1. Introduction

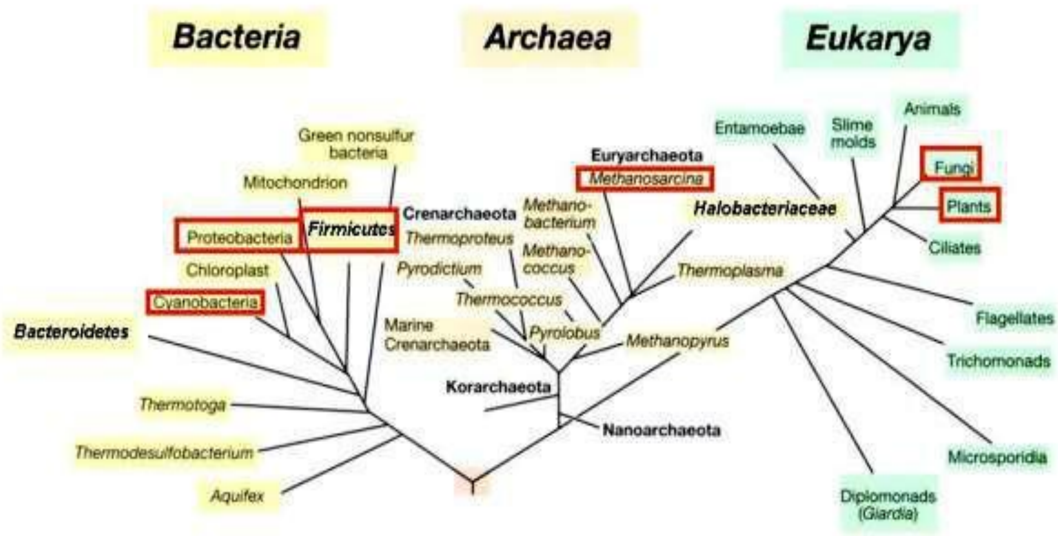
Hypersaline environments have very high salinity much higher than that of seawater, often close to or exceeding salt saturation. Hypersaline environments include acidic hypersaline lakes, deep ocean brine pools, alkaline hypersaline lakes, industrial effluents and solar salterns. Solar salterns are one of the extreme environments with high NaCl concentration which are used for commercial salt production. Ancient salt production technology involves concentration of sea water by allowing it to pass through a series of interconnected ponds (Javor, 2002). Though the crystallizer pond is saturated with NaCl, it is also rich in chloride, potassium and magnesium salts which provide the environmental conditions suitable only for specially adapted halophiles.

Halophiles are group of microorganisms which includes bacteria, archaea and fungi. The halophiles are classified into three, based on their salt tolerance: viz, slight halophiles (2-5% NaCl), moderate halophiles (5-20% NaCl) and extreme halophiles (20-30%) (Oren, 2002). The halophilic microorganisms differ from other microbial flora by having ability to balance the osmotic pressure and dentaturing effects of high salinity. The halophilic microorganisms use two strategies to withstand high salinity existing in the salterns viz., salt-in and salt-out strategy (Kushner and Kamekura, 1988).

During the last few years, there was an improvement in providing a systematic base for halophilic bacteria classification, together with the use of various phenotypic characteristic tests. However, the taxonomical classification of halophilic bacteria has not yet been much developed. The characterization of salt for optimal growth of halophilic bacteria is essential prior to classification. But that step is too costly and time consuming and it is limited only to the cultivable halophilic bacteria (Rafael *et al*., 2011). So far, the taxonomy of the halophilic bacteria was mainly based on morphology, biochemical characteristics, characterization of pigments and less attention was paid to molecular methods. The

16S rRNA based analysis has shown that halophilic eubacteria and the halophilic archaeabacteria are from different phylogenetic branches (Fig. 1).

Fig. 1: Phylogenetic tree depicting the relationship between microorganisms



The most outstanding biological phenomena in nature is the coloration of salt lakes that prevails in all the arid regions throughout the world. The bright orange, red to pink colour of the saltern is mainly due to the astronomical numbers of unicellular, microscopic salt loving halophilic archaea and bacteria that live in the salt crust and water. Halophilic bacteria thrive in saline lakes with salt concentrations of 15 to 30 per cent. Most of the halophilic bacteria show red, orange or pink colour due to the presence of carotenoids. The halophilic bacteria present in the salterns produce red to pink coloured pigments which is similar to that found in many colourful flowers and pink flamingos (Oren and Rodríguez-Valera, 2001).

Colour plays an important role in every part of our life that includes the food we choose, the clothes we wear, etc., (Downham and Collins, 2000; Manikprabhu and Lingappa, 2013). Since pre historic times, the pigments have been used as coloring agents for various purposes. Chinese used plants, barks and insects as source of pigments that has been traced back more than 5,000 years. During the Indus valley civilization (2500 BC), henna, saffron, colourful flowers and other plants were used as pigment sources. (Gokhale *et al*., 2004; Aberoumand, 2011).

Sir William Henry Perkin developed mauvine, the first synthetic color in 1856 and this started a revolution in the history of colorants (Walford, 1980). Since then, several synthetic colours have been used for many purposes; due to the ease of production, superior coloring ability and less expensive nature. But after a long time, synthetic colourants have proved to be toxic which ultimately led to several adverse effects on health and environment (Downham and Collins, 2000).

In order to protect themselves from excessive heat and sun light, halophilic bacteria produce variety of pigments such as carotenoids, prodiginines, phenazines and quinones. The carotenoids pigments from halophilic bacteria have several biotechnological applications. Carotenoids such as astaxanthin, lutein, salinixanthin, beta carotene are used as food colorants, as animal feed supplements and as anti-oxidative agents. Recently they have been used as nutraceuticals, cosmetics and pharmaceuticals (Garrido-Fernandez *et al*., 2010; Jaswir *et al*., 2011). Prodigiosin acts as a potential therapeutic molecule, especially as anticancer, immuno-suppressor agent. Prodigiosin also has got insecticidal, anti-malarial, antibacterial and antifungal activitiy (Harris *et al*., 2004; Kamble and Hiwarale, 2012).

It is clear from the literature that the halophilic bacterial pigments are still largely untapped when compared to other halophilic forms such as fungi, archaea and algae. India is the third largest producer of solar salt production and about 1,45,308 hectares are used for salt production (Salt Department, 2013 – 2014). Studying this kind of unexplored econiches can help us to find novel organisms with unique biotechnological applications. Using bacteria for pigment production has numerous advantages over fungi such as short life cycle and ease for genetic modification.

However, most of bacterial pigments are still at the research and development stage. Eventhough, the huge area is under salt production, the halophilic biodiversity in Indian salterns that too particularly the present study site, Markkanam, Tamil Nadu, India are not documented. Therefore, it is essential to study and analyze the biodiversity of this solar saltern which would help us to understand the distribution, physiology and ecological roles of halophilic bacteria.

Thus, work on halophilic bacterial pigments production should be intensified to explore novel pigments with unique biological properties and to make them available on the market. Hence, the present study focuses on the solar saltern in Marakkanam, Tamil Nadu, India for novel bacteria with following objectives:

- To isolate and characterize pigment producing halophilic bacteria by morphological and biochemical methods from Marakkanam salt pan, Tamil Nadu, India.
- To extract bacterial pigments and evaluate antioxidant activity of pigments
- To assess the cytotoxicity of pigment extracts from selected halophilic bacteria
- To characterize and identify selected pigment producing halophilic bacteria by molecular properties including 16S rRNA gene analysis
- To optimize pigment production condition in *Bacillus licheniformis* S15 by statistical method, RSM (Response Surface Methodology)
- To perform mass multiplication, extraction, purification of pigment from *Bacillus licheniformis* S15 and characterize by FT-IR, Mass Spectroscopy and NMR spectral analysis.
- To analyze the bioactivity of the purified pigment from *Bacillus licheniformis* S15