**Isolation and characterization of halophiles microorganisms from solar salterns of Trapani, Sicily.**

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**Highlights**

* Halophiles microorganisms are very interesting for biotechnological applications.
* These organisms were isolated and characterized from solar salterns in Sicily.
* Tested samples showed potential cosmetic applications.

**1. Introduction**

Halophiles are those organisms that can grow optimally at high salt concentrations and, hence populate hypersaline environments such solar salterns, salt lakes and Dead sea.

Hypersaline environments are inhabited by various microorganisms belong to all the three domains of living organisms: Archaea (*e.g.* Halophiles), Bacteria (*e.g.* cyanobacteria) and Eukarya (*e.g.* microalgae). The most known and representative microalgae that can be found in high salt concentration are two species of the flagellates green algae *Dunaliella*: *D. viridis* e *D. salina*. The latter is one of the most studied microalgae for their ability to accumulate large amount (up to 14% of dried biomass) of *β*-carotene under extreme environmental [1]. Although *D. viridis* does not accumulate that large amount of *β*-carotene, it can produce oxygenated carotenoids and grow faster than *D. salina* [2]. Similarly to *Dunaliella* species many diatoms, can tolerate high salt concentration and have been found in both solar salterns and very saline and polluted lakes [3]*.* Moreover, amongst hypersaline bacteria we can find both photosynthetic and heterotrophic bacteria*: i)* the cyanobacteria *Cyanothece* and *ii)* *Salinibacter rubens*. Several strains of *Cyanothece* group isolated from hypersaline habitats have been characterized for the production of exopolysaccharide (EPS) with biotechnological application [4]. Moreover, halophiles organisms under stress condition such as high salinity and high light intensity accumulated large amount of carotenoids in order to protect the cell against light damage and oxidative stress. Due to the accumulation of these carotenoids, the solar salterns during the summer season are frequently pink-red colored [5]. These carotenoids show beneficial effects for human health, hence the production of halophilic microorganisms’ carotenoids is of increasing interest for the blue economy.

**2. Methods**

The sampling site was the natural area “Saline di Trapani e Paceco”, formed by several pans dedicated to the extraction of sea salt in the province of Trapani, city on the west coast of Sicily. Here, the different salt concentration in these pans allows the proliferation of different halophiles microorganisms visible by changing color from white to pink or red. These organisms were isolated with serial dilution methods in both liquid and solid artificial seawater with different salt concentration (from 20 to 200 g/L of NaCl). The species identification of isolates halophiles were done by Colony PCR using different molecular markers (*i.e.* 16s, ITS, 18S). The cells were grown under different culture conditions in order to stimulate the production of secondary metabolites. High light intensity and salinity (*i.e.* 100 μmol m-2 s-1 and 200 g/L NaCl) was used as stress condition to stimulate the synthesis of interesting molecules (*e.g.* carotenoids) that can have a beneficial effect on humans. At the end of growth experiment, the cells were collected by centrifugation and the pellet was freeze-dried. Freeze-dried biomass was extracted with an hydroalcoholic solution (Eth/H2O 3/1, v/v) and the obtained cell extracts were tested in biological assay including antitumor and cell repair activity on human cell.

**3. Results and discussion**

New strains of microorganisms (bacteria and microalgae) have been isolated from red and pink coloured pan present in the saltworks of “Saline di Trapani e Paceco”: *D. viridis*, *Nitzschia dubiiformis, Salinibacter rubens* and *Cyanothece sp.* All the cell extracts did not show any antitumor activity on human cell (*i.e.* HT29 and PC3) at all tested concentration. *D. viridis* extracts from low light and salinity condition showed cytotoxic effects on human epithelial cell BEAS-2 cells increasing with high concentration. The same effect was not showed in the *D. viridis* grown under high light and salinity condition probably for the production of secondary metabolites with beneficial effect. Almost all samples showed a moderate repair activity on human epithelial cell line BEAS 2B, especially at medium and low concentrations (*i.e.* 10 and 1 µg ml-1).

**4. Conclusions**

This research aims to promote the sustainable production of biomass with high biological value, thanks to the selection of halophiles microorganisms. Here, new strains of hypersaline microorganisms (bacteria and microalgae) were isolated from solar salterns at the natural reserve of “Saline di Trapani e Paceco”. Thanks to the use of several molecular markers, it was possible to identify the species of isolated microorganisms. Cell extracts did not show significant cytotoxicity neither on normal nor tumour mammalian cells tested. Almost all samples showed a moderate repair activity showing a potential use of these strains in cosmetics.

**References**

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