Chebotar *et al*., 2001). In sunflower roots, *Brevibacterium* colonize near the root zone in the form of groups while this strain colonizes near the root zone of wheat singly instead of in groups. From this it is observe that the colonization of this strain is not specific but its behavior change from crop to

crop. In case of *Bacillus cereus,* high population of cells were present on the rhizoplane and near the root zone of sunflower.

From the results of both crops it was observed that the number of *Ochrobactrum intermedium* cells were more where some root exudates seen. It is now well recognized that interaction of bacterium and plant is mutual, both partner support each other. Bacteria take their carbon and energy source from the compound (root exudates) released by plant roots. In return of it bacterial strains help plant growth by different mechanisms mainly by producing phytohormones, by fixing atmospheric nitrogen, phosphate solubilisation (El-Komy *et al*., 2003). The high root colonization potential of *Bacillus cereus* in both crops (*Triticum aestivum* and *Helianthus annuus*) might be due to its soil residing nature as compared to the other two strains (*Ochrobactrum intermedium* and *Brevibacterium*) which were isolated from waste water. Colonization of plants roots by soil born or introduced bacteria are an important strategy in the establishment of effective plant-bacterial interaction. So keeping in view the importance of these bacterial strains (*Ochrobactrum intermedium*, *Brevibacterium* and *Bacillus cereus*) for the growth promotion of various crops and their perfect colonization behavior in the rhizosphere and rhizoplane of roots make them as useful innocula for nutrient deficient crops instead of using chemical fertilizer.

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