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INDUSTRIAL IMPORTANCE OF HALOPHILIC BACTERIA

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ABSTRACT

Halophile is a collective name ascribed to organisms capable of surviving in hypersaline environments by their ability to maintain osmotic balance. They are capable of doing so by accumulating salts such as KCl and NaCl within their cytoplasm to such a concentration that it becomes isotonic with the environment. Both eukaryotic and prokaryotic group of organisms have been identified under the category of halophiles. During the last few decades, in the wake of the growing industrial utility of extremophilic microbes, there has been great interest among researchers to explore hypersaline environments for halophilic bacteria with some industrial utility. They are known to produce a variety of products such as light receptor proteins, bioplastic, enzymes, biosurfactants etc. This article highlights some of the latest biotechnological applications of halophilic bacteria.

KEY WORDS: halophile, bacteria, biotechnological applications

INTRODUCTION

Halophiles, the salt loving life forms are known to require high salt concentration (5-30%) for their growth and development. Halophiles refer to a broad group of organisms whose members are distributed to all three domains of life: Archaea, Bacteria and Eukarya (Oren, 2002). Halophiles have been discovered all over the globe in different habitats such as salt marshes, sub terranean salt deposites, dry soil, salted meats, hypersaline sea and salt evaporation ponds.. Majority of them have been reported from ecological zones of Antarctic lakes. However, a number of alkaline hypersaline soda brines are also known to exist in tropical zones. The halophiles are divided into three major categories- slightly halophilic, moderately halophiles and extremely halophilic. Slightly halophilic or halotolerant organisms grow optimally at a concentration of 2-5% (0.2-0.85 mol/l), moderate halophiles at a concentration of 5-20% (0.85-3.4 mol/l) and extreme halophiles at a concentration of 20-30% (3.4-5.1 mol/l) of NaCl, respectively (Dasarma and Arora, 2001).

Among the three categories, moderately halophilic bacteria are known to adapt themselves to survive under hypersaline environment as well as slightly saline environment (Galinski, 1993). This group of bacteria were found to have more industrial applications than other two groups owing to their property to thrive in both the hyper saline and slightly saline conditions (Vreeland, 1992).

Maintenance of life by halophiles in hypersaline environment

Halophilic microorganisms have adapted different mechanisms to tolerate the extremely saline conditions (Oren, 2002). Their proteins have unusual amino acid compositions rendering the molecules stable and active at high ionic strength. They have excessive number of acidic amino acid residues on their surface due to which hydrated ions bind significantly to these proteins, thus reducing the hydrophobicity and preventing precipitation. This mechanism helps halophiles survive at high salt concentration (Gomes and Steiner, 2004).

The second strategy is known as the 'salt-in' strategy in which the halophiles take in lots of K^+ and Cl^- ions and keep Na^+ concentration low in the cytoplasm. This helps maintain high osmotic pressure from inside the cell and maintain an osmotic balance in an environment of high NaCl. The aerobic halophilic archaea belonging to the *Halobacteriales* sp. accumulate KCl at concentration at least as high as the NaCl concentration in their surrounding medium. Among eubacteria, 'salt-in' strategies are observed only in *Halanerobials* sp. in which mostly fermentative or homoacetogenic anaerobes are presents. The pressure of KCl in high amount inside the cytoplasm is required for reaching adaptation of all proteins to enable all intracellular enzymatic systems to be active at high salt concentration (Oren 1999; Oren, 2000).

The third strategy involves osmotic adaptation. In this method the cell drips out all the salts from the cytoplasm as much as possible and takes up the organic solutes from the environment to the cytoplasm to provide osmotic balance. A variety of compounds such as glycerol, amino acids and derivatives such as glycine, betaine and ectoine, simple sugars, sucrose and trehalose are utilized by the cell to make this strategy effective (Galinski, 1995).

Industrial Applications of halophiles:-

The ability of halatolerant or halophillic microorganism to survive in saturated salty water makes them different from other microorganisms. The survival conditions have induced several biochemical and physiological changes in this class of microorganisms helping them to produce products of unparallel counterparts. Because these products can only be derived from halophiles, they have unique industrial uses too. Some of the halophilic products and their industrial uses are discussed below.

a) Bactereorhodopsin production

Bacteriorhodopsin (BR) and halorhodopsin are membrane bound retinal pigments found in some extremely halophilic archaea. BR is useful as it helps the organisms to utilize and use light energy to generate chlorine gradients. This is important for various biogenetic processes.(Oren, 1994; Lanyi, 1995). BR is a 26.5 KDa protein and is known to have different types of properties such as proton motivation, photo electric effect and other photochemical properties. Owing to these properties, it has several applications in many different fields. It is used in holography as a photo sensitive and erasable material for optical information recording and processing. Other applications include their use in the designing of spatial light modulators, artificial retina, neural network, optical computers and volumetric and associative optical memories. It is also known to be used as photochromic material in optical switches, logic gates (AND and OR gates) and optical computer (Rao et al., 1998). It is used as a bioelement in a motion sensor (Ackley and Shieh, 1998) and also as an image sensor in a bio computer (Kikura, 1998). Another application of BR is in the renewal of biochemical energy i.e. the back conversion from ADP to ATP. This is particularly useful in a system where large amount of energy is consumed by the biochemical processes in the form of ATP (Grob, 1997).

b) Biosurfactant production

Biosurfactants are important compounds for remediation of oil polluted environment. They work by decreasing the surface tension of the pollutants in an oil-polluted environment and thus increasing its solubility, resulting in an increase in the mobility of hydrophobic hydrocarbons leading to enhanced rate of its degradation. Biosurfactants are mostly used to remove the hydrocarbon pollution and for cleaning the storage oil tank in which large amounts of hydrocarbons is deposited. There are reports for its use for the purpose of pollution treatment in the marine environment (Banat, 2000). The biosurfactants from halophiles can also be used for *in situ* microbially enhanced oil recovery (MEOR). Resistance to the prevailing harsh conditions at the recovery sites helps them to easily cope with high temperature, high salinity and anaerobic conditions of oil and petroleum reservoirs. *Bacillus licheniformis JF-2* under anaerobic conditions produces a biosurfactant 'Lychenisn' a cyclic lipopeptide (Thomas, 1993). Another surfactant is Lychenisin A which is produced by *B.licheniformis BA S 50* and found to be thermo as well as halotolernant capable of growing under both aerobic and anaerobic conditions. (Yakimov, 1995.

c) Liposome production

There is growing use of liposome mediated drug and cosmetic transport to specific target site in the human body. Halophilic bacteria belonging to *Halobacterium cutirubrum* sp. produces the ether linked lipids instead of normal ester linked fatty acid derivatives. The importance of ether linked lipid is that it is esterase resistant and has high stability against chemicals and hence greater half life (Galinski, 1992; Choquet,1999) Owing to its greater stability it is used in designing drugs and cosmetics that can be stored for a comparatively longer period (Choquet,1999).

d) Bioplastic production

Some of the halophiles are reported to accumulate a compound Polyhydroxy alkanoates (PHA) inside their body and its properties are comparable to that of polyethylene and polypropylene (Steinbuchel, 1997). The plastic made by these compounds known as thermoplastics, is biodegradable and have excellent properties such as high strength and low melting point, similar to polypropylene. Efforts are underway to use the thermoplastic of an archea *Haloferax mediter* to replace the thermoplastics made by its nonthermophilic counterparts so as to be used for the production of bottles for cosmetics (Oren, 2002)

e) Food supplements and food colorants

Long chain polyunsaturated fatty acid (PUFA) are now a day essential in our diet to minimize the fat on the body. However, the large scale availability of PUFA to meet our requirement had been a challenge as the process of obtaining PUFA from fish is hard to scale up. Alternative to fish PUFA producers came to be seen in the form of halophilic bacteria which are recently reported positive for this activity. It is easy to produce, purify and get good quality and yield of PUFA from the halophilic especially from Antarctic marine bacterial species *Shwenella* and *Colwellia* (Nichols *et al*., 1993 &1999). â-Carotene is used in the food industry as a natural food colorant. It is also used in cosmetics, multivitamin preparation and health food products.

Canthaxanthin is used in cosmetics to prevent the skin damage after long exposure to sun light. Various halophilic bacteria are reported to produce it in an extracellular manner. An extremely halophilic bacterial strain was reported to produce 2.06 mg total carotenoids/g cells dry weight including 0.06 mg â-carotene and 0.7 mg canthax anthin in the presence of 25% (w/v) NaCl (Asker and Ohta, 1999).

f) Enzyme production

A variety of hydrolytic industrial enzymes such as xylanases, amylases, proteases and lipases active at high salt concentration have been reported to be produced by halophiles belonging to the genera *Acinetabacter*, *Haloferax*, *Halobacterium*, *Halohabdus*, *Halobacillus*, *Bacillus* and *Micrococus* (Gomes and Steiner, 2004; Joshi et al., 2007). Most of the halophilic enzymes have been extracted from the moderately halophilic bacteria. However, it is the archaeal domain that provides the main source of extremely halophilic enzymes. Other enzymes such as Nuclease H was reported to be produced by *Micrococcus Various* subsp. *Halophilus* for subsequent use in the production of flavouring agent 5′-guanylic acid and 5′-inosinic acid (KameKura, 1982). Some salt resistant halophilic bacteria have been reported to produce amylase from *Bacillus* sp. which is used in treatment of effluents containing starchy or cellulosic residues (Khire, 1992).

A thermostable type-I DNA topoisomerase has been isolated and purified from the hyperthermophilic methanogen *Methanapyrus kandleri* (Slesarev, 1997) *w*hich is active over a wide range of temperature and salt condition. It does not require magnesium or ATP for its activity which makes manipulations on DNA more convenient and efficient. It is also used for modeling novel drugs.

g) Bioremeditation:-

Bioremediation is another area where novel applications have been sought and suggested for halophilic microbes. There are many ecosystems which are polluted by the oil spills and halophiles have evolved as a remedy for this problem. The reverse micelles encapsulating halophilic extremozymes may be used to reduce or eliminate environmental hazards resulting from accumulation of toxic chemicals and other hazardous wastes (Gomes and Steiner, 2004). Halophiles and Halotolerant bacteria have been proved to be very effective in treating the oil spill in marine and arctic environment has been seen (Deille, 1998; Margesin,1999).

CONCLUSION

Although a lot of attention has been paid worldwide to explore halophilic bacterial diversity and look for their biotechnological potential, there are many terrains on the earth still left untouched for any such investigation. Careful investigation has to be done for suggesting novel applications for halophiles. Metagenomic tools can also be applied to unravel the real potential of these salt loving organisms.

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the pungent smell of formaline acts as hindrance to its users. Most of the seed production units are not equipped with gator rookery sprayer and face-mask required for indoor spraying of formaline. In its absence knapsack sprayer is used. The spraying done with this sprayer is generally not complete and foolproof as the person engaged for this purpose cannot withstand the pungent smell of formaline for longer time. In this way the germs are not killed and all efforts go waste. Now a day it is a practice to use humidifier for disinfection. But it is also not available with every unit. Moreover, tasar grainages are made of mud walled houses with tiled roof where foolproof sealing and airtight condition cannot be maintained to achieve best disinfection result.

So there has always been a need for alternate or foolproof means of disinfection. The flame gun comes as an easy answer to this problem. Here fire works as disinfectant. The high temperature of the flame does not spare any of the pathogens which are killed instantly as the fire is the supreme purifier. The efficacy of flame gun is time tested. The kerosene oil was used earlier in flame gun (Yadav and Mathur, 2000). Its availability and purchase is a big problem for government units. So even interested to use flame gun it becomes difficult to practice with it.

INNOVATION

It is worthwhile to mention here as to how the innovation of Modified LPG flame gun was made. The LPG flame gun was first developed by Raj Narian et al(2001). The authors used heating torch, connecting pipe, regulator and LPG cylinder to develop it. During the year 2007 the first author was transferred to BSM&TC Boirdadar, Raigarh, Chhattisgarh. At this centre the use of kerosene flame gun was in practice. However, during 2008 the purchase of kerosene oil from open market at Raigarh became very difficult as it was not available. Likewise, the heating torch was also not available in the market and the procurement of it from Delhi or other places would have wasted much time. The other parts like regulator, connecting pipe and LPG cylinder required for the apparatus were easily available in the local market and purchased. As the disinfection of grainage buildings was urgently required, so an idea came to the mind of first author to replace the heating torch of LPG flame gun with the one used earlier in kerosene flame gun and see the result. It was surprising to see that this rearrangement of different parts started working well and in this way an invention was made.

Modified LPG flame gun

The modified LPG flame gun consists of:

1. Heating torch fitted earlier in kerosene flame gun,
2. Connecting pipe,
3. Regulator, and
4. LPG cylinder.

Heating torch: Heating torch is the actual apparatus. It is one meter in length. One end is connected to the rubber pipe and the other end is fixed with burner. At the beginning of the torch on the main body is a regulator knob. This regulator knob is used to open and close the flow of gas.

Connecting pipe: It is a rubber tube connecting the heating torch with regulator. The tube is tightly fitted with heating torch on one side and regulator on the other with help of clips. The connecting pipe of five-meter length is found suitable for flame gun. However, it can be purchased of any other desired size, which is easily available in the market.

Regulator: It is the commercial regulator and fits in the LPG cylinder. It has screws to fix with the cylinder as also to regulate the pressure of the gas. The regulator is connected to heating torch with the help of connecting tube.

LPG cylinder: It is the normal 14.2 Kg LPG cylinder and fitted with the regulator. However, the other different sizes of cylinder available in the market can also be fixed with above regulator through an adopter, which is easily available in the market.

The above four parts joined together make modified LPG flame gun.

How does the modified LPG flame gun work?

First of all heating torch is connected with the regulator through connecting pipe. Now the regulator

regulator knob. At the end of the work or day the gas needs to be closed from the cylinder with the help of regulator so that the gas of the pipe can also be used. For flame gunning of the field, any surface of the room or rearing /grainage equipments the flame gun is held in one hand and the heating torch is slowly moved from one end of the disinfecting material to the other. In this way the entire material is flame gunned. The rearing trays, rearing and grainage room, appliances, rearing field and tree trunk can be conveniently flame gunned in this manner. All the three types of flame guns viz., kerosene flame gun, LPG flame gun and modified LPG flame gun are shown in plate-1 whereas it's functioning in plate-2. The disinfection done with flame gun is simple and foolproof. It requires no chemicals hence ecofriendly.

The flame gun can be used to disinfect rearing trays, rearing room, grainage room, appliances and for disposal of grainage waste i.e., burning of dead, rejected and examined moth and diseased layings. In tasar culture where silkworm rearing is conducted outdoor the flame gun can be used to disinfect field and tree trunk. After a crop is over the flame gun can be used to burn silkworm litter on the ground. Besides Mulberry and Tasar, it can conveniently be used in Eri and Muga sector also.

It takes eight hours time to empty one cylinder. Flame gunning of a model tasar grainage is conveniently completed within one hour with this machine. So the cost of disinfection comes to Rs. 44/- only for a tasar grainage, which is quite economic and foolproof than any other means of disinfection involving chemicals. For field use two workers are required per day with one set of apparatus, one for moving the heating torch and the other for displacing the cylinder. In this way five cylinders and ten mandays are required to complete the flame gunning of one hectare plantation and refilling charge of five cylinder comes to Rs. 1750/-. The cost of mandays is not calculated as every unit is having sufficient numbers of permanent skilled farm worker. Likewise, the cost of flame gunning of rearing trays, room, grainage room and other appliances can also be calculated which is always less than the cost of any other chemical being used. One thing is certain that flame gun fully, serves its objectives i.e. it kills the pebrine spore completely and thus contain the disease incidence to the maximum extent. Bansal et.al. (1997) also proved the effectiveness of flame gunning in killing the pebrine spore.

Merits of Modified LPG Flame gun

- Useful application of kerosene flame guns lying unused since long at different centres.
- The heating torch of modified flame gun being one meter in length and attached with handle, the person can use the flame gun while standing and there is no need for additional handle.
- Its flame being wider, the disinfection work is completed quickly.
- The consumption of gas is more or less similar to LPG flame gun.
- The modified LPG flame gun is quite convenient, economic and can effectively be used in seed production units of Mulberry, Tasar, Eri as well as Muga culture.

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