

Halobacterium sp. NRC-1: A Review

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Abstract

Halobacterium sp. NRC-1 is the halophilic bacterium. This halophile has a great potential to survive on high salinity conditions. The genome of this bacterium has sequenced. The studies have done at metagenomics level, which include the Identification and Molecular Characterization. Identification of this extreme halophile was done by 16S ribotyping and PCR amplification using universal primers FD1 and rP1. The sequences than compared with already sequenced genomes of halophiles. The post genomic tools and microarrays were applied for sequencing. *Halobacterium* sp. NRC-1 transcriptional profiling showed that it is highly responsive to the environment and give specific responses at gene expression level. There is a need for extensive research at metaproteomic level. To study the expression and function of proteins, whole protein profiling of this specie need to be studied.

Keywords: *Halobacterium* sp. NRC-1, Transcriptional profiling, metaproteomics.

1. Introduction

When protoplasmic components become resistant to salt, this phenomenon is called halotolerant. The protoplasm can tolerate the imbalance in ion exchange during salt stress. The toxic effect and osmotic pressure can increased the ion concentration [1]. The bacteria or other microbes that tolerate the salinity of 100 g/L are considered as halophiles. On the basis of varying salt concentration the halophiles are divided in slight, moderate and extreme halophile [2]. The hypersaline environment contains low water and high ionic components. Halophiles adopted various mechanisms to cope with the high salinity conditions. The cell membrane of halophiles contains chromoprotein which acts as transmembrane proton pump [3]. Gram negative halophiles have been studied in more detail as compared to gram positive halophile bacteria.

Nesterenkonia halobia (formerly *Micrococcus halobius*), a moderately halophilic gram-positive coccus that was described on the basis of a single strain. A study of a large number of gram-positive moderately halophilic rods that were able to produce endospores led us to describe a new species, designated *Bacillus salexigens*. Halophiles have a great potential to be utilized in

biotechnology. The β -carotenoids can be produced from these halophiles. These microbes are very effective in the treatment of waste water [4]. They have also tolerance to degrade organic and inorganic pollutants [5].

These bacteria can be very helpful in genetic engineering as their genetic studies have revealed that they contain large dynamic plasmids having multiple transposable insertion sequences [6]. Phenotypic and morphological tests are not very prevalent these days; the identification is based on 16S rRNA ribotyping.

Halobacterium sp. NRC-1 is an extreme halophile (with a 4.3 M NaCl optimum) that grows best heterotrophically in a rich organic broth. This organism's metabolism is versatile in addition to its aerobic metabolic capacity; it possesses facultative growth capabilities through anaerobic respiration by arginine fermentation utilizing dimethyl sulfoxide (DMSO) and trimethylamine *N*-oxide (TMAO). It also has phototrophic capability through the light-driven proton pumping activity of the retinal protein, bacteriorhodopsin, which is organized into a two-dimensional crystalline array in its purple membrane. *Halobacterium* sp. NRC-1 produce motile cells and hollow protein structures. Relevant to the study of

genetic regulation, *Halobacterium* sp. NRC-1 responds to many environmental effectors, including high and low temperatures and salinities, and ultraviolet (UV) and ionizing radiation [6].

2. Genome Sequencing

The halophilic bacteria by using 16S ribotyping or PCR amplification by using universal primers FD1 and rP1. These genes sequences could be compared with other gene sequences that are already available on genBank database. To find the closely related sequences these sequences can be compared by using genBank [8].

Halobacterium sp. NRC-1 is the one of the completely sequenced species of halophiles. The post genomic tools were applied and a micro array was also done. This halophile has a great potential to survive on high salinity conditions. *Halobacterium* sp. NRC-1 transcriptional profiling showed that it is highly responsive to the environment and give specific responses at gene expression level.

Responses to changes in salt conditions appear to be designed to minimize the loss of essential ionic species and abate possible toxic effects of others, while exposure to temperature extremes elicit responses to promote protein folding and limit factors responsible for growth inhibition. This work lays the foundation for further bioinformatic and genetic studies which will lead to a more comprehensive understanding of the biology of a model halophilic Archaeon. Some post genomic methods were developed after the complete sequencing of the *Halobacterium* sp. NRC-1. A facile gene Knockout system was developed for reverse genetic analysis.

The genome sequencing of *Halobacterium* sp. NRC-1 invented genes that are involved in maintaining the intracellular ionic conditions that are suitable for growth. These genes include the coding of active K⁺ transporters. *kdpABC*, an ATP-driven K⁺ uptake system, and *trkAH*, low-affinity K⁺ transporters driven by the membrane potential. The coding genes for Na⁺ efflux is done by *NhaC* proteins and unidirectional Na⁺-H⁺ antiporter

Interestingly, several of these genes, including those coding for *kdpABC*, *trkA* (three copies), and *nhaC* (one copy) were found on the extrachromosomal pNRC200 replicon [9].

3. Protein profiling

The fermentation of *Halobacterium* sp. NRC-1 includes anaerobic respiration using DMSO and TMAO as electron acceptor by using arginine deaminase pathway.

The capability for anaerobic growth is likely advantageous for the organism in its natural habitat as high salt concentrations and elevated temperatures, together with

high cell densities, reduce the availability of molecular oxygen. In a recent study of anaerobic respiration in *Halobacterium* sp. NRC-1, bioinformatic and transcriptional analyses, and gene knockouts showed it to harbor a bifunctional DMSO/TMAO reductase that is encoded by the *dmsREABCD* operon. This reductase is more closely related to NarG-type nitrate reductases than to bacterial DMSO/TMAO reductases, although phylogenetic analysis was inconclusive about its evolutionary origin. Whole-genome oligonucleotide microarray studies showed that the transcript level of the *dms* operon is strongly induced under anaerobic conditions [7].

Halophilic archaea are very beneficial to bio remediate of hyper saline salt environment. Two-dimensional gel electrophoresis and mass spectrometry are widely used now days for protein profiling. The genome profiling of *Halobacterium* species NRC-1 provide a guideline for protein profiling of other related species. Proteomics and genomics become coupled and become a latest tool to analyse the genes and protein function. Bioinformatics facilitate to predict the function of proteins in halophiles [10].

4. Low and high salinity effect on genes

This bacterium (*Halobacterium* sp. NRC-1) grows in the hypersaline environments. Their growth show variable pattern in different salt concentrations. The cells have ability to cope with high salts as well as mild salt conditions. KCl is the major compatible solute for this specie of halophile. The intracellular concentration of KCL is almost equal to the external concentration of NaCl. The predicted proteins genome wide has a median pI of 4.9 with presence of negative charges on proteins surface. The main characteristic of these negatively charged proteins is to permit the hydration in low quantity and maintain the function of cytoplasm with this low water activity.

High salt concentrations are also known to convert some DNA sequences, e.g. alternating (CG)-repeats, from the right-handed B to the left-handed Z form, facilitated by negative superhelical stress. Some compatible organic solutes are also be utilized in response to osmotic stress [9].

5. Conclusion

Halobacterium sp. NRC-1 has completely sequenced genome. The studies of these halophilic bacteria are extensive and have done on metagenomics and proteomics level. The function of different genes that cope the high salt conditions has been studied. The different operons model for this bacterium is also widely studied. The information about its genes and important protein functions can be used to study the various halophilic closely related species. Major research is needed in metaproteomic

levels of these halophiles. The protein profiling and expression can be done by using 2-D gel electrophoresis and mass spectrometry.

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