

Federal State Budget Educational Institution of Higher Professional Education
"National Research Saratov State University named after N.G.
Chernyshevsky"

Singed by candidate

Ibrahim Mohamed Ibrahim Ibrahim

**STRUCTURAL AND FUNCTIONAL CHARACTERISTICS OF SURFACE
GLYCOPOLYMERS OF MICROORGANISMS ISOLATED FROM
HYPERHALINE ENVIRONMENTS AND DETERMINATION OF THEIR
BIOTECHNOLOGICAL POTENTIAL**

Specialty: 03.02.03 – Microbiology

Dissertation for the degree of
Candidate of Biological Sciences
(Ph.D. in Microbiology)

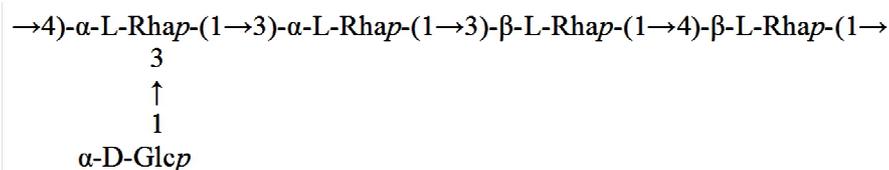
Scientific supervisor: **Prof. Dr. Konnova Svetlana Anatolevna**
Head of department of biochemistry and biophysics
Doctor of Biological Sciences, Professor

Saratov – 2019

Abstract

A total of 49 isolates as exopolysaccharide producers were isolated from Brine, multicolor solar salt, saline soil, saline mud and raw salts (Lake Qarun, Fayoum, Egypt and Lake Elton, Volgograd, Russia) using S-G agar supplemented with sugars and different salt concentrations. Based on the growth on different concentrations of salt (NaCl) all isolates were characterized into four groups. Seventeen promising isolates were selected. Based on the analysis of the nucleotide sequence of the 16S rRNA genes and the phenotypic and physiological characteristics, the seventeen promising isolates were represented in 10 major genera; *Bacillus*, *Salinibacillus*, *Virgibacillus*, *Piscibacillus*, *Halobacillus*, *Marinococcus*, *Chromohalobacter* and *Halomonas* as bacteria, and archaea was represented in *Haloterrigena* and *Haloterrigena*. The sequences of 16S rRNA genes for 17 isolates were registered in the Gene Bank at the National Center for Biotechnological Information (NCBI website: <https://www.ncbi.nlm.nih.gov>). Five strains were deposited in the Russian Collection of Agricultural Microorganisms (RCAM) of the All-Russian Research Institute for Agricultural Microbiology (ARRIAM), St. Petersburg. Nine strains were selected and their optimum conditions for EPS production were determined. It was shown that under optimal conditions, the highest EPS yields were observed for strains; *Chromohalobacter salexigens* EG1QL3 (13.7 g/l) and *Bacillus licheniformis* EG1QL30 (9.3 g/l). It was found that the two fructans (polysaccharides of fructose) dominate the formation of the EPSs of strains *Chromohalobacter salexigens* EG1QL3 and *Bacillus licheniformis* EG1QL30, which were homo-polysaccharides with a repeating unit of the following structure: $\rightarrow 6\text{-}\beta\text{-D-Fruf-(2}\rightarrow$ or Levan. It was observed the presence of teichoic acid in the EPSs of strain *B. licheniformis* EG1QL30, which was Poly-galactosylglycerophosphate with the following structure: $\text{-(6-}\alpha\text{-D-Galp-(1}\rightarrow\text{1)-sn-Gro-(3-P)-n}$. This polymer with this structure was first discovered among bacterial polysaccharides. O-specific polysaccharide (OPS) was isolated from the bacterial lipopolysaccharide (LPS) of *Halomonas*

ventosae RU5S2EL, with a new structure for bacterial polysaccharides. OPS consists of pentasaccharide repeating units of the following structure:



EPS solutions showed high emulsifying activity against hydrophobic substances, with high emulsion stability over time. It was observed that the most emulsifying activity (E24%) against kerosene by EPS solutions of *H. ventosae* RU5S2EL and *H. caseinilytica* EG33S7QL strains was about 80%. The emulsions of EPSs solutions of strains *H. caseinilytica* EG33S7QL and *Ht. saccharevitans* EG3QL57 with kerosene showed the highest stability over time. Multi-resistances were detected to seven of the nine strains producing polysaccharides against the influence of heavy metal ions. The tested strains showed stability (resistance) against studied heavy metal ions in the following order: Cu > Ni > Pb > Zn ≥ Cd. It was found that the high concentrations of heavy metals resulted in a decrease in the accumulation of biomass in addition to a decrease in EPS production, but at the low concentrations of heavy metals (0.2 mm) in the growth medium, it led to an increase in the EPS production by the most strains. Halophilic EPS-producing strains (*Halomonas caseinilytica* EG33S7QL, *Halomonas ventosae* RU5S2EL, *Chromohalobacter salexigens* EG1QL3, *Bacillus licheniformis* EG1QL30, *Bacillus subtilis* EGP5QL12, *Halobacillus dabanensis* EG1HP4QL, *Salinibacillus aidingensis* EG2QL8 and *Haloterrigena saccharevitans* EG3QL57) showed ability to use crude oil as a sole source of carbon. *H. caseinilytica* EG33S7QL showed a high degradation activity (68%) for crude oil in presence of salt in 12 days, while the degradation activity of residual strains from 23 to 34% of crude oil.