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Selection rhizosphere-competent microbes for development of microbial products as biocontrol agents

A.V. Mashinistova (1), A.A. Elchin (1), N.V. Gorbunova (1), V.S. Muratov (1), K.A. Kydralieva (2), B.M. Khudaibergenova (2), V.P. Shabaev (3), and Sh.J. Jorobekova (2)

(1) Institute of Applied Biochemistry and Machine-Building JSC "Biochimmash", K.Tsetkin 4, Moscow 125299, Russia, (2) Institute of Chemistry and Chemical Technology, NAS, Laboratory of Biophysical Chemistry, Bishkek, Kyrgyzstan (k_kamila@mail.ru, + 996 312 243607), (3) Institute of Physico-Chemical and Biological Problems of Soil Sciences of RAS, Pushchino, Moscow region

Rhizosphere-borne microorganisms reintroduced to the soil-root interface can establish without inducing permanent disturbance in the microbial balance and effectively colonise the rhizosphere due to carbon sources of plant root exudates. A challenge for future development of microbial products for use in agriculture will be selection of rhizosphere-competent microbes that both protect the plant from pathogens and improve crop establishment and persistence.
In this study screening, collection, identification and expression of stable and technological microbial strains living in soils and in the rhizosphere of abundant weed - couch-grass Elytrigia repens L. Nevski were conducted. A total of 98 bacteria isolated from the rhizosphere were assessed for biocontrol activity in vitro against phytopathogenic fungi including Fusarium culmorum, Fusarium heterosporum, Fusarium oxysporum, Drechslera teres, Bipolaris sorokiniana, Piricularia oryzae, Botrytis cinerea, Colletothrichum atramentarium and Cladosporium sp., Stagonospora nodorum. Biocontrol activity were performed by the following methods: radial and parallel streaks, "host – pathogen" on the cuts of wheat leaves. A culture collection comprising 64 potential biocontrol agents (BCA) against wheat and barley root diseases has been established. Of these, the most effective were 8 isolates inhibitory to at least 4 out of 5 phytopathogenic fungi tested. The remaining isolates inhibited at least 1 of 5 fungi tested. Growth stimulating activity of proposed rhizobacteria-based preparations was estimated using seedling and vegetative pot techniques. Seeds-inoculation and the tests in laboratory and field conditions were conducted for different agricultural crops – wheat and barley. Intact cells, liquid culture filtrates and crude extracts of the four beneficial bacterial strains isolated from the rhizosphere of weed were studied to stimulate plant growth.
As a result, four bacterial strains selected from rhizosphere of weed - couch-grass Elytrigia repens L. Nevski were chosen as a core of collection of 98 pure cultures with high fungicidal and plant growth-stimulating potentials. Partial determination of nucleotide sequence of 16S ribosomes of tested bacteria indicated that Pseudomonas and Bacillus species were the most dominant bacteria exhibiting biocontrol activity. Typing of bacterial strains was performed on the basis of partial determination of nucleotide sequence 16S ribosome of the studying strain. For this purpose polymerase chain reaction (PCR), using specific primers was provided with chromosomal DNA of bacterial strain under study. After determination of nucleotide sequences of the obtained PCR-fragments, the data obtained was compared with the sequences available in the bank of data (GENEBANK: http://www.ncbi.nlm.nih.gov), with the aim to determine close related strain to the organism under study. When the level of homology exceeded the level of 98%, one could conclude that the strain under study was identical to the available in the bank of data. Amplification and sequencing of gene 16S pDNA was performed using universal for the majority of prokaryotes primers. Thermopolimerase Long PCR Enzyme Mix «Fermentas», dNTP -«Fermentas» was used for amplification. While performing PCR, reagent concentrations corresponded to the protocols described in a set Long PCR Enzyme Mix «Fermentas». DNA separation from the sample was performed with DNeasy Plant Mini Kit «QIAGEN». DNA separation from gel was performed with QIAquick Gel Extraction Kit«QIAGEN».
Phylogenetic affinity was determined on the basis of the comparison of nucleotide sequence – 400 nucleotides that approximately corresponded to the positions from 500 to 907 nucleotides by nomenclature of E.coli. Primary analysis of the similarity of nucleotide sequences of genes 16S DNA of the strains under study was performed

on the basis of data Genbank. Sequences were aligned according to nucleotide sequences of those bacteria, which had the highest degree of homology with the strains under study, applying the program ClustalX 1.83. Building of rootless phylogenetic trees of the studying bacteria was carried out with the help of the program Njplot.

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