

Salt-tolerant mechanism and application of salt-tolerant bacteria

Fupeng Yu¹, Chen Zhao^{3,4}, Kunlun Li², Le Su¹, Song Zhang¹, Qiulin Yue¹, Lin Zhao^{1,*}

¹State Key Laboratory of Biobased Material and Green Papermaking, Shandong Provincial Key Laboratory of Microbial Engineering, Qilu University of Technology (Shandong Academy of Sciences), Jinan 250353, China

²Jinan Hangchen Biotechnology Co., Ltd., Jinan 250353, China

³National Engineering Laboratory of Crop Stress Resistance Breeding, Anhui Agricultural University, Hefei 230036, People's Republic of China

⁴Shandong Food Ferment Industry Research & Design Institute, Qilu University of Technology (Shandong Academy of Sciences), Jinan 250353, P.R. China

*Corresponding author: sdilizhaolin@163.com

Keywords: Salt-tolerant bacteria, salt-tolerant mechanism, bio-industry technology.

Abstract: With the gradual acceleration of socialization and industrialization, salt-tolerant microorganisms have received more attention due to their unique salt-tolerance mechanism. It has a wide range of applications in Benedictine, cosmetics, wastewater treatment, agricultural soil and other industries. However, the current understanding of salt-tolerant microorganisms is still at a basic stage. The salt tolerance mechanism of salt-tolerant bacteria has not been fully confirmed. The current research on the salt tolerance mechanism mainly focuses on the cell external structure osmotic mechanism of phallic enzymes, compatible substances and ion transport across membranes. This article mainly reviews the salt-tolerant mechanism of salt-tolerant bacteria and its application prospects in social and industrial applications.

1. Introduction

Salt-tolerant bacteria, refer to bacteria that have a certain tolerance to salt Manacle concentration, mainly rt grow in high salt concentration environmental conditions, such as saline soil, salt lakes, salt farms [1]. For a long time, salt-tolerant bacteria have attracted people's research interest with their unique adaptive mechanism. Most of salt tolerant bacteria are hetero trophic classification. They have a wide range of carbon sources and are suitable for alkaline environments. Specifically, it can be divided into the following 5 categories [2] Table 1. The metabolic types of salt-tolerant bacteria are diverse, including oxygen-producing and non-oxygen-producing photo trophic bacteria, aerobic hetero trophic bacteria, fermentation Bacteria, denitrify bacteria, sulfate reducing bacteria and methanol [3].

Salt-tolerant bacteria have a very special physiological structure and metabolic mechanism, so that they can tolerate high salt and hyper tonic environment, and also produce many biologically active substances with special properties [4]. At present, salt-tolerant bacteria can be used in fermentation production, have the ability to resist high salt concentration environmental stress, they also play an important role in the treatment of high-salt sewage; develop salt-tolerant enzymes for biological applications technical engineering.

Table 1. Classification of salt-tolerant bacteria.

Classification	Growth salt concentration/(mol/L)	Examples
Anglophile	<0.2	Freshwater microorganisms, common fungi
Mild Phallic bacteria	0.2-0.5	Most marine microorganisms
Moderate phallic bacteria	0.5-2.5	<i>actinomycete</i> ,
Extreme halophilic bacteria	2.5-5.5	<i>Lith bacterium</i> , <i>halophilic actinomycete</i> , <i>halobacterium</i>
Salt-tolerant bacteria	>4.3	<i>Staphylococcus epidermic</i> , some fungi, <i>algae</i>

2. Salt tolerance mechanism of salt-tolerant bacteria

2.1 Na⁺ export mechanism and Osmotic adjustment

Na⁺ is necessary for the growth of salt-tolerant bacteria [5]. Na⁺ must exist in the active transport system of amino acids and plays a role as an essential factor in the respiratory response of production capacity. Na⁺ can maintain the structural integrity of the cell wall of salt-tolerant bacteria, but excessive concentration will damage the cells.

For some extreme phallic bacteria and anaerobic phallic bacteria, the accumulation of K⁺ / Cl⁻ ions in the cells, the growth of certain phallic bacillus depends strictly on the presence of chloride [6], these bacteria rely on Cl⁻ to make up for the increased external salinity while regulating the normal physiological activity of the cells [7]. In some phallic bacteria, there is a relatively complete K⁺ transport system, and most of them are unidirectional transport proteins. Under the high salt environment, high acellular accumulation of K⁺ can be achieved to balance the acellular and extracellular osmotic pressure. Compared with Na⁺, K⁺ may regulate protein activity and fold more correctly.

2.2 Stabilization of the external structure of the cell

The cell wall of salt-tolerant microorganisms does not contain antiseptically or glucose components, but is replaced by lactoprotein carrying a large number of acidic amino acids on the surface. The acidic amino acids carried by lactoprotein [8] will form a negative shield on the surface to avoid damage caused by high pressure penetration. At the same time, compared with neutral phospholipases, with the increase in salinity, the cell membrane of phallic bacteria increases a relatively high proportion of anionic phospholipases, which is beneficial to maintain the hydration state of the cell membrane and better resist external osmotic pressure.

2.3 Compatibility substances

The concept of compatible solute was first proposed by Brown in 1972 [4]. When the microorganisms are in a high osmotic pressure environment, the cells lose water and shrink, and the normal physiological and biochemical functions of the cells will be affected [7]. To adapt to this condition, salt-tolerant microorganisms will accumulate a large amount of small molecule organic solutes such as sugar and amino acids to restore the cell volume and osmotic pressure to their original levels [9]. These organic solutes are affinity compatible solutes, compatible solutes. The accumulation process will not affect the normal physiological and metabolic functions of the cells. When the hypnotic conditions are restored, the compatible solutes in the salt-tolerant microorganisms will quickly decompose, thereby maintaining the balance of osmotic pressure and avoiding cell damage caused by the large amount of environmental moisture entering the cells. In addition to osmotic adjustment, compatible solutes also have functions such as resistance to drought, high temperature, low temperature, and radiation [10].

3. Application of salt-tolerant bacteria

At present, we are facing the continuous depletion of energy and the increasingly serious problem of environmental pollution [7]. Therefore, the production model of the chemical industry must be reformed and turned to industrial biotechnology. Because of its unique salt-tolerant characteristics, salt-tolerant bacteria can adapt to more extreme environments, so it has a wide range of Biotechnology applications, from agriculture to medical treatment [11].

3.1 Application of salt-tolerant bacteria in wastewater treatment and ecological restoration

The acceleration of the process of socialization and industrialization will discharge a large amount of high-salt organic wastewater, causing the problem of freshwater pollution to become more serious. Traditional methods for treating high-salt wastewater are mainly through physical and chemical methods, but they are energy-intensive and costly. Salt-tolerant microorganisms have natural advantages in the treatment of salt-containing wastewater. On the one hand, it can adapt to the environment of high salt permeation [12], on the other hand, salt-tolerant bacteria can degrade organic matter in wastewater, and at the same time have a good removal effect on cadmium ions, mercury ions and other heavy metal ions in wastewater [13]. The *in vitro* culture and domestication of these halophilic bacteria and halophilic bacteria make the biological treatment of high-salt organic wastewater have great application potential.

According to extensive reports, soil salinization is a major agricultural problem, especially in irrigated agriculture. About 20% of agricultural land and 50% of farmland in the world are under salt stress. Some halophiles have the ability to use oxyanions as terminal electron acceptors, *Halofuran* sp. D1227 and *Heliocal* sp. D1 were shown to degrade such aromatic compounds as benzoate, cinnamate, 3-phenylpropionate, and 4-hydroxybenzoic acid [14]. Moosejaw et al. found a moderately halophilic Gram-positive coccus designated as *Salini* coccus sp. strain QW6 showing high capacity in the removal of toxic oxyanions of tellurium under a wide range of factors [15]. Therefore, salt-tolerant microorganisms play an important role in the restoration of saline-alkali soils.

3.2 Application in the food field

From fermented foods and preserved foods, halo monas and other salt-tolerant bacteria can be isolated, which can convert nitrate into nitrite, so that the food has a good flavor and color. Combining some salt-tolerant bacteria with alkaline protease or flavor protease into fermented food can increase the content of volatile substances and free amino acids in the product [16], and improve the flavor of the product. In addition, a recent study used moderately halophilic bacteria, including halo mona elongate, for biological control of strawberry diseases caused by *botrytis cinerea*. Such bacteria may replace synthetic fungicides and can be used in commercial production under storage and greenhouse conditions. Some halophilic lactic acid bacteria are an important microbial composition of the starter for the production of fermented foods. They can improve the quality of fermented food products or shorten the fermentation cycle. At the same time, these lactic acid bacteria can regulate the normal gastrointestinal flora, control endotoxins, and inhibit harmful microorganisms. Various probiotic effects such as production of substances and substances are of great significance to the human body's nutrition, physiological functions and immune response.

3.3 Bioactive substances produced by salt-tolerant microorganism

3.3.1 Cytocompatibility solute

In order to adapt to the dry osmotic environment, salt-tolerant bacteria will produce compatible solutes to adjust the osmotic balance. Among them, extreme halophilic bacteria will produce tetrahydro pyrimidine, which is commonly used in the medical and cosmetic industries as a protein stabilizer and drying protectant [17]. At present, the most widely compatible solute is examined, which was first discovered in the halophilic phototrophic bacteria *Salina*. It is currently widely used in industrial technology to protect many unstable enzymes and nucleic acids from high salinity and heat. The harmful effects of denaturation, drying and freezing. Recent studies claim that exorphin can

counteract the effects of ultraviolet and ultraviolet induction and accelerated aging, so it is added to the dermatological cosmetic preparations by the cosmetic industry as a moisturizer in cosmetics for care dry, irritated and aging skin [2].

3.3.2 Production of PHA by salt-tolerant bacteria

PHA is a polyester substance that can be synthesized by many microorganisms. Due to its excellent plasticity and biocompatibility, PHA can be used in the production of a variety of plastics and biological materials, such as medical materials, film products, disposables and packaging materials and other fields have broad application prospects and are currently the most versatile bioplastics [18]. At present, more and more salt-tolerant have been discovered and proved to produce PHA, such as *Bacillus*, *Halophiles*, *Halo monas* and so on. The high-salt and high-permeability environment will prevent the contamination of other miscellaneous bacteria, making long-term continuous cultivation possible. Secondly, seawater can be used as a saline medium, thereby reducing the consumption of fresh water. Recently, Boruc et al. discovered in 2017 that PHA accumulation can protect cells from the effects of excessive osmotic pressure [17]. Sedale et al. also discovered in 2019 that PHA can regulate osmotic pressure fluctuations [19], suggesting that PHA accumulation is an additional adaptation to high-salinity environments Strategy. Halophilic bacteria are a low-cost industrial biotechnology platform.

3.3.3 Salt-tolerant proteolytic enzymes

Salt-tolerant bacteria produce a variety of extracellular proteolytic enzymes, which can also maintain stability under high salt conditions. The protease primary sequence produced by salt-tolerant bacteria has a high content of acidic amino acids and is exposed to the outside and can tolerate organic solvents, and catalyse hydrolysis reactions. Halophilic amylases are produced by halophilic bacteria and include the genus *Chromophiles*, *Halo bacillus*, *Halo bacillus oweniid*, *Micrococcus halo coccus* and *Streptomyces* [20]. Amylase is widely used in food, fermentation, textile and paper industries. Protease-producing halophilic archaea include *Halofuran*, *Halobacterium*, *Antriea*, *Heliacal*, and *Natron bacterium* [21]. Proteases are widely used in detergent additives, feed, food processing, medicine, and leather manufacturing. Halophilic microorganisms can also produce lipase, agarose, chitinase, pectinase, pullulans and other hydrolytic enzymes, which are essential for biotechnology applications. In recent years, the research focus on the extracellular proteases of salt-tolerant bacteria has turned to its enzymatic properties and metabolic regulation [22,23], trying to find proteases suitable for industrial production. Producing proteins by halophilic microorganisms has bright prospects. The cost of harvesting halophilic cells for production is high; for example, harvesting *Daniella* cells for biofuels [24] and glycerol is expensive, so production may not be able to compensate for the cost of cell harvesting and final production cost.

In addition to being a potential salt tolerance mechanism, carotenoids have important functions in colorants, antioxidants [25,26], anti-cancer, heart disease prevention, and immunity enhancement, and are widely used in the food, medical, pharmaceutical and cosmetic industries, the synthesis of carotenoids has always attracted attention.

3.4 Prospect

In recent years, halophilic microorganisms have been made progress. Because of its unique salt tolerance mechanism, it is becoming more and more attractive to industrial biotechnology. Halophile biotechnology potential description and understanding of current destinations. The use of genetic means, heterologous expression and other means to transform and expand the production and applicable environment of salt-tolerant microbial products is a new trend in the development of salt-tolerant bacteria resources [27]. Nevertheless, our understanding of salt-tolerant bacteria is still at an early stage, and we have limited understanding of its physiological, metabolic, genetic and other characteristics. Therefore, the deeper the understanding of salt-tolerant bacteria, the easier it is to commercialize in large-scale industrial reductions. Salt-tolerant bacteria, with bright prospects for production [28].

4. Conclusions

As a special strain, salt-tolerant bacteria can survive in extreme environments. At present, it has become a hotspot in microbial research, and its salt-tolerance mechanism has become a research hotspot. It is increasingly attractive to industrial biotechnology. Consequently, halophiles are becoming classics for industrial efforts to meet growing market.

Demands with rapid and reliable metabolic engineering tools and approaches. However, the current understanding of salt-tolerant bacteria is not very deep, and the knowledge of halophilic bacteria including physiology, metabolism, enzymes and genetics is still very limited. Some salt tolerance mechanisms such as K^+ have limited cognition. The industrialization process is still immature, and how to reduce the production cost of salt-tolerant bacteria is currently crucial.

Acknowledgments

This work was supported by the Key Technology Research and Development Program of Shandong Province [grant numbers 2019QYTPY024, 2019YYSP019]; Key Technology Research and Development Program of China [grant number 2020-CXY45]; Spring Industry Leader Talent Support Plan [grant numbers 2017035, 2019042]; Shandong Taisha Leading Talent Project [grant numbers LJNY202015, tscy20180507]; Shandong Provincial Natural Science Foundation (No. ZR2020QC008); and Science, Education, and Industry Integration Innovation pilot project at Qilu University of Technology (Shandong Academy of Sciences) [grant numbers 2020KJC-YJ01, 2020KJC-GH10].

References

- [1] Debbie M F, Wahab R A, Hypo F. Halophiles: biology, adaptation, and their role in decontamination of hypersaline environments [J]. *World J Microbial Bioethanol*, 2016, 32 (8): 135.
- [2] Yin Jinn, Chen Jinn-Chun, Wu Qigong, Chen Guo-Qigong, *Coming Stars for Industrial Biotechnology*, *Biotechnology Advances* (2014).
- [3] Moksha N, Chaudhari B, Patil U. Operative utility of salt stable proteases of halophilic and halotolerant bacteria in the biotechnology sector [J]. *Int J Biol Macrogol*, 2018, 117 (1): 493 - 522.
- [4] Wang, Y., et al. Zhuhai, challenges and opportunities [J]. *Cur. Open. Biotechnology*. 2014, 30: 59 – 65.
- [5] Xu liang Zhuang, Zhen Hana, Zhuhai Zhu Hai in decontamination by halophilic microorganisms in saline wastewater and soil [J]. *Environmental Pollution*, 2010, 158: 1119 - 1126.
- [6] Liu C, Baffle D K, Zhang M, et al. Halophile, an essential platform for bioproduction [J]. *Journal of Microbiological Methods*, 2019, 166: 105704.
- [7] Zhang X, Lin Y N, Chen G Q. Halophiles as chassis for bioproduction [J]. *Adv BISYS*, 2018, 2 (11): 1800088.
- [8] Hamlet Inga, Müller Volker. Molecular Mechanisms of Adaptation of the Moderately Halophilic Bacterium *Thiobacilli*'s halophiles to Its Environment [J]. *Life*, 2013, 3: 234 - 243.
- [9] Uma G, Babu M, Prakash V S G, et al. Nature and bioprospecting of haloalkaliphilic: a review [J]. *World Journal of Microbiology and Biotechnology*, 2020, 36 (5): 66.
- [10] Kulich, Terry Massachusetts. Molecular aspects of bacterial pH sensing and homeostasis [J]. *Nat Rev Microbiol*, 2013, 9: 330 - 343.
- [11] Sium, S. H.; Müller, V. Regulation of osmoadaptation in the moderate halophile *Halo bacillus* halophiles: Chloride, glutamate and switching osmolyte strategies. *Saline Syst*. 2008, 4, 4.

- [12] Sium, S.; Pfeiffer, F.; Palm, P.; Ramp, M.; Schuster, S.; Müller, V.; Westervelt, D. Chloride and organic osmolytes: A hybrid strategy to cope with elevated salinities by the moderately halophilic, chloride-dependent bacterium *Halo bacillus halophiles*. *Environ. Microbial.* 2012.
- [13] Vaidya, Shivani, Duvet. Distinct Osmoadaptation Strategies in the Strict Halophilic and Halotolerant Bacteria Isolated from Lungu Salt Water Body of North West Himalayas [J]. *Current Microbiology an International Journal*, 2018.
- [14] Harding T, Simpson A G B. Recent Advances in Halophilic Protozoa Research [J]. *Journal of Eukaryotic Microbiology*, 2017.
- [15] Karan R, Capes M D, Disarm S. Function and biotechnology of extremophilic enzymes in low water activity [J]. *Aquatic Biosystems*, 2012, 8 (1): 4.
- [16] Spitzer J: From water and ions to crowded biomacromolecules: In vivo structuring of a prokaryotic cell [J]. *Microbial Mol Boil R* 2011, 3: 491 - 506.
- [17] Ortega G, Lain A, Taddeo X, López-Méndez B, Castano D, Millet O: Halophilic enzyme activation induced by salts [J]. *Scientific Reports* 2011, 1: 6.
- [18] Disarm S, Disarm P. Halophiles and their enzymes: Negativity put to good use [J]. *Current opinion in microbiology*, 2015, 25: 120 - 126.
- [19] Yu H Y, Li X. Characterization of an organic solvent-tolerant thermostable glucoamylase from a halophilic isolate, *G. sp. SK71* and its application in raw starch hydrolysis for bioethanol production [J]. *Biotechnology Progress*, 2014, 30 (6): 1262 - 1268.
- [20] C. Binninger, et al., The more adaptive to change, the more likely you are to survive: Protein adaptation in extremophiles, *Semin Cell Dev Boil* (2018).
- [21] Boil, Haokip, Haokip and functional insights into thermally stable cytochrome *c'* from a thermophile, *Protein Sci. Publ. Protein Soc.* 2017, 26: 737 – 748.
- [22] Deng, et al. "Mechanism of Thermal Adaptation in the Lactate Dehydrogenases." *Journal of Physical Chemistry B Condensed Matter Materials Surfaces Interfaces & Biophysical* (2015).
- [23] Sibel A, Nusrat S. Salt inhibition on anaerobic treatment of high salinity wastewater by up flow anaerobic sludge blanket (UASB) reactor [J]. *Desalination and Water Treatment*, 2016, 57: 12998 - 13004.
- [24] Moksha N, Chaudhari B, Patil U. Operative utility of salt-stable proteases of halophilic and halotolerant bacteria in the biotechnology sector [J]. *Int J Boil Macromol*, 2018, 117 (1): 493 - 522.
- [25] Hu X, Li D, Qipao Y, et al. Salt tolerance mechanism of a hydrocarbon-degrading strain: Salt tolerance mediated by accumulated betaine in cells [J]. *Journal of Hazardous Materials*, 2020, 392:122326.
- [26] Lawrence A, Balakrishnan M, Valsalva V N, et al. Functional characterization of a major compatible solute in Deep Sea halophilic eubacteria of active volcanic Barren Island, Andaman and Nicobar Islands, India [J]. *Infection, genetics and evolution: journal of molecular epidemiology and evolutionary genetics in infectious diseases*, 2019, 73.
- [27] Gul, Nadia, Schuurman-Wolters, Gea, Kurosawa, Akira, teal. Functional characterization of amphipathic α -helix in the osmoregulatory ABC transporter *Oputa* [J]. *Biochemistry*, 2012, 51 (25): 5142 - 5152.
- [28] Wang K, Liu Y, Dong K, et al. The effect of NaCl on proline metabolism in *Saussure Amara* seedlings [J]. *African Journal of Biotechnology*, 2011, 10 (15): 2886 - 2893.