TITLE: OBTAINING COMPETENT CELLS OF PAENIBACILLUS ELGII AC13

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ABSTRACT:

Paenibacillus is a bacterial genus that produces many bioactive molecules such as antimicrobials, enzymes, and plant growth promoters. Few species of this genus were genetically manipulated for future biotechnological purposes. In the present work, we have used Paenibacillus elgii AC13 which shows antimicrobial activity, mainly due to the production of lipopeptides by non-ribosomal synthesis. Products of this synthesis are secondary metabolites that exhibit a remarkable array of biological activity and many of them are clinically valuable antimicrobial, antifungal, antiparasitic, antitumor and immunosuppressive agents. However, there are no protocols for the genetic manipulation of Paenibacillus elgii. The objective of the present work was to obtain P. elgii AC13 competent cells for genetic transformation. First, it was necessary to obtain selection markers for transformants, since this species is naturally resistant to several antibiotics. In this study, kanamycin, ampicillin and chloramphenicol were tested in various concentrations. Two protocols were then compared to obtain electrocompetent cells for transformation of this bacteria. Both protocols were optimized and variables such as: optical density, culture medium, incubation conditions, and wash solution were systematically tested. The plasmids used were pUC19 and pAD123. The preparation of these plasmids was carried out by cellular transformation of Escherichia coli XL1 blue and ER2925 respectively. For the resistance test, ampicillin and chloramphenicol were chosen in the concentration of 25 µg/mL. It was initially possible to obtain P. elgii AC13 transformants from one of the electrotransformation protocols, but they were not stable in subsequent cultures. It is still necessary to perform screening tests to confirm that the plasmid used in the transformation has actually been incorporated.

Key-words: Paenibacillus, selection antibiotic, competent cells, transformation.

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