ABSTRACT of

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Present study was conducted on plant-microbe interactions in wheat (*Triticum aestivam* L.) under wheat-rice and wheat-cotton rotations. Soil samples were collected from rhizosphere of wheat and used for isolation of bacteria on LB medium as well as on selective media for phosphate solubilizers and nitrogen fixers. A total of 29 isolates were obtained and identified on the basis of 16S rRNA gene sequence analysis as *Azospirillum* (2 strains), *Acinetobacter* (2 strains), *Actinobacteria* (one strain), *Arthrobacter* (3 strains), *Bacillus* (5 strains), *Enterobacter* (3 strains), *Microbacterium* (2 strains), *Pantoea* (one strain), *Pseudomonas* (4 strains) and one strain each of the genera *Sphingobacteria*, *Terribacillus* and *Xanthomonas*. In the present study, *pqqE* (a gene known to have role in P-solubilization) was PCR amplified and sequenced. Sequence analysis of *pqqE* gene amplified from *Arthrobacter* sp. WP-2, *Pseudomonas* spp. T-27 and NN-4 showed maximum (80-81%) sequence similarity with that of *Pseudomonas putida*. Nucleotide sequence of *pqqE* obtained from *Pantoea* sp. WP-5 showed maximum sequence similarity (84%) with that of *Klebsiella pneumonia* strain. The bacterial isolates were characterized for their plant growth promoting traits like P-solubilization and plant growth hormone production. P-solubilization activity was detected in 12 bacterial strains in Pikovskaya medium supplemeneted with sucrose, maltose, glucose or galactose as single C-source. Among the tested strains, high P-solubilization activity in pure cultures of *Arthrobacter* sp. WP-2 (206.8 µg/mL), *Azospirillum* sp. WS-1 (218.1 µg/mL), *Bacillus* sp. T-34 (298.3 µg/mL), *Enterobacter* spp. T-41 and T-42 (292.2 µg/mL and 215.9 µg/mL), *Pantoea* sp. WP-5 (310.5 µg/mL) and *Pseudomonas* sp. T-27 (211.9 µg/mL) was detected. These strains produced organic acids like acetic acid, citric acid and gluconic acid in growth medium containing insoluble phosphate (TCP). Phytohormone (IAA) production was determined which showed maximum IAA production in *Bacillus* sp. T-34 (31.2±3.3 µg/mL), *Enterobacter* sp. T-41 (26.4±3.2 µg/mL), *Pseudomonas* sp. WP-1 (24.9±3.6 µg/mL), *Arthrobacter* sp. WP-2 (23.6±2.5 µg/mL) and *Azospirillum* sp. WS-1 (11.1±2.1 µg/mL). The bacterial isolates were tested as single-strain inocula for wheat seedlings in sand culture. *Azospirillum* sp. WS-1, *Bacillus* sp. T-34 and *Enterobacter* sp. T-41 showed maximum increase in plant dry weight by 26%, 28% and 15%, respectively over non-inoculated control. In pot experiments in which non-sterilized soil was

used, maximum increase in straw weight of plants inoculated with *Azospirillum* sp. WS-1 (19%), *Bacillus* sp. T-34 (22%) and *Enterobacter* sp. T-41 (17%) over non-inoculated control was recorded. Data on grain yield indicated best performance (i.e. % increase over control) of *Arthrobacter* sp. WP-2 (15.3%), *Azospirillum* sp. WS-1 (9.3%), *Bacillus* sp. T-34 (14.8%), *Enterobacter* sp. WP-8 (12.3%), *Enterobacter* sp. T-41 (13.1%), *Pantoea* sp. WP-5 (15.3%) and *Pseudomonas* sp. NN-4 (13.3%). A field experiment was conducted to study the effect of bacterial inoculation on wheat grown under wheat-rice crop rotation. Maximum increase in grain yield was recorded when *Azospirillum* spp. WS-1 and WB-3, *Bacillus* sp. T-34 and *Pantoea* sp. WP-5 were applied as single-strain inocula. In field experiments conducted during 3 consective years under wheat-cotton rotation, maximum increase in grain yield of wheat due to inoculation with *Enterobacter* sp. T-41 (11.6%), *Arthrobacter* sp. WP-2 (7%), *Azospirillum* sp. WS-1 (13.6%) and *Bacillus* sp. T-34 (11.9%), *Pantoea* sp. WP-5 (10.5%) and *Pseudomonas* sp. NN-4 (12.8%) was recorded. Bacterial population determined at different growth stages of inoculated plants indicated maximum number of bacteria (10^8-10^9 cfu/g dry soil on LB and 10^3-10^4 cfu/g dry soil on Pikovskaya medium) at booting stage. In rhizosphere (rhizosheath) of wheat, four dominant colony types were detected and the representative isolates from these colony types were identified using 16S rRNA gene sequence analysis as *Arthrobacter, Acinetobacter, Bacillus* and *Enterobacter* strains. Rhizosheath formation was observed in wheat grown under wheat-rice and wheat-cotton rotation. Organic acids (acetic acid, citric acid, malic acid and oxalic acid) and sugars (sucrose and glucose) were detected in rhizosheaths of wheat. Diversity of bacteria in the rhizosheath of wheat under both crop rotations was studied through direct soil DNA analysis of 1*6S rRNA* using barcoded pyrosequencing. From the soil samples a total of 57,638 cleaned sequences were obtained with read length of 319 bp and out of these, 46,971 sequences were classifiable when grouped on the basis of 97% similarity level. It was observed that 48.8% sequences were obtained from wheat-cotton rotation while 51.2% from wheat-rice crop rotation. It was noted that out of total 46,971 sequences, 11,729 (24.97%) showed 97% similarity with phylotypes having PGPR activity. The results showed that in wheat-cotton cropping system, *Proteobacteria* were dominant (25.12%), followed by un-classified bacteria (20.5%), *Actinobacteria* (17.7%), *Chloroflexi* (10.3%), *Firmicutes* (9.75%), *Acidobacteria* (4.98%), *Planctomyctes* (3.57%), *Bacteriodetes* (2.97%), *Cynobacteria* (1.55%), *Verrucomicrobia* (1.25%) and *Nitrospora* (0.88%). Results from wheat-rice cropping system showed that

Proteobacteria were dominant (35.72%), followed by un-classified bacteria (17.1%), *Actinobacteria* (13.6%), *Firmicutes* (9.02%), *Chloroflexi* (7.5%), *Bacteriodetes* (6.48%), *Acidobacteria* (3.82%), *Planctomycetes* (2.02%), *Verrucomicrobia* (1.75%), *Nitrospora* (0.75%) and *Cynobacteria* (0.62%). It was also observed that out of 495 different phylotypes detected, 280 phylotypes were common in both the crop rotations while 96 were only abundant in wheat-rice rotation and 41 were only present in wheat-cotton rotation system and remaining phylotypes were those which were rarely present in the sequence data obtained in the present study. Diversity of diazotrophs was determined in rhizosheath of wheat under both crop rotations by *nifH* sequence analysis amplified from soil DNA. A total of 72,033 cleaned *nifH* sequences were obtained with read length of 339-345 bp and out of these, 41,287 sequences were classifiable when grouped on the basis of 91% similarity level. The results showed that in wheat-rice cropping system, *Proteobacteria* were dominant (61.3%), followed by un-classified bacteria (12.77%), *Cynobacteria* (11.98%), other bacteria (9.22%), *Chlorobi* (1.71%), *Firmicutes* (1.62%), *Verrucomicrobia* (0.72%), *Euryarchaeota* (0.32%), *Spirochaetes* (0.22%), *Actinobacteria* (0.07%) and *Fibrobacteres* (0.05%). Results from wheat-cotton rotation indicated that *Proteobacteria* were dominant (59.85%), followed by *Cynobacteria* (22.57%), un-classified bacteria (8.12%), other bacteria (7.18%), *Firmicutes* (1.45%), *Chlorobi* (0.25%), *Verrucomicrobia* (0.27%), *Spirochaetes* (0.15%) and *Fibrobacteres* (0.13%). Analysis of data showed that 41,287 sequences were grouped at 91% similarity level into 26,05 operational taxonomic units (OTU). Sequence analysis indicated presence of *nifH* sequences belonging to 150 different nitrogen fixing genera. Among these 150 genera, 22.6% genera were only present in soil samples from wheat-rice rotation, 12.6% genera were found in wheat-cotton rotaion and 40.6% genera were commonly present in both the cropping systems