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Enhancement of alkaline protease production in Bacillus circulans using plasmid transformation

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Abstract
Plasmid transformation is an efficient and crucial biotechnological tool that enables the enhancement of many important microbial characters that would be beneficial in a lot of industrial, agricultural and environmental applications. In the present study, five Bacillus species (B. subtilis, B. cereus, B. alvei, B. circulans and B. pumilus) were investigated. They were isolated from agricultural soils of different local arid environments of the Kingdom of Saudi Arabia, identified and characterized for their plasmid content. The main objective of the present study was to enhance the production of alkaline protease in Bacillus circulans (the recipient strain) through plasmid transformation from B. subtilis (the donor strain). All the tested Bacillus strains successfully produced unique multiple (3, 4 and 5) spontaneous antibiotic resistant mutants against chloramphenicol, neomycin, rifampicin, streptomycin, kanamycin and tetracycline and all of which were mutated to Rifr strains. B. pumilus showed the highest resistance against five of the six tested antibiotics while both of B. alvei and B. circulans showed the lowest resistance to only three of the tested antibiotics. Results revealed that B. subtilis was the best among the tested species concerning the production of alkaline protease (90.2 U/ml) while B. pumilus was the lowest in activity (40.3 U/ml). Screening of plasmid content revealed the presence of one or two mega indigenous plasmids in all the tested species. The four transformant strains BC1, BC2, BC3 and BC4 resulting from plasmid transformation exhibited significant increases in the activity of alkaline protease and recorded 2.31- to 3-fold increases compared to the parent B. circulans cells and 2.11- to 2.75-fold increases compared to the donor cells of B. subtilis. They also acquired antibiotic resistance to tetracycline and chloramphenicol that was completely absent in the parent cells of B. circulans. Results revealed that plasmid transformation among the tested Bacillus spp. is a powerful technique that can be efficiently exploited to enhance alkaline protease production in the transformed Bacillus spp. compared to their wild strains and we recommend using the improved transformant strains for commercial and industrial purposes.

Author Keywords
Alkaline protease; B. subtilis, B. circulans; Enhancement; Plasmid transformation

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