Biotechnological potential of solar salt works: focus on Squarebop I bacteriorhodopsin

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The biomass of the hypersaline water of coastal saltern crystallizer ponds is constituted by red extremely halophilic microorganisms, rich of carotenoid derivatives, and is generally considered a renewable source of valuable bio-products [1-5].

Most of extremely halophilic microorganisms inhabiting the hypersaline saltern ponds belong to the Archaea domain.

Extremely halophilic archaea offer a multitude of actual or potential biotechnological applications, containing in their cells interesting metabolites, such as osmotically active substances (so-called compatible solutes), exopolysaccharides, special membrane lipids, proteins and enzymes.

For example, the compatible solutes play the role of maintaining a positive water balance in the cell and are compatible with the cellular metabolism in high intracellular concentration. These compatible solutes have been found to be excellent stabilizers for biomolecules [6-8]. Ectoine and its derivatives have been used as moisturizers in cosmetics [9] and the exopolysaccharide mannosylglycerate has showed a very high stabilizing effect on several enzymes subjected to heating or freeze-drying [10].

Among extremely halophilic Archaea, *Halobacterium salinarum* has been intensively studied in the course of the last four decades because it expresses the photo-activated membrane protein bacteriorhodopsin [11], which is an analogue of the rhodopsin of animal eye. Bacteriorhodopsin is a photo-activated proton pump, generating a proton gradient which is used by the organism to synthesize adenosine triphosphate; in brief, bacteriorhodopsin is used by the bacterium to directly convert sunlight into chemical energy. Due to its high thermal and photochemical stability, bacteriorhodopsin is considered a promising biological material for photonic device applications, such as holography, spatial light modulators, artificial retina, volumetric and associative optical memories.

Recently we have considered the possibility to use renewable resources of coastal salterns as starting material for the production of bacteriorhodopsin and we showed that, by starting from the concentrated biomass solubilized in detergent, we can collect pure fractions of a bacteriorhodopsin homologue after one-step affinity

chromatography (12). A microfiltration process has been designed to concentrate saltern biomass as starting material to isolate pure bacteriorhodopsin.

As regards the nature of the bacteriorhodopsin homologue isolated from the concentrated biomass of the saltern of Margherita di Savoia (Italy), we have been able to show that it corresponds to Squarebop I protein, the light-activated proton pump of the square halophilic archaeon *Haloquadratum walsbyi* [13-14]. No evidence for the presence of other bacteriorhodopsin-like proteins, such as archaerhodopsins [15-17] and cruxrhodopsins [18,19] was found in the environmental sample analyzed in the present study.

It is indeed well known that mature saturated brine (crystallizers) communities are largely dominated by these square halophilic microorganisms [20]. On other hand the peculiar morphology of *Haloquadratum walsbyi* (square flattened cells of 2–5 μm sides) could favor its enrichment by microfiltration; smaller rod and spiral-shaped cells might in part pass through a 0.2 μm pore size filter, while the square-shaped cells are mostly retained.

Environmental PCR and cloning techniques retrieved the genes encoding for the bacteriorhodopsin of *Haloquadratum walsbyi* in different saltern ponds of Alicante, in Spain , while similar metagenomic studies of the saltern of Margherita di Savoia, in Italy, are not yet available. Information on microorganisms present in that saltern community of brines has been obtained by lipidomic studies [21-22].

Interestingly, the lipid pattern of concentrated biomass showed lipid components not degraded after the long concentration process; in particular the lipid profile of concentrated biomass is very similar to that present in the biomass before the microfiltration process and to that of the membranes isolated from *Haloquadratum walsbyi* laboratory cultures also, as expected considering the abundance of this archaeon in the crystallizer ponds (23). Reported biochemical data clearly show that bacteriorhodopsin can be isolated from the concentrated biomass of salterns with its *annulus* of phospholipids and glycolipids; it is well known that the association with specific lipids is crucial for the functioning of an integral membrane protein. Finally the assay used to test the functionality of isolated and purified Squarebop I bacteriorhodopsin revealed that the final product of the concentrated biomass still retains its photoactivity; the photocycle of saltern bacteriorhodopsin is very similar to that of *Haloquadratum walsbyi* Squarebop I bacteriorhodopsin [13].

In conclusion, it is possible to concentrate saltern biomass by using a bioreactor; the final mud still contains many valuable biochemical components of cells of extremely halophilic organisms, similar to those isolated from fresh cell cultures. Despite the fact that the red water processing is quite long, we have shown that the functional Squarebop I bacteriorhodopsin and not degraded archaeal lipids are still present in the concentrated biomaterials and can be extracted at a reasonable yield.

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