Title:	Anaerobic haloalkaliphilic electrotrophs: Nitrate and Sulphate reducing microorganisms possessing extracellular electron transfer capabilities from a highly saline-alkaline environment
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Abstract:	Electromicrobiology deals with the study of extracellular electron transfer (EET) processes between microorganisms and insoluble, solid-state electron donors or acceptors, and their roles in different environments. Different microorganisms are known to possess EET capabilities to fulfil their respiratory and metabolic requirements in various environments. EET seems to be a widespread metabolic trait by which microorganisms use multivalent metal ions associated with minerals and other insoluble compounds as either a sink or source for the electrons. It is classified into two types, viz. outward EET (from cells to electron acceptor) and inward EET (from an electron donor to cells). The microorganisms bearing such unique capabilities are termed as Electroactive Microorganisms (EAMs). Based on the type of EET, EAMs are further categorized into two groups, namely, exoelectrogens and electrotrophs. The microorganisms possessing outward EET are termed as exoelectrogens, whereas the microorganisms which take up electrons from an extracellular electron donor to uphold their metabolic processes are termed as electrotrophs. Exoelectrogens and outward EET mechanisms have been well documented and understood, whereas very little is known about the electrotrophs and the inward EET mechanisms they possess. Furthermore, not much research has been conducted on the electrotrophic microorganisms. Only a handful of pure cultures of electrotrophs are known to date, including, nitrate or sulphate reducing microorganisms. More importantly, the extreme environments have been barely explored for such microorganisms, which can be of interest for biotechnological applications. This study aimed at investigating the EET capable anaerobic nitrate-reducing bacteria (NRB) and sulphate-reducing bacteria (SRB) from a hypersaline-alkaline soda lake (Lonar Lake, Maharashtra, India), which is known to host a wide diversity of haloalkaliphilic microorganisms. Two different approaches were used for this purpose. The first one was based on the use of electrochemical cultivation, wherein the electrode, i.e., cathode poised at a specific electric potential, was used as the source of electrons. The second approach involved the enrichment in serum flasks with soluble electron donor source and further testing of the enriched culture for its ability to draw electrons from the cathode via EET for growth. A highly saline (20 g NaCl/L) and alkaline (9.5 pH) growth medium supplemented with either acetate (10 mM) or bicarbonate (10 mM) was used for enrichment experiments. The successful enrichment of NRB was achieved via both the enrichment approaches. However, SRB was enriched via the electrochemical approach only. Particularly the increase viin the cathodic reduction current confirmed the enrichment of both electrotrophic NRB and SRB. The cyclic voltammetry recorded with the enriched NRB revealed two redox-moieties with the formal potentials of -0.622 V and -0.433 V vs. Ag/AgCl. Further, the decrease in nitrate and sulphate concentrations in the electrochemical reactors confirmed the growth of NRB and SRB, respectively. In the case of serum flasks, the increase in optical density, and the decrease in nitrate concentration confirmed the enrichment of NRB. Its EET capability of this culture remains to be checked via the electrochemical cultivation approach. Microscopic analysis of the enriched cultures revealed the abundance of oval-shaped cells in all cases. These results indicate the successful enrichment of the electrotrophic NRB and SRB from the sediment samples of an extreme halo-alkaline environment. Their EET capabilities will be confirmed via additional electrochemical tests. Further characterization of the enriched cultures through 16S rRNA metagenome sequencing is envisioned to understand the dominant haloalkaliphilic electrotrophs. It will be followed up by isolation of the novel electrotrophs and identification and characterization of the observed redox-moieties involved in the inward EET processes. Detailed understanding of the haloalkaliphilic electrotrophic microorganisms is expected to increase our existing knowledge of this novel microbial group and electron uptake electromicrobiology mechanisms, discipline and which have developing biotechnologies.
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