Biodiversity as a source of innovation and development: the Trapani and Marsala salt works

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The coast from Trapani to Marsala has been influenced by the presence of salt works for sea salt production for over 2000 years. This activity has had a significant impact on both the territory and its local population, influencing the areas geomorphological, ecological, economic and ethno-anthropological points of view.

It has been suggested that salt harvesting in the area began with the Phoenicians. The first historical record however, dates back to 1145 by the Arab geographer El Idrisi (Bufalino, 1983).

The choice of this area for sea salt production, presumably was determined by the combination of a number of factors; such as the morphology of the coast (flat and low), climatic characteristics (dry and windy), the presence of artisan skills for the construction of windmills. Finally, the high productivity of the fishing industry in this area led to a ready market for the sea salt to preserve the caught fish.

We can find evidence of the abundance of fishery resources in the rock art of the Cave of Genovese (on the Island of Levanzo 20km off the coast of Trapani). Where carvings dating back 11000-12000 years ago (late Paleolithic) and paintings dating back to 5000-6000 years ago (late Neolithic) depicting numerous marine animals have been found (Figure 1).

Figure 1 – Painting of the Cave of Genovese on the Island of Levanzo, off the coast of Trapani.

Further, evidence of the abundance of fish in the area and its strong relationship with sea salt harvesting can also be found in the pits for making garum (a salted and fermented fish sauce made in Roman times) and the more recent “*tonnara*”, trap nets utilized to catch blue fin tuna, which would have been preserved with sea salt (Figure 2).


Figure 2 – The buildings and a traditional boat of the “*tonnara*” industry on the Island of Favignana (left) and blue fin tuna fishing in the trap net used in “*tonnara*”, off the coast of the Island of Favignana (right).

Over the centuries the surface occupied by salt works underwent cyclic variations. Along with fishing, sea salt production had a major impact on the local economy.

Today the salt works cover an area of 1,200 ha. The majority of salt works in this area are still exploited, but a few are now abandoned or utilized as extensive aquaculture basins (Figure 3). About 75 % of this surface is exploited for salt harvesting with an annual production of approximately 90,000 t of sea salt, however, nowadays, sea salt is no longer a major factor for the local economy.

Nonetheless the geometry of the basins and the windmills still make a unique landscape in this part of Sicily (Figure 3).


Figure 3 – Salt harvesting in salt work “Ettore Infersa” (left). The lagoon Stagnone of Marsala and the Salt work “San Teodoro”, utilized for fish farming (right).

Along the coast from Trapani to Marsala there are two types of salt works, those characterized by industrial exploitation and small artisanal family-run salt works (Figure 3).

Industrial salt works of Sosalt Spa occupy the major part of salt works surface area (800 ha) producing almost 80% of sea salt.

Sosalt spa utilizes two production sites, one on the Island “Isola Lunga” in the lagoon of Stagnone and one near the city of Trapani where it also owns a sea salt processing plant (Figure 4).

Figure 4 – Processing plant of the industrial salt work (left). Salt harvesting in an artisanal salt work (right).

Sea salt harvesting in the artisanal salt works of Trapani has remained tied to traditional techniques to cultivate the salt. Until the end of the last century the salt was almost exclusively harvested by hand, with a large experienced workforce (Figure 4). The experienced workforce was needed as the manual harvesting requires some knowledge/skill to avoid collecting the mud that lies under the salt layer.

During the 90s to counter a growing financial crisis in the sector, determined by the limited profit margins, lack of workforce and high labor costs a process of “artisanal technological innovation" was started. This resulted in some prototypes of salt harvesting machines (Figure 5).

Figure 5 – Artisanal mechanization of salt harvesting in the salt works of Trapani. Ph A. Battaglia (left), D. Culcasi (right).

The interest in innovation of the salt works farmers is confirmed by the "willingness to experiment", as in the case of the successful pilot trial currently in progress with the cooperation of the industrial salt works. This is being carried out to verify the possibility of using brine from the desalinization plant of Trapani (Figure 6) to improve the salt production.

Furthermore, it is worth stressing that a patent for the technique to produce Water-soluble natural integral sea salt tablets (Figure 6), prepared as single unit doses simple and ready to use (Daidone, 2005).

Figure 6 – Desalination plant of Trapani (left) and water-soluble natural integral sea salt tablets (right), ph http://www.flamingosalt.it/.

At the end of the 20th century and the beginning of the 21st century the most significant innovation resulting in an increased income has involved the Trapani salt works, and is represented by the possibility to maximize the main wealth of these unique artificial ecosystems: "the biodiversity", consisting of four levels: genes, species, ecosystems and functions (Nunes and van den Bergh, 2001).

Keeping in mind that the salt works are industrial environments, generated and maintained by the labor of man, these extreme environments have a peculiar biodiversity that is determined by the gradients of ecological conditions and their seasonal variations, arising by the cycle of sea salt production and harvesting

The salt works of the Province of Trapani, are characterized by high biodiversity not only in terms of genes and species, but also in terms of ecosystems and functions, *i.e.* landscape and cultural and ethno anthropological heritage.

To protect the high ecological and cultural value, unique biodiversity and ethno-anthropological heritage, the salt works of the Province of Trapani have now been protected through the institution of two Natural Reserves:

1) the Regional Natural Reserve “Isole dello Stagnone di Marsala”, established in 1984, with DA 215/04.07.1984, managed by Province of Trapani;

2) the Regional Natural Reserve “Saline di Trapani e Paceco”, established in 1995 with DA n. 257/44/11.5.1995, management by World Wildlife Fund WWF.

Figure 7 – The “challenge” for the management of the natural reserve.

These two Natural Reserves are very peculiar, they exist because man has built these artificial environments to exploit the natural resources and will exist as long as man governs and uses these resources.

For these reasons, they represent a big challenge for the institutions and the persons appointed to manage the reserves and to govern inevitable conflicts (Figure 7).

An example: since the establishment of the reserve and the disappearance of hunting, we have witnessed the increase in the bird population and among these the populations of piscivorous birds (Figure 8).

In the first storage basins of the salt works of Trapani euryhaline fish species are reared by extensive techniques. However piscivorous birds cause considerable damage to the fish production (Figure 8).

Figure 8 – Cormorants in a salt work basin (left). Sea bream with a wound caused by a bird's beak (right).

According to the derogation provided by the Council Directive 2009/147/EC (Birds Directive), the salt farmers in order to protect farmed fish are permitted to put in place bird scaring systems such gas cannons. However these systems can have a significant impact on other bird species (Russel *et al.*, 2012).

Over the years other less invasive defense systems have been tested (Figure 9), which should be promoted and recommended to the salt workers, this will ensure and improve the sustainability of human activities (Santulli, 2009).

Figure 9 – Net pens installed in rearing basins to prevent the underwater hunting by cormorants.

This experience suggests that it is necessary to implement management policies that consider the preeminent sustainable exploitation that is essential for the survival of these artificial ecosystems.

In spite of the conflicts arising from the protected environment being an artificial environment, and for its preservation, it is essential that man continues to produce and harvest the salt. The formation of two natural reserves has led to many positive effects on the conservation of salt works, biodiversity, and the local economy.

It has to be considered that the most significant effect of the reserve institution is undoubtedly represented by the increase in the number of tourists, which has increased significantly around the salt works of Trapani. Tourists are attracted by the unique features of the territory and also by the peculiar biodiversity associated with these environments.

Figure 10 – Red water of the basins of a salt work on Isola Lunga, in the Stagnone Lagoon, one of the most distinctive aspects of the salt work landscape

Among the "exploited" aspects of the biodiversity of salt works of Trapani some are "consciously exploited" such as the bird community that inhabits the salt works. Whilst others are "unconsciously utilized" such as the halophilic microorganisms that turn the waters of the salt works red (Figure 10).

A detailed description of the biodiversity of the Natural Reserve of the salt works of Trapani and Paceco was made by Troia (2006). Troia (2006) published a review from several authors' works, to describe the different aspects of the biodiversity.

The biodiversity of the Trapani salt work has been also reviewed by Giordano and Coll. (1998), Gianguzza and Coll. (2003), Mannino (2010) and by Mazzola and Coll. (2010).

The birdlife gives an immediate idea of biodiversity and immediately impress visitors to the salt works.

Trapani and Paceco the number of species increased from 113 in 1996 to 200 in 2005 for both wintering and nesting birds' species (Giordano *et al*., 1998; Troia, 2006).

Among the species listed in Annex 1 of the EC Birds Directive (79/409/EEC), we can find *Botaurus stellaris*, *Egretta garzetta*, *Casmerodius albus*, *Platalea leucorodia*, *Phoenicopterus roseus*, *Himantopus himantopus*, *Larus genei*, *Sternula albifrons* and finally *Recurvirostra avosetta* (Figure 11), which has been adopted as a flagship species of the Reserve the salt works of Trapani and Paceco (Figure 11).

Figure 11 – *Recurvirostra avosetta* (left) WWF logo (right).

The area of the salt works of the Province of Trapani represents an important habitat for large numbers and many species of water birds (Troia, 2006~~)~~ similar to other man-made Mediterranean salt works(Birtas *et al*., 2011).

The effect of the institution of the two Natural Reserves on the number of bird species and individuals was particularly evident. In the reserve of the salt works of

The areas surrounding the basins are also populated by a rich fauna, less visible and less eye-catching, but no less significant in terms of biological importance (Troia, 2006).

Among insects there are many endemic and rare species such as *Cephalota circumdata imperialis, Cephalota litorea goudoti,* and *Pterolepis elymica* that are found in the salt works.

The small Limantride, *Teia Arcerii dubia* is particularly vulnerable as well as rare, because females are apodal and apterous.

It is believed that among insects there are still many unknown species (Troia, 2006).

Figure 12 – Female of *Platycleis (Decorana) drepanensis* (Ph. B. Massa).

Recently, in fact, an interesting endemism has been described: a grasshopper (Figure 12), discovered in 2006 within the Reserve, is endemic *Platycleis (Decorana) drepanensis* (Massa *et al*., 2006).

Two species of water beetles are also particularly interesting, *Potamonectes cerisyi*, and *Hydroporus limbatus* (Troia, 2006).

Diversity of the organisms inhabiting the water of salt work basins of Trapani and Marsala is greatly influenced by the salinity gradient. It is evident the progressive reduction of the number of euryhaline species with increasing salinity.

The first storage basins are usually used for the breeding of fish (*Dicentrarchus labrax* and *Sparus aurata*). In these basins wild resident fish species are present, such as *Syngnathus abaster*, *Atherina boyeri*, *Aphanius fasciatus* (Troia, 2006; Mazzola *et al*., 2010).

However little is known about other organisms, such as polychetes (Cicciari *et al.*, 1996), crustaceans (Cicciari *et al*., 1997) or mollusks (see Gianguzza *et al*., 2003 for a review).

Following the salt work basins gradient, at higher salinity the number of species is strongly reduced. These basins are characterized by the presence of *Aphanius fasciatus* and subsequently, at higher salinity by *Artemia salina* (Troia, 2006)

Salt works flora includes a small number of species characterized by a high degree of specialization.

Approximately 450 species of salt-tolerant plants are adapted to the extreme environment of the salt marshes, where the plants that survive are those tolerating or even requiring a high salt concentration for their growth and development. Along salt work banks there are *Salicornia radicans*, *Halimione portulacoides*, *Suaeda vera*, *Inula critmoides*, *Atripex halimus*, *Halocnemum strobilaceum*, *Arthrocnemum glaucum.* These plants are able to survive in these extremely high saline conditions through various mechanisms for adaptation and specialized physiological and biochemical defense mechanisms (Troia, 2006).

The presence of some endemic species is particularly noteworthy, such as *Limonium densiflorum*, *Calendula maritima*, *Anthemis intermedia*, *Limonium ferulaceum Limonium avei*, *Limoniastrum monopetalum*, *Halopeplis amplexicaulis* and *Cynomorium coccineum* (Troia, 2006).

A large number of these plants have been used for food and in traditional phytotherapy (Troia, 2006).

In the first storage basins of the salt works, where salinity is lower than 80‰, 70% of the vegetated surface is occupied by two seagrasses that are adapted to these extreme conditions: *Ruppia cirrhosa* and *Cymodocea nodosa* (Mannino *et al*., 2006). The remaining 30% is characterized by the presence of macroalgae belonging to 47 taxa, including *Chaetomorpha*, *Chondria*, *Cladophora* (Troia, 2006, Mannino, 2010).

At the highest salinity the waters of the salt work basins of Trapani is populated by numerous species of halophilic unicellular eukaryotes (*Dunaliella salina*) and prokaryotes (bacteria and archaebacteria), that, in spite of their crucial role in the production of salt, have received little attention (Margheri *et al*., 1987; Troia, 2006).

In fact, even if the raw material for sea salt production is sea water, the quality of produced salt crystals, in terms of density, inclusions, dimension, color, can be different in different crystallization basins of the same salt work, in different harvesting seasons and among different salt works. The halophilic microorganisms of the waters of salt works, as well as ensuring the spectacular phenomenon of “red waters”, determined by the accumulation in their cells of β -carotene and bacterioruberin (Oren *et al*, 1992; Oren and Dubinsky, 1994; Oren and Rodríguez-Valera, 2001; Oren, 2009; Oren 2010a), are also involved in the crystallization process, influencing the quality of the salt crystals (Figure 13).

Figure 13 – Basins of a salt work of Trapani characterized by different color, determined by different microbial populations and different crystallization in terms of quality and quantity.

Salt works therefore could be considered a super organism (Riggio, 2009) a ”giant bioreactor”, where extremophilic microorganisms, their metabolites, with biological processes associated with them and with the biological processes that they determine, significantly influence the formation of halites in the crystallization basins (Tackaert and Sorgeloos, 1993; Davis and Giordano, 1996; Popowski Casan and Sanchez Lorenzo, 1999; Javor, 2002; Liu *et al.,* 2002; Sundaresan *et al.,* 2006; Davis, 2009; Oren, 2010a; 2010b).

It has been demonstrated that halophiles present in salt work basins, directly or indirectly, can be involved in:

- initiation of the crystallization process (Castanier *et al*., 1999),
- acceleration of the formation of crystals (Norton and Grant, 1988);
- determination of the size and the number of halites (Lopez-Cortes *et al*., 1994).

In red water of crystallization basins, bacterial cells can reach and exceed the remarkable concentration of 10^7 - 10^8/ml (Oren, 2009). When the sodium chloride crystallizes and precipitates, it can incorporate the brine and then halophilic archaebacteria and bacteria that can reach up to 10^5-10^6 CFU per gram salt (Bírbír and Sesal, 2003). Within halites, they can survive for long periods (Norton and Grant, 1988) up to several tens of thousands of years (Lowenstein *et al*., 2011).

The biodiversity of the microbial community entrapped in fluid inclusions of halites can provide clear added value to sea salt, being an infallible tool for the certification of the geographical origin of sea salt.

There is a growing need for a pan-European identification system that validates the origin of food products, and strengthens consumer confidence and enables the identification and control of products intended for human consumption (Schwagele, 2005). In this context it has been demonstrated, by molecular biology techniques, already used for the characterization and geographical traceability of many foods (Luykxe and van Ruth, 2008). It will be possible to implement a traceability of sea salt, through utilizing the strong geographical connotation of this product which ties incontrovertibly to the production area. This can lead to a strong positive effect on the value of traditional food productions and the territory that utilize sea salt.

Dufossé and Coll. (2013) demonstrated that it is possible, utilizing 16S rDNA profiles generated by PCR DGGE, to characterize the bacterial community present in salt crystals that results different in salt from different producing areas.

These markers were proposed as a new traceability tool to certify the origin of salts, which allows tracing back the packaged salts from store shelves to their geographic origin (Dufossé *et al.,* 2013).

The same results have be obtained analyzing organic volatile products and Carotenoid-derived aroma of salt crystals and of *fluer de sel*, by gas chromatography–mass spectrometry (Silva *et al*., 2009; 2010; Donadio *et al.,* 2011). These compounds are produced by the microbial community of the salt works and they vary in salts from different origins, and have been proposed as chemical biomarkers to trace geographic origin of marine salt.

Sea salt from Trapani, recently included in the register of protected designations of origin and protected geographical indications, will surely have significant benefits from the use of these tools.

These results strongly suggest that biodiversity of salt work can be profitably used to increase the income of economic activities linked to the exploitation of salt works, by taking advantage in a sustainable manner of the peculiar biological characteristics of extremophilic organisms living in these environments.

However, there may also be other uses of biodiversity, aimed at the conservation and exploitation.

For the salt pans of Trapani there is a paradigmatic example.

One of the reasons that led to the establishment of the reserve is the presence of an endemism of great ecological value: the Sea marigold, *Calendula maritima* Guss (Figure 14), a critically endangered species (de Montmollin and Strahm, 2005). The Italian National Research Council, the University of Palermo , IUCN plant conservation programs in the Mediterranean, Conservatoire botanique national de Brest and WWF started a conservation program for this species. The project is also supported by the Klorane Institute, which is aware of the fragility of plant resources and operates in close cooperation with botanical conservatories against the disappearance of plant species in the world.

Figure 14 – *Calendula maritima.*

The conservation measures taken by the project are: *in situ* micro Reserves and *ex situ* conservation and population reinforcement, within the project GENMEDOC, an inter-regional network of Mediterranean seedbanks (Cardona *et al*., 2013).

Among the interests for the conservation and the protection of this critically endangered species, it have to be stressed that *C. maritima* belongs to the genus Calendula (Asteraceae), that comprises other species with numerous pharmacological effects, already exploited, in particular of *C. offincinalis* (Arora *et al*., 2013). Under this respect, we still have little information about *C. maritima*.

Terrestrial and aquatic biological communities of salt works are strongly influenced by environmental factors, in particular the high salinity that has a negative effect, reducing both species richness and diversity (Williams, 1998) compared to the sea and the adjacent areas.

The organisms that are able to survive in extreme conditions are known as "extremophiles", "halophiles" in the case of organisms adapted to live in very high salt concentrations.

Their ability to survive in these extreme conditions (Figure 15) is determined by the presence of complex systems of biochemical defense, such as compatible solutes, antioxidants, carotenoids and polyphenols that play a crucial role in stress management and adaptation and in scavenging free radicals and oxidative stress, often related to UV exposure (Margesin and Schinner, 2001; Oren, 2010b).

Oxidative stress in human is recognized to be at the basis of numerous pathological conditions (i.e. skin-ageing, Alzheimer, cardiovascular disease, melanoma, etc.).

For this reason studying extremophile organisms and the components of their defense mechanisms can be useful to better understand the molecular mechanisms of resistance to oxidative stress and to develop industrial biotechnological applications in pharmaceuticals, nutraceutical and cosmeceutical sectors, utilising compounds that the extremophiles produce for their adaptation to these extreme conditions.

Figure 15 – *Limonastrium monopetalum* along the banks of a salt work (Ph. M. Aleo)

Keeping in mind these considerations, we focused our attention on some extremophiles living in the salt-works of Trapani, to study some bioactive compounds that they produce and to verify their possible industrial application. Their utilization has to be sustainable and tightly linked to the territory.

Among the halophilic organisms we investigated: the plant Pickleweed (*Arthrocnemum glaucum and Halocnemum strobilaceum*), microalgae (*Dunaliella salina*) and halophilic bacteria and archaebacteria.

The bioactive properties of the molecules we extracted from these organisms were evaluated on seafood products, for nutraceutical applications, and *in vitro* for pharmacological and cosmeceutical applications.

1. BIOACTIVE COMPOUNDS FROM EXTREMOPHILES AND NUTRACEUTICAL APPLICATION

Halophytes, extremophile perennial plants growing along salt marshes, salt lakes and salt works all over the world, have strong antioxidant properties due to the production of secondary metabolites, produced to counteract environmental stressors, represented by variations of salinity, temperature, soil composition. Among secondary metabolites, phytosterols and phenolic compounds, mainly flavonoids and phenolic acids, are particularly abundant (Kim *et al*., 2011).

These compounds are responsible of the strong antioxidant activity (Rhee *et al*., 2009; Sung *et al*., 2009). Some halophytes have been used as a food by coastal people and in traditional medicine to treat a variety of diseases, like gastroenteric disorders, diabetes, asthma, hepatitis, hyperlipidaemia and cancer (Rhee *et al*., 2009; Sung *et al*., 2009; Essaidi *et al*. 2013). Due to these properties, halophytes are considered a promising source of functional foods, pharmaceuticals and cosmetics.

In the food industry, in particular, natural polyphenols are preferred to the synthetics ones, because are considered safer than synthetics and, further, they demonstrate equivalent ability to inhibit tissue oxidation, allowing to a broad range of applications (Ngo *et al*., 2011). Among these, the utilization in the seafood industry represent one of the most promising possibility, due to the increasing number of scientific evidence that demonstrate the ability of natural polyphenols in preserving seafood quality.

Fish, are a primary source of omega-3 polyunsaturated FAs (PUFAs), which provide many health benefits in humans (Arab-Tehrany *et al*., 2012), but at the same time they are very susceptible to lipid oxidation. This degradation leads to loss of quality, shortening shelf-life, decreasing consumer acceptability, reducing both the nutritional value and safety of the products. Methods for preventing or retarding the oxidation of sea food include storage at low temperature, appropriate packaging, glazing with various chemicals and incorporation of antioxidants (Ngo, *et al.* 2011).

Furthermore, modified atmosphere packaging (MAP), in which air in the packaging is substitute with a mixture of bacteriostatic and inert gases, is a widely used technique, effective in preserving the quality and extending the shelf-life of many fish products (Mastromatteo *et al*., 2010).

We investigated the effects of coupling MAP with polyphenols obtained from a halophyte species of salt works of Trapani, *Halocnemum strobilaceum*, on quality and shelf-life of a fisheries species common in the Mediterranean region, *Coryphaena hippurus* (common dolphin-fish).

In *H. strobilaceum*, the presence of strong antioxidants polyphenols, such as flavonoids, caffeic acid esters and cumarins, was previously demonstrated (Miftakhova *et al*., 2001).

Common dolphinfish, a cosmopolitan species very appreciated in Mediterranean, is characterized by high fillet yield, good taste and low price, but it is available in a restricted period during the year, limiting the fresh consumption.

Our results demonstrate that antioxidant treatment of dolphinfish shelf life coupled with MAP, significantly increases, respect untreated control, improves the sensory properties, such as colour, odour and general aspect, and ameliorated the levels of some biochemical markers.

In particular, the treatment with MAP and polyphenols extracted from *H. strobilaceum* was able to ensure the most effective protection against lipid peroxidation, respect to the control group, as demonstrated by the variation of markers related to this event (Messina *et al.,* submitted).

2. BIOACTIVE COMPOUNDS FROM EXTREMOPHILES AND PHARMACEUTICAL APPLICATION

Microorganisms that survive in extreme ecological conditions, such as high temperatures, high salt concentrations or extreme pH levels, have developed unique physiological and biochemical characteristics that make them a potentially valuable resource in the development of new biotechnological processes and industrial applications such as new products pharmaceuticals, cosmetics, nutritional supplements, molecular probes, and enzymes (Margesin and Schinner, 2001; Oren, 2010b).

Halophilic archaebacteria living in high salinity basins of salt works produce high levels of carotenoids (bacterioruberin), in response to high light intensities (Ben-Amotz *et al.*, 1983 Oren *et al.*, 1992; Jehlička *et al.*, 2013). These molecules exert many functions within the bacterial cells, protecting organisms from excessive irradiance and allowing the use of light as source energy (Oren *et al.*, 1992; Oren, 2010).

Carotenoids, lipid-soluble antioxidants, responsible for the yellow and red colours in many vegetables, have been shown in humans to prevent many types of cancer and cardiovascular diseases related to oxidative stress, thus these compounds have a considerable potential for use in pharmacology (Alquéres *et al.*, 2007).

The term oxidative stress identifies a pathological condition caused by the impairment of the physiological balance between the production and the elimination of oxidants, free radicals. Free radicals are unstable molecules, searching of its chemical balance through acquisition of missing electrons from other molecules that, in turn, become unstable and seek another electron from other molecules, thus triggering a chain mechanism.

Some oxidizing species, known as reactive oxygen species (ROS), are products of normal metabolic activity. In physiological concentration, they play important functions at cellular and systemic level. These metabolites react with various organic substrates mainly by oxidation, determining a broad range of structural and functional damages that can alter or compromise many biochemical patterns in cells, tissues and organs (Ames, 1983, 1992).

An excess of ROS contributes to the processes of aging and is implicated in the development of chronic diseases, neurodegenerative diseases, diabetes, cardiovascular diseases (Halliwell and Gutteridge, 1990; Benzie, 2000). ROS can oxidize lipids, causing lipid peroxidation, can oxidize protein substrates, resulting in the wrong folding and thus causing a structural or enzymatic dysfunction, depending on the type of protein. Still, they can cause damage to the DNA of various types ranging from the skeleton to the nitrogenous bases, resulting in modification of gene expression.

From the biochemical point of view, antioxidants act preventing or scavenging free radicals, thus the interest in natural sources containing these chemical compounds is increasing.

Natural antioxidants, such as phytochemicals have the ability to modulate many signalling pathways, they interact with growth factor receptors, influencing cell survival, cellular signalling cascades, and modulating cell cycle regulatory molecules, leading to inhibition of growth and/or apoptotic death of tumour cells (Mo *et al*., 1999).

The utilization of a natural antioxidants needs further studies, in order to verify *in vitro*, the effects of the exposure in terms of toxicity, antioxidant properties, induction of apoptosis and inhibition of proliferation and molecular basis of the reported effects.

Halophilic archaea and other halobacteria where extracted from salt crystals, they were isolated and cultivated and then identified by 16S rRNA gene sequence analysis.

Halobacterium salinarum, was chosen on the basis of both their growth rate and production capacity of carotenoids, and mass cultivated in a 14l bioreactor.

Bacterioruberins were extracted by supercritical fluid extraction and tested *in vitro*, both in normal and cancer cell lines, to evaluate the dose-dependent toxicity, the inhibition of oxidative stress and the inhibition of proliferation.

In normal cells, the pre-treatment with bacterioruberins extracted from the halobacteria of the salt work of Trapani and Marsala, protected cells from ROS production and subsequent mortality induced by oxidative stress.

In cancer cells, the treatment with increased concentration of these bacterioruberins, induce a significant decrease of cell viability in a time-dose dependent manner of up to 50%. Furthermore, the analysis of cell death shows that cancer cells undergo programmed cell death (apoptosis) that is one of the required molecular mechanisms for the identification of anticancer drugs.

Because the involvement of oxidative stress was demonstrated in all stages of carcinogenesis (Valko *et al*., 2006), attention is growing to the direct and indirect antioxidant capacity of many phytochemicals.

Intake of phytochemical antioxidants through diet may prevent or reduce oxidative damage and could reduce the risk of chronic diseases and cancer.

Pharmaceutical application of natural compounds in the fight against cancer is also a very fascinating suggestion.

But it is known that from the discovery of the potential anticancer activity of a natural compound to its pharmacological application against cancer can take several decades of experimentation.

However, there are other applications, less fascinating from the scientific point of view, but that may have more immediate and profitable applications in cosmeceutical industry.

3. BIOACTIVE COMPOUNDS FROM EXTREMOPHILES AND COSMECEUTICAL APPLICATION

Cosmeceutics seem to be one of the most promising industrial applications of halophilic bacteria (Kim *et al*., 2008). Its remarkable economical potential is confirmed by the great success of the ectoine (Kunte *et al*., 2014), a compatible solute produced by moderately halophilic bacteria. Ectoine has been utilized for skin protecting cosmetics due to its ability to protect the skin from the effects of UVA-induced cell damage (Buenger and Driller, 2004) and its activity against skin ageing (Heinrich *et al*., 2007).

Human skin is constantly exposed to UV rays in sunlight. This triggers a stress condition, and over time a series of cellular changes. The photoaging is a complex process that gradually leads to skin alterations as a result of intense and repeated exposure to the sun or UV rays. Major skin changes induced by UV are represented by the appearance of deep wrinkles and skin blemishes stimulated at the biochemical level by ROS-induced activation.

Extremophile organisms have a high biodiversity due to the extreme conditions of the habitat in which they live causing them to produce bioactive molecules (such as carotenoids and polyphenols). These compounds can act as inhibitors against these metabolic patterns inducing photoaging and protect against the ultraviolet radiation (Singh and Gabani, 2011).

In addition to antioxidant and antitumor activities, bacterioruberins obtained from H. salina*rum* can exert potential photoprotective and anti-photo-aging effects. These properties can be useful in the prevention of cellular damage induced by ultraviolet radiation; continuous exposure to UV rays (both UVA and UVB), which may lead to the development of skin cancer and other numerous complications. Mainly related to ROS production.

Experimental evidence confirmed the importance of natural antioxidants from marine organisms or extremophiles in preventing processes related to photoaging. This reduces the occurrence (in a dose dependent manner) of factors promoting skin damage (Ryu *et al*., 2009).

Most of the studies aimed to evaluate the potential of bioactive compounds have been conducted *in vitro*. Cultured cells represent an excellent model system to investigate the role that natural compounds have in enhancing the endogenous antioxidant power and in the inhibition of elastase and tyrosinase (the key enzymes involved in the process of photodamage).

We have shown that polyphenols extracted from halophytes and bacterioruberins from halophilic microorganisms are able to stimulate the endogenous antioxidant such as SOD and CAT. Furthermore polyphenols and bacterioruberins can inhibit the production of ROS in cell cultures that have been in an induced oxidative stress condition.

Furthermore both classes of antioxidants were able to reduce the levels of elastase and tyrosinase, responsible of wrinkle and blemish formation.

Recently, our studies on stem-cells *in vitro* have also shown that bacterioruberins from halobacteria have a significant anti-wrinkle effect by stimulating cell renewal (the main mechanism involved in the anti-aging effect in cosmetics).

Treatment of these cell lines with bacterioruberins showed a significant induction of molecular markers related to cell differentiation and the anti-wrinkle effect.

Figure 16 – Artisanal (left) and touristic (right) salt harvesting in a salt work of Trapani.

On the basis of these results and their potential industrial applications, it can be suggested there is a beneficial approach to biodiversity exploitation.

The study of biodiversity to protect and at the same time take advantage of an ecosystem in a sustainable manner, may produce innovation, development and generate income; part of which will be reinvested in the study and protection of biodiversity.

Our results indicate the possibilities to use some aspects of the Trapani salt works biodiversity, through innovative and sustainable technologies for the sea food industry and with nutraceutical and cosmeceutical application.

The technologies we propose, based on utilization of salt crystals and salt work biodiversity, enable the development of new goods and services in accordance with the innovation policy of the European Commission, and its strategy to boost the industrial production through one of the six key enabling technologies (KETs): “industrial biotechnology”.

Development of blue biotechnologies, together with the production of salt (one of the oldest and most sustainable exploited natural resources), will promote the conservation of salt works and of their biodiversity, along with providing jobs and growth for our country. This could help in addressing some of the major concerns for citizens in Europe by providing environmental protection and sustainable products.

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