

# Beneficial Microbes: Food, Pharma, Aqua & Beverages Industry

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## Evidence for plasmid-conferring salt in root associated bacteria from halophytes (*Haloxylon recurvum* and *Atriplex amnicola*) and non-rhizospheric soil samples

Salma Mukhtar<sup>1</sup>, Muhammad S Mirza<sup>2</sup>, Samina Mehnaz<sup>1</sup> and Kauser A Malik<sup>1</sup><sup>1</sup>Forman Christian College, Pakistan<sup>2</sup>National Institute for Biotechnology and Genetic Engineering, Pakistan

Main purpose of this research was to investigate the evidence for plasmid conferring salt tolerance in rhizospheric bacteria isolated from *Haloxylon recurvum* and *Atriplex amnicola*. 22 strains were selected after screening of salt tolerance. Selected strains were characterized morphologically, biochemically and identified by PCR amplification of specific 16S rRNA gene sequences. Plasmid curing of isolates was done by heat shock method to study the effect of plasmid conferring salt tolerance. These plasmids were isolated and transformed into *E. coli* and growth response of original strains and transformed *E. coli* was compared at 1.5-4M NaCl concentration. Almost all strains showed optimum growth at 1-3.5M NaCl. These strains were related to *Bacillus* spp., *Pseudomonas* sp., *Streptomyces* sp., *Halomonas* spp., *Citricoccus* sp., *Halobacterium* sp., and *Kocuria polaris*. Cured isolates showed no growth in halophilic medium but grew well on LB medium. Biochemical and morphological characterization of cured strains was done and compared with the original strains. Various biochemical changes were observed after plasmid curing. Certain traits other than salt tolerance were plasmid linked and on curing, either the loss of the complete plasmid or a part of the plasmid resulted in the loss of specific characteristics. Mostly transformed *E. coli* were able to grow up to 3M NaCl concentrations. Different salt tolerant will be characterized and can be used to develop transgenic plants having salt tolerance.

salmamukhtar85@gmail.com

## Characterization of indigenous *Bacillus thuringiensis* strains based on their *cry* genes and Cry protein profile

Shakila Sultana, Asaduzzaman Shishir, Shakila Nargis Khan and Md Mozammel Hoq

University of Dhaka, Bangladesh

*Bacillus thuringiensis* (*Bt*) is widely used biopesticide. The aim of this study was to characterize indigenous *Bt* strains by their Cry protein profiling and to establish a correlation between the *cry* genes and their Cry protein profile. Through analyzing 21 strains by SDS-PAGE at 20, 40 and 72 hours, prominent protein bands were assigned presumptive Cry protein classes based on their molecular weight. From the whole cell protein profile at different time, it was observed that the molecular weight of the Cry proteins obtained from the indigenous *Bt* strains were in the range from 17 to 165 kDa. Cry1A class was the most prevalent among the strains. Cry2, Cry3, Cry6, Cry9 and cytotoxin class of endotoxins were also frequently found. *Bt* strains harboring potential Cry toxin classes (Cry1, Cry2, Cry3 and Cry9) were subject to PCR for the confirmation of the presence of respective *cry* genes. Twelve of the isolates showed the presence of Cry1A toxin and *cry1A* gene. Four isolates were found to contain Cry3 toxin and *cry3* gene, four isolates showed the presence of *cry9* gene and only one isolate showed the presence of the *cry2* gene only. Thus correlation between the *cry* gene and Cry protein class was found. From the chronological protein profiles of the isolates it was presumed that protease activity might be present that was degrading the Cry proteins gradually. Ten of the isolates showed increasing protease activity while their protein concentration decreased with time. Finally RAPD genotyping of the isolates showed close relationships among the strains that contained *cry1A* and *cry9* genes.

sultanashakila130@gmail.com

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