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PCR AMPLIFICATION OF *ure*C GENE OF ALKALINE *BACILLUS* ISOLATES FROM SOIL RICH IN CALCITE

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Introduction

Ureolysis is controlled by genes that are responsible and specific for targeted metabolic activity. The urease production and activity are controlled by the structural *ure*A, *ure*B, and *ure*C genes, which encode enzyme subunits (γ, β, α-). Additionally, functional genes (*ure*D, *ure*E, *ure*F, *ure*G) are required to encode proteins that actively influence enzyme maturation. The three basic structural subunits form the (UreABC)3 complex, while the functional genes create the UreDFG complex which is sequentially organized around (UreABC)3 complex. The best confirmation of urease activity in bacteria is considered to be the presence of all three structural genes. However, detection of ureolytic activity is often based on the amplification of the *ure*C gene, which is a response to the main genetic structural α- subunit. The reason for choosing the *ure*C gene as a genetic indicator is the fact that the expression of the *ure*C gene significantly increases during ureolytic activity, while the level of *ure*A and *ure*B gene activity does not change.

Results and discussion

According to the obtained results of PCR amplification, it can be concluded that the targeted gene for the ureolysis process was successfully amplified from the genomes of all selected *Bacillus* isolates. The expected PCR fragment for the *ure*C gene has about 340 bp. In the case of the reference strain *S. pasteurii* DSM 33, the size of the amplified fragment of the *ure*C gene was 342 bp, while the size of the amplified fragment of the same gene in the case of the natural isolates was 334, 341, 343, 341 and 344 bp for *B. muralis, B. lentus, B. simplex, B. firmus* and *B. licheniformis*, respectively. The obtained sizes of *ure*C gene fragments coincide with the fragment size of 344 bp of the same gene amplified from the genome of *S. pasteurii* and 340 bp amplified from the genome of isolates belonging to the genera *Bacillus, Virgibacillus*, and *Lysinibacillus*.

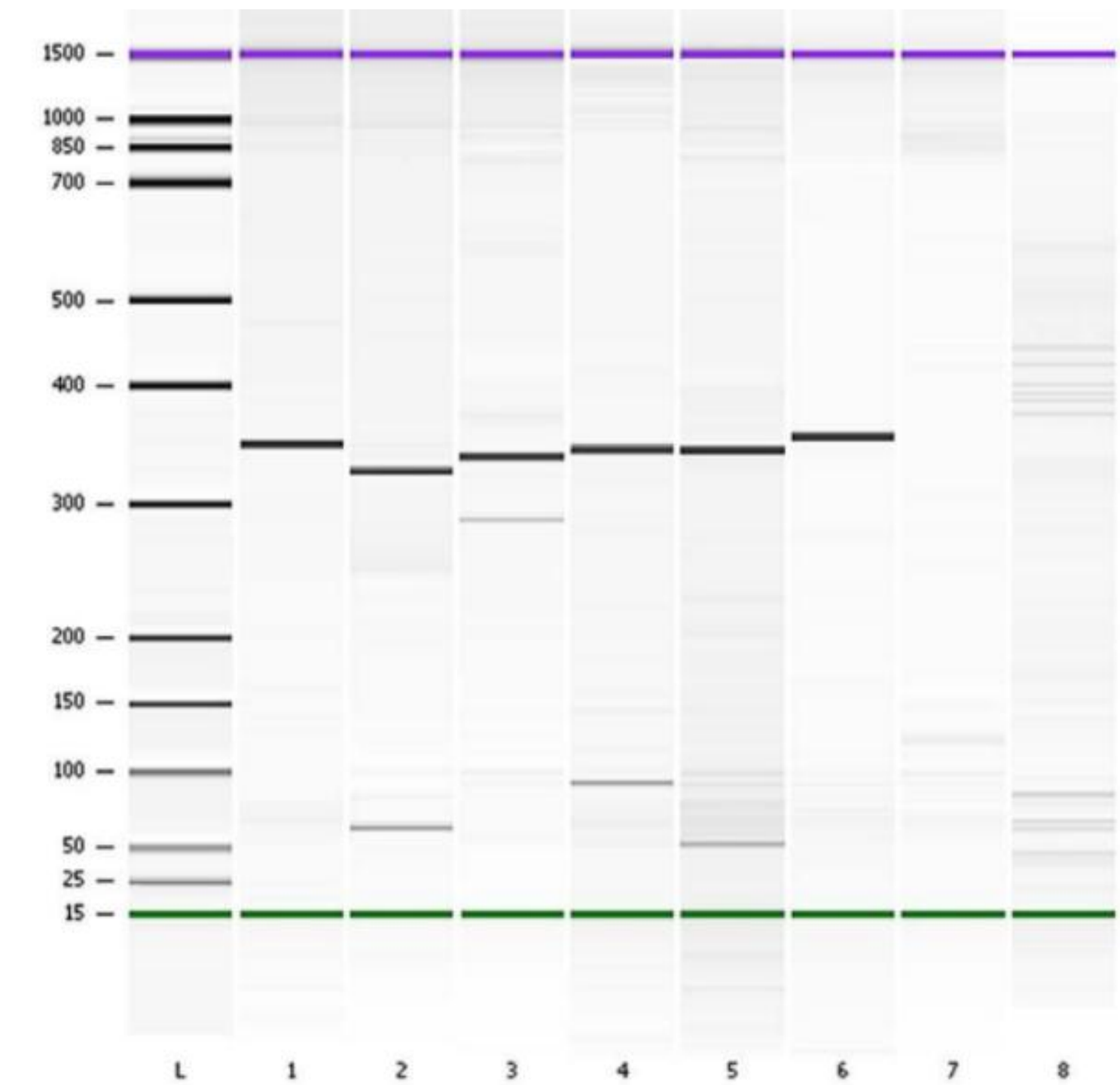

Figure 1. PCR amplification of *ure*C gena on lab-on-chip electrophoresis (L – standard; 1 – *S. pasteurii* DSM 33; 2 – *Bacillus muralis*; 3 – *B. lentus*; 4 – *B. simplex*; 5 – *B. firmus*; 6 – *B. licheniformis*; 7 – *B. pseudofirmus* DSM 8715; 8 – primer control)

Objectives

This study aimed to amplify the *ure*C gene, as a marker for urease presence in bacteria, using the PCR technique. Comparative analysis of PCR amplification was done for five *Bacillus* isolates from alkaline soils previously described as ureolytic bacteria based on highly efficient urea hydrolysis in laboratory conditions, as well as control ureolytic and non-ureolytic bacteria.

Materials and methods

In this study, for test bacteria are chosen Bacillus strains (*B. muralis, B. lentus, B. simplex, B. firmus, and B. licheniformis*) which are previously isolated as ureolytic and alkalophilic/alkaloresistant bacteria from alkaline and calcite-rich soils. For positive control reaction, DNA of *Sporosarcina pasteurii* DSM 33 which contains the ureC gene was used, while negative control was DNA extracted from non-ureolytic *Bacillus pseudofirmus* DSM 8715. All bacterial strains were grown at 30 °C on Columbia blood agar for 18 hours. The genomic DNA was extracted by a DNA Isolation Kit following procedure for Gram-positive bacteria, while the quantity of extracted DNA was determined by NanoDrop™ One Microvolume UV-VIS spectrophotometer. PCR amplification of ureC gene in the genome of tested bacteria was conducted using L2F (59-ATHGGYAARGCNGGNAAYCC-39) and L2R (59-GTBSHNCCCCARTCYTCRTG-39) primers. The final PCR mix (a total volume of 20 µl) involved DNA template (100 ng), PCR Master Mix (10 µl), primers (0.5 µmol per primer), DNA polymerase (0.5 µl), and H2O. The ureC gene was amplified under the following conditions: 5 min at 94.5 °C (initial denaturation); 30 cycles for 1 min at 94 °C, 1.5 min at 55.7 °C (primer annealing), 2 min at 72 °C (primer extension), and 10 min of final extension at 72 °C. All chemicals, as well as a spectrophotometer, are made by Thermo Fisher Scientific, Waltham, MA USA. Visualization of the PCR products (expected length 300-400 bp) was done by capillary Lab-on-a-Chip electrophoresis at 2100 Bioanalyzer (Agilent Technologies, USA).

Conclusions

Detection of the *ure*C gene provides basic information on the existence of a fully functional operon that carries information on the synthesis of the urease enzyme. Furthermore, among the structural genes for urease, the *ure*C gene is the largest coding structural subunit of urease, and more importantly, there are a large number of highly conserved regions suitable for PCR primer binding. According to the gained results, the *ure*C gene confirms the previously mentioned facts, because it is successfully amplified in all tested bacteria during this study.

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