



读书报告

黄建蓉

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Extremophiles

Applications



Part 1 Biotechnological applications of extremophiles,extremozymes
and extremolytes

Part 2 Halophilic microorganism resources and their applications
in industrial and environmental biotechnology



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MINI-REVIEW

Biotechnological applications of extremophiles, extremozymes and extremolytes

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Fabio Fava¹**

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极端微生物，极端酶，极端代谢产物在生物技术上的应用



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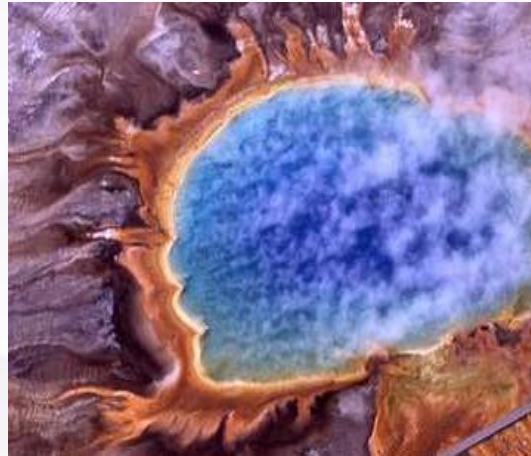
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Introduction

In the last decade, attention to extreme environments has increased because of interests to isolate previously unknown extremophilic microorganisms in pure culture and to profile their metabolites.



Extremophilic microorganisms thrive in the harsh environments where other organisms cannot even survive.

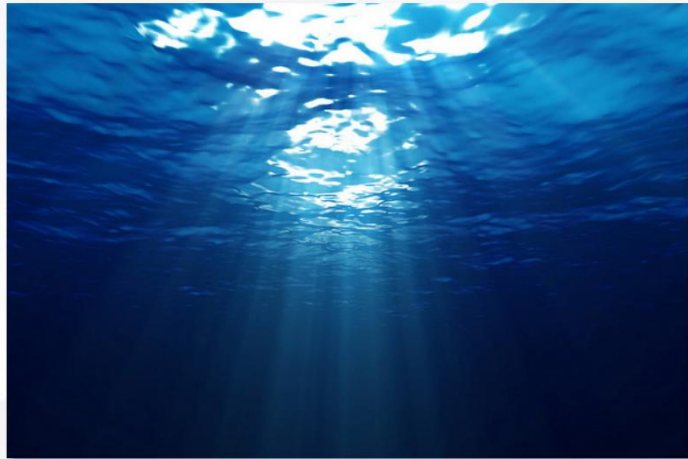
极端微生物	
thermophiles	halophiles
psychrophiles	barophiles/piezophiles
acidophiles	metalophiles
alkalophiles	radiophiles

These microorganisms produce extremophilic enzymes(extremozymes) and protective organic biomolecules (extremolytes) that convey characteristics for survival in extreme environmental conditions.



Ecology and classification of extremophiles

Extremophiles are organisms that have adapted to thrive in ecological niches that are uninhabitable to others, for example, deep-sea hydrothermal vents, hot springs, solfataric fields, soda lakes, inland saline systems, solar salterns, hot and cold deserts.





Ecology and classification of extremophiles



Psychrophiles are extremophiles that are adapted to extreme cold, and **halophiles** describe those that thrive in the presence of high salt concentrations; each type of microorganism uses different survival strategies to be successful in their environment.

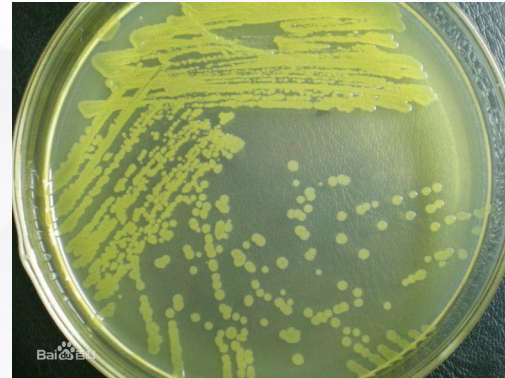




Ecology and classification of extremophiles



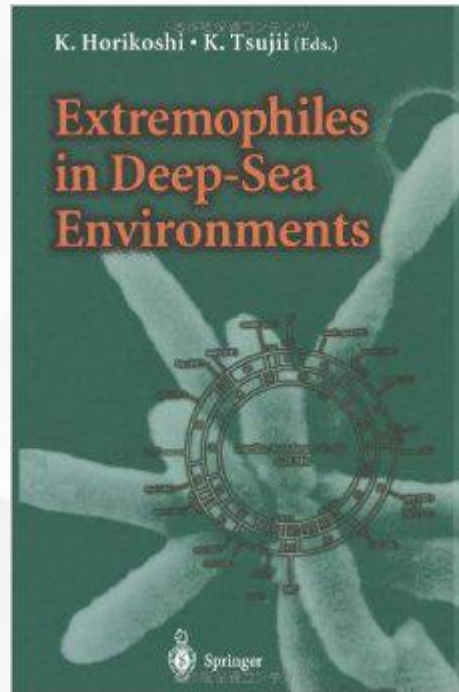
Adaptations for acidic environments, **acidophiles** are also typically adapted to environments with high temperatures, high salinity, or heavy metal concentrations because these conditions often co-occur; **alkalophiles** thrive in alkaline environments such as gypsum-based soils or soda lakes and are often halophiles.





Ecology and classification of extremophiles

Deep-sea and deep subsurface environments host **piezophiles** (barophiles), a group of extremophiles that produce **compatible solutes** and **polyunsaturated fatty acids** and form **multimeric** and **antioxidant proteins** that enable them to survive under extremely high hydrostatic pressures.





Potential applications of extremophilic/extremotolerant biocatalysts



Entire microbial cells

- 1、 because of their adaptation to high concentrations of heavy metals, **metalophiles/acidophiles** are currently being used for bioremediation and biomining(Navarro et al., 2013; Johnson 2014; Orell et al., 2013);
- 2、 **radiophiles** are suited for application in the management of nuclear waste-polluted environments(Brim et al., 2003; Appukuttan et al., 2006);
- 3、 applications can also be envisaged in agriculture where **desert bacterial extremophiles** that are able to cope with low water activity conditions can be used to improve the management of water by plants under drought stress(Marasco et al., 2012; Rolli et al., 2015);



Potential applications of extremophilic/extremotolerant biocatalysts



Extremozymes

1、 Enzymes produced by **psychrophiles** have been shown to display high catalytic efficiency in the detergent and food industries and for the production of fine chemicals (Cavicchioli et al.,2011);

Halobacterium sp. NRC-1 be active in high-salinity environments (with maximal activity in either 4 M NaCl or KCl) across a wide temperature range (−5 to 60°C) functionality is conserved in the presence of 10–20 % (v/v) organic solvents, including methanol, ethanol, n-butanol, and isoamyl alcohol, suggesting its suitability for the synthesis of oligosaccharides under low water activity and cold temperatures.

2、 **halophilic** enzymes: the extremotolerant cellulases produced by *Paenibacillus tarimensis* L88, have been shown to have high functionality across a broad pH range (3.0 to 10.5), at high temperatures (80 °C) and high salt concentrations (up to 5-M NaCl) (Raddadi et al., 2013).



Extremolytes and their biotechnological applications



Extremolytes are organic compounds that can constitute up to 25 % of dry cell weight accumulated in microorganisms exposed to stressful environmental conditions.

The **bacterioruberin**(**菌红素**) produced by radioresistant microbes (*Halobacterium* and *Rubrobacter*) has been suggested to have application in preventing human skin cancer because it participates in repairing damaged DNA strands caused by ionizing UV radiation (Singh and Gabani, 2011).





Extremolytes and their biotechnological applications

Extremolytes can inhibit protein misfolding and/or aggregation and, hence, are interesting candidates for the development of drugs for several diseases (Ryu et al., 2008; Faria et al., 2013; Kanapathipillai et al., 2005).

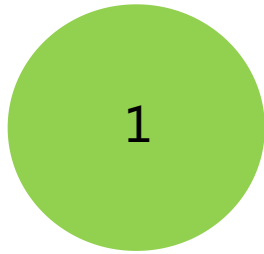
Furthermore, extremolytes have the potential for application in the food industry for the production of functional foods (Cencic and Chingwaru, 2010).



Conclusion

In conclusion, extremophilic/extremotolerant microorganisms are sustainable resources that could be better exploited in several biotechnological sectors towards the development of a bio-based economy.





Halophilic microorganisms comprise a heterogeneous group of microorganisms and require salts for optimal growth. They have been isolated from diverse salinity environments, varying from natural brines, hypersaline lakes to saturation salinities. Diverse halophilic population includes Archaea, Bacteria, and Eukarya.

常见嗜盐菌: *Halobacterium*, *Halomonas* and *Salinibacter*



2

Halophilic microorganisms are a potential source of extremozymes called **halozymes** like proteases, amylases, nucleases, lipases, cellulases, xylanases, catalases, and esterases, which are capable of functioning under high concentrations of **salt**, wide range of **pH values**, and **temperatures** at which other proteins will usually precipitate or denature.



3

These halozymes have been commercialized in various industries including food, baking, feed, chemical and pharmaceutical, paper and pulp, detergent, leather industries, fish sauce and soy sauce preparations, saline waste water, and oilfield waste treatment.



Review

Halophilic microorganism resources and their applications in industrial and environmental biotechnology

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嗜盐微生物资源和它们在工业、环境生物技术上的应用



objective

- (i) potential biotechnological applications of bioactive compounds, compatible solutes and some novel hydrolytic enzymes;
- (ii) recent efforts on discovery and utilization of halophiles for biotechnological interest;
- (iii) future perspective of aforementioned points.





Antioxidant Pigments

抗氧化色素

Families *Haloferacaceae* and *Halobacterium*, inhabit the extremely halophilic environments.

Their enzymes generally tolerate or require 4–5 M salt.

In addition to these adaptabilities, the extremely halophilic Archaea are also capable of producing extraordinary red pigments known as **carotenoid compounds (类胡萝卜素)** to **combat the high salt and intense UV radiation.**

It was shown that these colored pigments have strong antioxidant, immune boosting activities and likely protecting premature ageing.





Antioxidant Pigments



Although many halophilic Archaea produce carotenoids, the halophilic Eukarya *Dunaliella salina* is the only halophilic organism **used in industry** for the production of carotenoids.

Carotenoids are colorful natural products responsible for yellow, orange, red, to purple colors. They are extensively used as dyes and functional ingredients in food products including cosmetics.

In addition, extraction and purification of carotenoids are quite simple by direct lysis under hypoosmotic condition.



Mycosporine-like Amino Acids

类菌胞素氨基酸

In the past few decades, one major concern was the increase in the **solar UV radiation** reaching to the Earth's surface due to industrially released chemicals. Marine organisms are a group having direct impact from detrimental effects on the solar UV radiation.

Accumulating evidence has shown that the synthesized secondary metabolite compounds such as **scytonemins** and mycosporinlike amino acids (**MAAs**) and **non-enzymatic antioxidants** (i.e., carotenoids) are crucial for **scavenging ROS** (活性氧清除) generated due to UV exposure.



Mycosporine-like Amino Acids

Biosynthesis and accumulation of MAAs were mostly reported in microorganisms that thrive in hypersaline conditions such as marine **cyanobacteria** and **eukaryotic algae**.

It was shown that the MAAs protect the cell by **absorbing UV radiation** and **dissipating the energy as heat without generating ROS**.

It was shown that **halophilic cyanobacterial community** inhabiting a gypsum crust accumulated large concentration of MAAs (at least 98 mM) . High accumulation level is one of merits using halophilic strains that would be exploited in a large amount for industrial applications.



Compatible Solutes

相容性溶质

Compatible solutes are a group of low-molecular mass organic compounds that act as osmolytes to help organisms survive under various stress conditions.

Metabolic accumulation of compatible solute is often regarded as the second line of defense. This strategy is one of the foremost tolerance mechanism adopted by halophiles.

The most predominant solutes found in moderate halophiles are the **amino acid derivatives glycine-betaine** (氨基酸衍生物甘氨酸甜菜碱) and **ectoine** (四氢嘧啶).



Compatible Solutes

For industrial application, production of **ectoine** is promising.

It has shown that *Halomonas elongata* was employed to produce ectoine via the so-called milking process.

Milking process was carried out by using high-cell density cultures and then subjected to hypoosmotic condition. Consequently, ectoine was released into medium. Using this process, approximately 80% of the cytoplasmic ectoine was released to the culture medium. Finally, recovery, purification and crystallization of ectoine were conducted.



四氢嘧啶



Novel Hydrolytic Enzymes

新型水解酶

Halophilic microorganisms have been perceived as **potential source** of enzymes for various biotechnological applications.

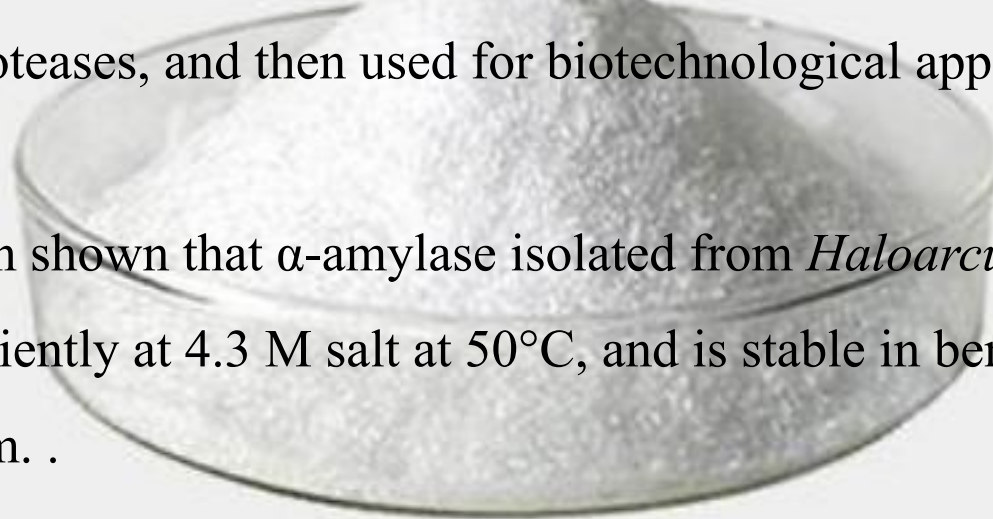
It has been shown that many enzymes derived from halophiles can **function** under harsh chemical and physical conditions.



Novel Hydrolytic Enzymes

Several **halophilic hydrolases** have been described including amylases, lipases and proteases, and then used for biotechnological applications.

It has been shown that α -amylase isolated from *Haloarcula* sp. functions efficiently at 4.3 M salt at 50°C, and is stable in benzene, toluene and chloroform. .





Future Perspectives

The recent availability of various **complete genome sequences** of halophiles together with advances in **omics technologies** would further provide new opportunities for exploration, discovery and identification of unique properties and/or novel biomolecules derived from halophiles in the future.





THANKS

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