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Title:	Haloalkaliphilic cable bacteria: Investigations of sulphide-oxidizing filamentous microorganisms from a highly saline and alkaline environment
Authors:	Singh, Ramandeep
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Abstract:	Electron transfer is an integral part of the aerobic and anaerobic respiratory processes in different life forms. Microorganisms gain energy by transferring the metabolically produced electrons to the terminal electron acceptors through a series of electron transfer chain components located in the cell membrane. In aerobic conditions, oxygen acts as the ultimate electron acceptor, while in anaerobic conditions, microorganisms use different soluble or insoluble electron acceptors, other than the oxygen. These include, for instance, fumarate, Fe (III) or Mn (IV), sulphate and nitrate. In the case of insoluble or solid-state electron acceptors, some microorganisms use a unique mode of electron transfer referred to as extracellular electron transfer (EET) to achieve their respiratory processes in anaerobic conditions. Such microorganisms are termed as Electroactive Microorganisms (EAMs). A select filamentous microbial group named as cable bacteria has been reported to achieve its respiratory processes by using distantly placed electron donor and acceptor molecules, for instance, at anoxic-oxic interfaces. They perform a unique mode of EET, referred to as Long Distance Electron Transfer (LDET), to link sulphide oxidation to oxygen or nitrate reduction reactions separated over centimetre distances. The study of microorganisms capable of EET and LDET provides important insights into the dynamics of electron transfer processes and their interactions with the environment, e.g., biogeochemical cycling of various elements. They can also be explored for some applications. For instance, the nanowires produced by such microorganisms bear high conductivity, comparable to copper wire, thus making them a potential candidate for the development of bio-electronics. These microbes can reduce the sulphide toxicity in soils and aid in agriculture. They can also outcompete methanogens in anaerobic environments, and thereby affect the release of methane - a potent greenhouse gas. The cable bacteria have been studied mainly from the marine and freshwater habitats so far. To broaden the understanding of the cable bacteria in extreme environments, this study focused on investigating them in a haloalkaliphilic environment. To this end, two different cultivation approaches, namely, serum flask and electrochemical, were used for the enrichment of sulphide-oxidizing cable bacteria in a highly saline (20 g/L) and alkaline (pH 9.5) growth medium. It helped to understand the microbial growth pattern with soluble and insoluble terminal electron viiacceptors. The electrochemical cultivation technique resulted in the enrichment of filamentous bacteria but at different length distribution in the reactors. For instance, larger size filaments (up to 200 μm) were dominant in suspension, whereas smaller size filaments (in the range of 5-10 μm) were observed at the electrode surface. In the case of serum flask cultivation, mostly single cells and a few smaller size filaments were observed. The enriched bacteria were capable of linking sulphide oxidation with the electrode and nitrate reduction in electrochemical and serum flasks approaches, respectively. The light, fluorescence, and scanning electron microscopy observations revealed the presence of long filaments, thereby confirming the growth of haloalkaliphilic filamentous bacteria. This study, for the first time, reports on the electrochemical cultivation of filamentous microbes in the extreme saline-alkaline environment and thereby validates their presumably ubiquitous presence in diverse interfacial environments. It also opens up the possibilities of understanding their LDET processes and a role in the biogeochemical cycling of sulphur, nitrate, oxygen, and Fe-bearing minerals in extreme environments. Further characterization of the enriched filamentous bacteria via metagenomics approach would help to understand their phylogenetic lineage and functional genes.
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