TITLE: METHODS OF DNA EXTRACTION OF GRAM-POSITIVE BACTERIA PRODUCING BIOPOLYMER ISOLATED FROM CAATINGA

AUTHORS: JAMBEIRO, I.C.A.; BARROS, Y.C.; LANDIM, L.F.; SOUZA, B.C.; SILVA, E.P.; SANTOS, A.F.J.

INSTITUTION: Universidade do Estado da Bahia-UNEB, Departamento de Tecnologia e Ciências Sociais, Campus III (Av. R. Edgar Chastinet, s/n - São Geraldo, Juazeiro - BA, 48900-000, Juazeiro-BA, Brazil).

ABSTRACT:

The bacterial cell wall presents characteristics such as elasticity, resistance to pressure and high temperature. The Gram staining method divides the bacteria into two groups, Gram-positive and Gram-negative, and this classification is related to the structure and permeability of the cell wall. The structure of the Gram-positive cell wall consists of a thick layer of peptidioglican, stiffness which gives rigidity to the wall. This feature can make the DNA extraction process more complex. Furthermore, the Gram-positive bacteria producing exopolysaccharides (EPS), have a higher level of complexity, because the EPS promotes cell adhesion forming aggregates, provides protection against sudden changes in the environment, resistance to desiccation and mechanical protection. The aim of this study was to test different DNA extraction protocols for Gram-positive bacteria producing EPS for amplification of 16S rRNA gene. Four Gram-positive EPS-producing bacteria isolated from the Cactaceae of the Caatinga were used. The isolates were incubated for 24 h in Nutrient Broth culture medium at 30 °C under constant stirring at 130 rpm. Five extraction protocols were used: P1: thermoblock thermal extraction with 0.05 M NaOH buffer and 0.25% SDS at 100 °C for 15 min; P2: thermal extraction in a water bath with 0.05 M NaOH buffer and 0.25% SDS for 2 h at 100 °C; P3: thermal extraction in Tris-EDTA buffer adding an extraction step with chloroform-phenol (1:1); P4: phenol-chloroform-isoamyl alcohol extraction (25:24:1); P5: extraction by adding 3 µL Proteinase K (20 mg/mL) to 567 µL TE and 30 µL SDS (10%). After extractions, the DNA was analyzed in 1% agarose gel electrophoresis. The protocols P1, P3 and P5 were efficient for extraction of only 1 isolate, protocol 2 extraction was efficient for 2 isolates already with protocol 4 was extracted the DNA of all samples. PCR performed using the primer pair (Y1 and Y3 - 1500 bp) showed that the genetic material obtained by protocols 1, 2 and 4 was satisfactory. Protocols 3 and 5 evidenced the presence of substances that may have inhibited the PCR reaction. Thus, P4 was shown to be the most effective for extracting genetic material from Gram-positive bacteria producing EPS native to Caatinga.

Keywords: microorganisms, semiarid, exopolysaccharide, genetic material

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