Global Journal of Science Frontier Research: C
Biological Science
Volume 19 Issue 1 Version 1.0 Year 2019
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Use of NMR to Determine Compatable Solutes in Halophilic Bacteria Isolated from Highly Saline Areas

By Reda Hassan Amasha
King Abdulaziz University

Abstract- Ten halophilic bacteria (two Gram-negative) belonging to the Halomonadaceae and (eight Gram-positive), belonging to the Bacillaceae, were isolated fromtheRed Sea, Arabian Gulf and Dead Sea using a high salinity medium, followed by identification using 16S rRNA.Four of the isolates were designated on the basis of their tolerance to highsalinity. The isolates respectivlyexhibited 97% homology to Halomonasaquamarina, 97% homology to Sediminibacillus sp., (Red Sea), 94% homology to Halobacillus sp., (Arabian Gulf) and 98% homology to Halobacillusdabanensis(Dead Sea). 1H-NMR spectroscopy was used to determine the osmolytes accumulated by H. aquamarina, Sediminibacillus sp.,Halobacillus sp. and H. dabanensis grown in a saline nutrient medium at varying concentrations of NaCl and a range of organic sources. In the case of H. aquamarina, betaine and ectoine concentrations increased at high salinities. In contrast, betaine was found when casein and peptone were used as nutrient sources, while ectoine was produced in the presence of peptone.

Keywords: halophiles, compatible solutes, nuclear magnetic resonance spectroscopy (NMR), 16S RRNA gene sequence.

GJSFR-C Classification: FOR Code: 060599

Strictly as per the compliance and regulations of :

Use of NMR to Determine Compatable Solutes in Halophilic Bacteria Isolated from Highly Saline Areas

Reda Hassan Amasha

Abstract- Ten halophilic bacteria (two Gram-negative) belonging to the *Halomonadaceae* and (eight Gram-positive), belonging to the *Bacillaceae*, were isolated fromtheRed Sea, Arabian Gulf and Dead Sea using a high salinity medium, followed by identification using 16S rRNA.Four of the isolates were designated on the basis of their tolerance to highsalinity. The isolates respectivlyexhibited 97% homology to *Halomonasaquamarina,* 97% homology to *Sediminibacillus* sp., (Red Sea), 94% homology to *Halobacillus* sp., (Arabian Gulf) and 98% homology to *Halobacillusdabanensis*(Dead Sea). ^{1}H-NMR spectroscopy was used to determine the osmolytes accumulated by *H. aquamarina*, *Sediminibacillus* sp.,*Halobacillus* sp. and *H. dabanensis* grown in a saline nutrient medium at varying concentrations of NaCl and a range of organic sources. In the case of *H. aquamarina*, betaine and ectoine concentrations increased at high salinities. In contrast, betaine was found when casein and peptone were used as nutrient sources, while ectoine was produced in the presence of peptone. In the case of *Sediminibacillus* sp., betaine was the only osmolyte produced at high salinities, while betaine and ectoinewas produced when peptone and casein were used. In *Halobacillus* sp., betaine was the only osmolyte produced at high salinities, whereas betaine and ectoine were produced in the presence of peptone and casein. Finally, in the case of *H. dabanensis*, only betaine accumulated at high salinities and in the presence of all organic nutrient sources.

Keywords: halophiles, compatible solutes, nuclear magnetic resonance spectroscopy (NMR), 16S RRNA gene sequence.

I. Introduction

Halophilesgrow in hyper-saline concentrations and include representatives of the Eukarya, Bacteria, and Archaea (Rampelotto, 2010; Mohammadipanah, Hamedi and Dehhaghi, 2015). The pink-red color of hypersaline environments worldwide isdue to halophilic microorganisms, and the most generally observed halophiles either belong to the Archaea or to genera *Haloquadratum, Halobacterium, Halomonas* and *Salinibacter*, as well as the green alga, *Dunaliellasalina* (Ma *et al.*, 2010; Oren, 2011; Waditee-Sirisattha, Kageyama and Takabe, 2016). Halophiles can be divided into three main groups, based on their salt requirements; extreme halophiles prefer to grow at 5 M of NaCl, moderate halophiles at 3 M of Na Cl and slight halophiles at 1 M of Na Cl (Kanekar *et al.*, 2012; Ventosa *et al.*, 2015). Microorganisms, living in hypersaline environments, encounter at least two difficulties. Firstly, the presence of high concentrations of salts which affect protein function by precipitation. Archaea and bacteria, inhabiting high salinity environments are however, protected by possessing acidic proteins having a large number of negative charges, which allow then to function salinities more efficiently in these environments than do basic proteins. Secondly, because of increasing salinity, cellular water is lost into the external medium, resulting in likely dehydration, loss of turgor pressure and a reduction of cell volume. Halophilic microorganisms generally accumulate high concentrations of solutes into their cytoplasm (Ewert and Deming, 2013). Halo phlic bacteria, for example, accumulate organic solutes known as compatible solutes; these are the highly soluble, low-molecular weight organic compounds, and osmoregulatory compounds such as, amino acids and their derivatives, sugars, and polyoles (Kempf and Bremer, 1998; Santos and Galinski, 1998; Empadin has and Da Costa, 2006; Shivanand and Mugeraya, 2011).

Compatible solutes are accumulated in by either *de novo* synthesis, or uptake from the environment (Oren, 2002). The diversity of compatible solutes accumulated intracellularly can determine the level of halotolerance. Non-halophilic and slightly halophilic bacteria usually accumulate sugars (e.g. sucrose and/or trehalose),K^{+}, and amino acids (e.g. proline and/or glutamate), as compatible solutes, while moderately halophilic bacteria also accumulate glucosylglycerol, and halotolerant and the extremely halophilic bacteria accumulate ectoine, and quaternary ammonium compounds, such as glutamate betaine in addition to glycine betaine, as well as K^{+}, glutamate, sucrose or trehalose as and various other minor components (Detkova and Boltyanskaya, 2007).These compounds maintain the osmotic balance, stabilize biomolecules and protect the cell from environmental change (Detkova and Boltyanskaya, 2007). The composition of mixtures of compatible solutes varies in response to the growth phase and medium employed (Kempf and Bremer, 1998). Over recent years, detailed

Author: King Abdulaziz University, Faculty of Science, Department of Biology, Jeddah, Saudi Arabia, P. O Box: 42799 Jeddah 21551 Saudi Arabia. e-mail: ramashah@kau.edu.sa

studies have been conducted on the biosynthesis of compatible solutes and the regulatory pathway of these osmolytes. It has been shown that different intracellular osmolytes work in combination and are regulated by one another (DasSarma, 2015). Commonly accumulated compatible solutes in halophiles include sugars, amino acids, and their derivatives, including methylamines, as well as polyols; like: betaine, sucrose, trehalose, ectoine, glycine and glycerol. Some extreme halophiles, especially members of halobacteria, accumulate potassium chloride into their cytoplasm, untilthe internal concentration is similar to the external concentration of sodium chloride. Polyols are accumulated in halophilic fungi, whilst glycine, betaine and ectoine are accumulated in most halophilic bacteria. Compatible solutes of the Archaea generally resemble, in structure, bacterial compatible solutes, the key difference is that the majority of them carry a negative charge due to an excess of acidic over bases, which enhances solubility and promotes growth in low water activity conditions (Averhoff and Muiiller, 2010;Ewert and Deming, 2013; DasSarma, 2015).

The aim of the work reported here was to isolate bacteria from the Red Sea, the Arabian Gulf in Saudi Arabia and the Dead Sea in Jordan, and then identify any halophilic bacteria isolated, using 16S rRNA gene sequencing and then to use NMR to determine the types of compatible solutes accumulated by these halophilic bacteria when exposed to a range of salinity stresses and different organic nutrient sources.

II. Materials and Methods

a) Sites and Sampling

Samples were collected in May, 2016 (Shaban, 1437) and September, 2016 (Zu- Alhija, 1437). Three samples of water and three samples of sediment were aseptically collected from six different sites at the southern part of Red Sea (Site1, N:21°29'14.8", E:39°07'58.0"; Site2, N:21°29'05.8", E:39°08'004"; Site3, N:21°28'50.2", E:39°07'52.2"; Site4, N:22.144268, E:38.974901; Site5, N: 22.174521,E: 38.965919), at various depths (17m, 21m, 12m, 14m, 11m) with maximum distance estimated at nearly (1nmi =~1.852km), located in Jeddah city, Saudi Arabia. One sample of water and the other of sediment were collected from Coast of Arabian Gulf, located in Khobar city (N:28°24'01.2", E:49°18'28.6"), Saudi Arabia. Three samples of water, three samples of sediment and three samples of saline mud were also collected from two different sites at the northern part of Dead Sea (N:31°42'27.0", E:35°34'52.7") at two depths of (1.5m - 3m), located in Balqa province, Jordan. Recorded temperaturesat the sampling sites varied between 34°C, 38°C and 30°C, respectively.

Samples were placed in sterile plastic containers with a space of approximately 1inch left between the container lid, in order to leave an air space, and stored in an icebox; the samples were then transported to the laboratory for analysis.

b) Isolation, purification and preservation of halophilic bacteria

Forthe isolation of halophilic bacteria sediment and mud were suspended in dH_2O and the resulting suspension was serially diluted. Culture media were inoculated with 0.1ml (100μl) of the diluted solutions of each sample and was spread on the surface of the medium using a glass spreader. All plates were incubated at 37°C over a period of 72h. Colonies were picked off and transferred to fresh medium in order to obtain pure cultures which were purified using the same media from which they were isolated; all isolates were then stored at 4°C.Simultaneously, the isolates were grown in broth, and 1ml of cultures were transferred with 1ml of 50% glycerol for long preservation at (-20°C).The following media were used: Saline nutrient medium (Nieto *et al*, 1989), Zobell marine medium (Lee *et al.*, 2003), casein medium (Nieto *et al.*, 1989), seawater medium (Satbhai *et al.*, 2015),Luria- Bertani (LB) medium, modified M9 medium, and National Botanical Research Institute's phosphate (NBRIP) medium.

c) Determination of compatible solutes by using nuclear magnetic resonance spectroscopy (NMR).

NMR analysis was used to identify the compatible solutes accumulated by four halophilic bacterial strains *H .aquamarina*, *Sediminibacillus* sp. *Halobacillus* sp. and *H. dabanensis* when exposed to different salinity stress conditions (0.5 M – 3 M Na Cl). In this experiment, the ability of halophilic bacterial isolates to conduct *de novo* synthesis or uptake from the medium was also investigated by using different organic sources (e.g. yeast extract, peptone and casein) in a saline nutrient medium. The analysis of NMR was conducted in The University of Sheffield, Sheffield, United Kingdom.

III. Results and Discussion

The Red Sea and Arabian Gulf are saline habitats, which are also alkaline (pH8.39 - pH8.35) whereas the Dead Sea is a hypersaline and acidic region (pH6.03) making them harsh environments even for microorganisms. The main approach used for the selection of the halophilic strains was their ability to grow at a range of salinities in saline nutrient medium. Initial characterization of the isolates showed them to be halophilic bacteria. In order to identify the strains, molecular methods were used, specifically 16S rRNA sequencing. which is acknowledged as the method of choice for identifying novel isolates to the genus and particularly species level. Ten halophilic bacteria (two Gram-negative) belonging to the *Halomonas daceae* and (eight Gram-positive), belonging to the *Bacillaceae*,

were isolated from the Red Sea, Arabian Gulf and Dead Sea using a high salinity medium, followed by identification using 16S rRNA. Four of the isolates were designated on the basis of their tolerance to highsalinity. The isolates respectively exhibited 97% homology to *Halomonas aquamarina*, 97% homology to *Sediminibacillus* sp., (Red Sea), 94% homology to *Halobacillus* sp., (Arabian Gulf) and 98% homology to *Halobacillus dabanensis* (Dead Sea).

a) Accumulation of compatible solutes as a strategy for adapting to salinity stress by H. aquamarina

Compatible solutes present in *H. aquamarina* grown at different salinities were analysed by NMR techniques. Figure (1) shows the compatible solutes accumulated by *H. aquamarina* when the isolate was grown in a saline nutrient medium at pH 7.0 and was adapted at different salinities (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 M) NaCl. From the spectra shown in this figure, it is clear that when *H. aquamarina* was subjected to different salinity stresses, different amounts of compatible solutes (e.g. betaine and ectoine) were produced. In *H. aquamarina*, the concentration of betaine rises with salt concentration up to about 2 M. The minimum amount of betaine was produced at 0.5 M NaCl. While the maximum amount of betaine production was at 2 M Na Cl. After this point, the concentration of betaine remained fairly constant at 2.5 and 3.0 M Na Cl. On the other hand, the concentration of ectoine started increasing at 3.0 M. Usually, the signals from leucine, valine and isoleucine methyls at about 1 ppm are the same in all spectra in a series.

b) Accumulation of compatible solutes as a strategy for adapting to difference of organic sources by H. aquamarina

It was necessary to determine whether these compatible solutes were synthesized or taken up from medium containing different organic sources. For this reason, *H. aquamarina* was grown insaline nutrient broth at pH 7.0 and 1 M Na Cl except that they were contained in each times with 5% different organic sources (e.g. peptone and casein) instead of yeast extract. From the spectra shown in Figure 2 it can be clearly seen that *H. aquamarina* accumulated significant quantities of betaine in presence the casein, followed by the peptone, and the yeast extract. On the other hand, considerable amounts of ectoine was accumulated by *H. aquamarina* in medium containing peptone (though maybe only 25% that of betaine), and the yeast extract contained some, while substitution of yeast extract to casein in medium led to an absence of ectoine. The other obvious variation is in the amount of acetate, which is notably high in the presence of yeast extract. It was concluded that betaine was synthesized by *H. aquamarina* while ectoine and acetate were up taken from medium.

c) Accumulation of compatible solutes as a strategy for adapting to salinity stress by Sediminibacillus sp

The spectrum of compatible solutes in *Sediminibacillus* sp. strain wasanalysed by NMR techniques at different salinity conditions. Figure 3 shows the compatible solutes accumulation by *Sediminibacillus* sp. when the strain was grown in a saline nutrient medium at pH 7.0 and was adapted at different salinities (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 M) NaCl. It is clear in this figure that when *Sediminibacillus* sp. grew in a saline nutrient medium almost no ectoine accumulated, even at the highest salt concentrations. On the other hand,the amount of betaine increases steadily as the salt concentration increased, so it appears that (in contrast to *H. aquamarina*) betaine is the only compatible solute. No other metabolites changes noticeably in concentration.

d) Accumulation of compatible solutes as a strategy for adapting to difference of organic sources by Sediminibacillus sp.

It was considered important to ascertain whether these solutes were synthesized *de novo* or taken up from medium. For this purpose, *Sediminibacillus* sp. was grown in media that were similar to a saline nutrient broth at pH 7.0 and 1 M Na Cl except that they were contained in each times with 5% different organic sources (e.g. peptone and casein) instead of yeast extract. Figure4 shows the compatible solutes accumulation by *Sediminibacillus* sp., it can be clearly seen that the almost same amount betaine was produced in media containing the casein and the peptone. While twice as much betaine was produced when it was in medium containing the yeast extract. On the other hand, the spectra of ectoine appeared in presence the casein, but very little was produced in the organic source others. Nothing else changed much. It was concluded therefore that betaine was synthesized by *Sediminibacillus* sp. unlike ectoine which was up taken from medium.

e) Accumulation of compatible solutes as a strategy for adapting to salinity stress by Halobacillus sp.

Compatible solutes present in *Halobacillus* sp. strain grown at different salinities conditions were analysed. (Figure 5) shows the compatible solutes accumulated by *Halobacillus* sp. when the isolate was grown in a saline nutrient medium at pH 7.0 and was adapted at different salinities (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 M) Na Cl. From the spectra shown in (Figure.5), it is clear that there is very little betaine was formed at 0.5M Na Cl, while at high concentrations of salt the amount of betaine changed in a rather an unpredictable way, possible because of contamination. Ectoine was present at the 1.5 M Na Cl, but not in noticeable amounts in any of the other concentrations of Na Cl. Contamination may have affected the metabolite profiles, as is apparent in

figure that there is quite a lot of acetate accumulated at the 0.5 M Na Cl, but no acetate at the 1 M Na Cl and no lactate at the 1 M and 1.5 M Na Cl. So it is clear that the only osmolyte is betaine.

f) Accumulation of compatible solutes as a strategy for adapting to difference of organic sources by Halobacillus sp.

Halobacillus sp. was grown in media that were similar toa saline nutrient broth at pH 7.0 and 1 M Na Cl except that they were contained 5% different organic sources (e.g. peptone and casein) instead of yeast extract. Based on the spectra shown in (Figure 6), it can be clearly seen that lower amounts of betaine were produced by *Halobacillus* sp. in medium containing casein, while twice this amount was formed when it was grown in medium containing the peptone. The maximum amount of betaine was produced in the medium containing the yeast extract. On the other hand, the amount of ectoine is similar to the concentration of betaine in the casein medium, but much less than in peptone, and less again in the presence of yeast extract. It was concluded that betaine was synthesized by *Halobacillus* sp. unlike ectoine which was up taken from medium.

g) Accumulation of compatible solutes as a strategy for adapting to salinity stress by H. dabanensis

Compatible solutes present in the *H. Dabanensis* strain grown at different salinities were analysed. (Figure7) shows the compatible solutes accumulated by *H. dabanensis* when the isolate was grown in a saline nutrient medium at pH 7.0 and was adapted at different salinities (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 M) Na Cl. From the spectra shown in Figure 6, it is clear that the only osmolyte produced by *H. dabanensis* is betaine. Betaine increased as the salt concentration was increased. It was predicted that some amino acids, like proline, would increase with increasing salt concentration, but this did not occur.

h) Accumulation of compatible solutes as a strategy for adapting to difference of organic sources by H. dabanensis

In this experiment, NMR was used to determine whether these solutes were synthesized *de novo* or taken up from medium. For this objective, *H. dabanensis* was grown in media that were similar to a saline nutrient broth at pH 7.0 and 1 M Na Cl except that they were amended with 5% different organic sources (e.g. peptone and casein) instead of yeast extract. Figure 8 shows the compatible solutes accumulation by *H. dabanensis*it is clear that the only metabolite to change is betaine. The peptone medium has significant amounts of betaine, followed by yeast extract and casein. It was concluded that betaine was synthesized by *H. dabanensis* and was not up taken from medium.

IV. Discussion

Halobacteria that are classified as Archaea, belonging to the family of *Halobacteriacea,* such as *Halococcus, Halorubrum, Halobacterium, Haloarcula, Haloferax, Haloterrigena,* and *Halobaculum,* which have been isolated from neutral hypersaline waters. *Halobacterium salinarum* has been found in sea food (Grant, 2004). Raghavan and Furtado (2004) found that some 5.5 $\times 10^3$ cells of halophilic archaea can be found in every gram of Indian Ocean sediments. The salt concentration in the cytoplasm of halophilic archaea is extremely high, for example; potassium accumulates internally at concentration of around 5 mol 1^{-1}, whereas, sodium accumulates in lower concentrations (DasSarma and Arora, 2002; DasSarma, 2012). Halophiles have purple membranes, which contain a" crystalline lattice of a chromo-protein, named as bacteriorhodopsin", which acts as a light-dependent trans-membrane proton pump.This membrane potential, which is generated, is used to reinforce a stage of phototrophic growth as well as the production of ATP (Fendrihan *et al.*, 2011). A large variety of methanogens have been isolated, such as, *Methanosalsus zhilinac, Methanohalophilus halophilus,* and *Methanohalophilus muhii,* from hypersaline and alkaline saline environments.

Methanohalobium evestigatum has also been reported as thermophilic halophiles (Kerkar, 2004). Green algae, such as *Dunaliella viridis, Dunaliella parva,* and *Dunaliella salina*, are also isolated at moderate level of salinity (Na Cl of 1 to 3.5M). In the main, use the polyols glycerol as the compatible solutes. A group of diatoms, such as *Navicula*sp., *Nitzschia*, and *Amphora coffeaeformis* have also been isolated from saline environments up to 2M of Na Cl. This group accumulates oligosaccharides and proline to maintain osmolality, as do protozoa like *Porodonutahensis* and *Fabreasalina.* Halotolerant yeast, such do *as Cladosporium glycolicum* has been found in the Great Salt Lake, while *Debaromyces hansenii* has been found in seawater, while halophilic fungi, such as *Basipetospora halophila* and *Polypaecilum pisce* have been found in sea food (DasSarma, 2012).Finally, twenty- six genera of fungi have isolated from the Dead Sea, including species of *Penicillium, Cladosporium, Aspergillus,* and *Chaetomium* (Oren and Cimerman, 2012).

a) Strategies used for osmo-adaptation in halophilic bacteria and archaea

Microorganisms of the three domains of life, which exist in such environments have to possess various mechanisms of osmoadaptation (Hänelt and Müller, 2013).Two osmo- adaptation mechanisms are known in halophilic microorganisms. Namely, a)"salt in cytoplasm mechanism" and b) the accumulation of

compatible solutes (osmolytes). The most commom strategy used by halophilic or halotolerant microorganisms, is to synthesize ectoine and glycine betaine as their main compatible solutes. Sugars including trehalose or sucrose are commonly observed as osmolytes by halotolerant microorganisms. Other compatible solutes, such as natural amino acids (e.g. proline and glutamate), polyols (e.g. glycerol and glucosylglycerol) and their derivatives (da Costa, Santos and Galinski, 1998; Empadinhas and da Costa, 2006), quaternary amines and their sulfate esters (e.g. choline-O-sulfate),sulfonium analogues (e.g. dimethylsulfoniopropionate and carnitine) and N-acetylated diamino acids and small peptides (e.g. N-acetylglutaminylglutamine amide and Nδ-acetylornithine) have also been identified in halophilic microorganisms (Kempf and Bremer, 1998.In the current study, out of fifty-eight bacterial isolates of this study, ten moderately halophilic bacterial isolates were identified using 16S rRNA analysis. The results were compared with these described in a range of identification schemes and the literature in general, (Hotl*et al.*, 1994; Liu *et al*, 2005; Tamegai *et al.*, 2005; Carrasco *et al.*,2008).

There is considerable interest in how halophilic bacteria protect themselves from the physical parameters to which they were exposed in hypersaline environments. It is well-known that the production of organic compounds, accumulated into the cytoplasm of halophilic bacteria (i.e. "compatible solutes") is the most important strategy which allows halophilic bacteria to adapt to extreme saline environment without interfering with their cellular metabolism. The accumulation of compatible solutes can determine the tolerant range of halophiles to salinity. Slightly halophilic bacteria usually accumulate sugars (e.g. sucrose and/or trehalose), in response to salt stress, while moderately halophilic bacteria accumulate glucosylglycerol, and extreme halophiles accumulate ectoine and quaternary ammonium compounds such as glutamate betaine and glycine betaine (Waditee-Sirisattha, Kageyama and Takabe, 2016). The production of these solutes has been studied using nuclear magnetic resonance (NMR) and high performance liquid chromatography (HPLC) (Ventosa *et al.*, 1998; Brill *et al.*, 2011). Nuclear magnetic resonance spectroscopy is a very useful and adaptable technique for investigating biological molecules and their interactions in solution (Fenn *et al.*, 2002). The accumulation of compatible solutes by *H. aquamarina,*, *Sediminibacillus* sp., *Halobacillus* sp. and *H. dabanensis* were determined during the present studies in a saline nutrient medium containing a range of salinities. It is clear that glycine betaine and ectoine are the main compatible solutes produced in response to varying salinity stress. In the case of *H. aquamarina* isolated from Red Sea, there is a clear relationship between the salt concentration in saline nutrient medium and the accumulation of betaine in the cells, the amount of betaine increasing with increasing salt concentration. The production of betaine then remained constant, while the amount of ectoine increased. The reverse occurred however, in the case of *Sediminibacillus* sp. where almost no ectoine was produced, even at the highest salt concentrations. The amount of betaine increases steadily as the salt concentration increased, showing that, in this case, betaine is the only compatible solute produced. In the case of *Halobacillus* sp. isolated from Arabian Gulf, little betaine is produced at 0.5 M NaCl, but at higher concentrations the amount of betaine changes in a somewhat unpredictable way, possibly due to contamination. While ectoine was produced at 1.5 M NaCl, little evidence of such production was seen at any other salt concentration. It is noteworthy that considerable acetate was accumulated at 0.5 M NaCl, but not at 1 M NaCl, and no lactate was present at 1M and 1.5 M NaCl, showing that in this case betaineis the only significant osmolite. In the case of *H. dabanensis* isolated from Dead Sea, the amount of betaine present increased steadily as the salt concentration was increased. It was expected that the concentration of amino acids, like proline, would increase with increasing with calt concentration, but no evidence of this was found, a fact which emphasizes that betaine is the only compatible solute produced by *H. dabanensis*.

The pathway of metabolites accumulation is critically important to ascertain whether the solutes are synthesized *de novo* or taken up from medium (Lamosa *et al.*, 1998). This study revealed an unexpected ability of halophilic bacterial strains to scavenge suitable components from the medium and to use them as compatible solutes, thus bypassing their synthesis and saving energy. It is noteworthy that no reports have been published on the ability of *H. aquamarina*, *Sediminibacillus* sp., *Halobacillus* sp. and *H. dabanensis* to derive such compatible solutes from the medium. The purpose of this study was to identify solutes accumulation in such species. Halophilic bacterial strains were grown in saline nutrient broth at pH 7.0 and 1 M NaCl containing 5% of a range of different organic source (e.g. peptone and casein) instead of yeast extract. *H. aquamarina*, accumulated large amounts of betaine in the medium, with lesser amounts in medium containing casein, peptone, and yeast extract respectively. It is clear that betaine was synthesized by *H. aquamarina*, since it is appeared in differing amounts, in all media. While large amounts of ectoine accumulated in the peptone medium, the amount seen was dramatically diminished in the presence of yeast extract, and none was produced in the casein amended medium. It can be seen therefore that ectoine was up taken from medium. In both casein

and peptone media, *Sediminibacillus* sp. accumulated nearly equal amounts of betaine, while the maximum amount of betaine was in the yeast extract. Thus, betaine was synthesized by *Sediminibacillus* sp. In addition, the accumulation of ectoine was strong in casein, but quite weak in the other media, therefore ectoine was up taken from medium. *Halobacillus* sp. accumulated much considerable amounts of betaine in the medium containing yeast extract, followed by the peptone, and the casein, thus betaine was synthesized by *Halobacillus* sp., whereas the amount of ectoine was similar to betaine in the presence of casein, but much less so in the presence of peptone, and less so with yeast extract; *Halobacillus* sp. therefore uptakes ectoine from the medium. In the case of *H. dabanensis*, significant amounts of betaine only accumulated in the peptone medium, followed by yeast extract and casein; betaine was therefore synthesized by *H. dabanensis* and was not up taken from the medium.

References Références Referencias

1. Averhoff, B., and Müller, V. (2010). Exploring research frontiers in microbiology: recent advances in halophilic and thermophilic extremophiles. *Research in Microbiology*, Vol. 161(6): 506- 514.
2. Brill, J., Hoffmann, T., Bleisteiner, M., and Bremer, E. (2011). Osmotically controlled synthesis of the compatible solute proline is critical for cellular defense of *Bacillus subtilis* against high osmolarity. *Journal of Bacteriology,* Vol. 193(19): 5335-5346.
3. Carrasco, I., Márquez, M., Xue, Y., Ma, Y., Cowan, D., Jones, B., and Ventosa, A. (2008). *Sediminibacillus halophilus* gen. nov., sp. nov., a moderately halophilic, Gram-positive bacterium from a hypersaline lake. *International Journal of Systematic and Evolutionary Microbiology,* Vol. 58(8):1961-1967.
4. Da Costa, M., Santos, H., and Galinski, E. (1998). An overview of the role and diversity of compatible solutes in *Bacteria and Archaea. Biotechnology of Extremophiles*, *Springer*: 117-153.
5. DasSarma, S., and Arora, P. (2002).Halophiles. *Encylopedia of Life Sciences*: 1-9.
6. DasSarma, S., and Das Sarma, P. (2012). Halophiles. *John Wiley & Sons, Ltd*.
7. DasSarma, S.,and Das Sarma, P.(2015). Halophiles and their enzymes: negativity put to good use. *Current Opinion in Microbiology*, Vol.25: 120-126.
8. Detkova, E., and Boltyanskaya, Y. V. (2007). Osmoadaptation of haloalkaliphilic bacteria: role of osmoregulators and their possible practical application. *Microbiology*, Vol. 76(5): 511-522.
9. Empadinhas, N., and Costa, M. S. d. (2006). Diversity and biosynthesis of compatible solutes in hyper/thermophiles. *International Microbiology*, Vol. 9:199-206.
10. Ewert, M., and Deming, J. W.(2013). Sea ice microorganisms: Environmental constraints and extracellular responses. *Biology,* Vol. 2(2): 603-628.
11. Fendrihan, S., Legat, A., Pfaffenhuemer, M., Gruber, C., Weidler, G., Gerbl, F., and Stan-Lotter, H. (2011). Extremely halophilic archaea and the issue of long-term microbial survival. *Reviews in Environmental Science and Bio/Technology*, Vol. 5(2-3): 203-218.
12. Fenn, J. B., Tanaka, K., and Wüthrich, K. (2002). The Nobel Prizein Chemistry. http://nobelprize.org/nobel_prizes/cgemistry/Laureates/2002/index.htm;accessed 6/16/09.
13. Grant, W. (2004). Life at low water activity. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, Vol. 359(1448): 1249-1267.
14. Hänelt, I., and Müller, V.(2013).Molecular mechanisms of adaptation of the moderately halophilic bacterium *Halobacillis halophilus* to its environment. *Life*, Vol. 3(1): 234-243.
15. Holt, J., Krieg, N., Sneath, P., and Staley, J. (1994). *Bergey's manual of determinative bacteriology* Williams and Wilkins. *Baltimore, MD*: 527.
16. Kanekar, P., Kanekar, S., Kelkar, A.,and Dhakephalkar, P.(2012). Halophiles–Taxonomy, diversity, physiology and applications. *Microorganisms in Environmental Management*, *Springer*: 1-34.
17. Kempf, B., and Bremer, E.(1998). Uptake and synthesis of compatible solutes as microbial stress responses to high-osmolality environments. *Archives of Microbiology*, Vol. 170(5): 319-330.
18. Kerkar, S. (2004). Ecology of hypersaline microorganisms. Marine microbiology: Facets and Opportunities. *National Institute of Oceanography*, Goa: 37-47.
19. Lamosa, P., Martins, L. O., Da Costa, M. S., & Santos, H. (1998). Effects of Temperature, Salinity, and Medium Composition on Compatible Solute Accumulation by *Thermococcus* spp. *Applied and Environmental Microbiology*, Vol. 64(10): 3591-3598.
20. Lee, Y., Kim, H., Liu, C., and Lee, H. (2003). A simple method for DNA extraction from marine bacteria that produce extracellular materials. *Journal of Microbiological Methods,* Vol. 52(2): 245-250.
21. Liu, W., Zeng, J., Wang, L., Dou, Y., and Yang, S. (2005). *Halobacillus dabanensis* sp. nov. and *Halobacillus aidingensis* sp. nov., isolated from salt lakes in Xinjiang, China. *International Journal of Systematic and Evolutionary Microbiology,* Vol. 55(5): 1991-1996.
22. Ma, Y., Galinski, E. A., Grant, W. D., Oren, A., and Ventosa, A. (2010). Halophiles 2010: life in saline environments. *Applied and Environmental Microbiology*, Vol. 76(21): 6971-6981.

23. Mohammadipanah, F., Hamedi, J., and Dehhaghi, M. (2015). Halophilic Bacteria: Potentials and Applications in Biotechnology. *Halophiles*. *Springer,* Cham: 277-321.
24. Nieto, J., Fernandez-Castillo, R., Marquez, M., Ventosa, A., Quesada, E., and Ruiz- Berraquero, F. (1989). Survey of metal tolerance in moderately halophilic eubacteria. *Applied and Environmental Microbiology,* Vol. 55(9): 2385-2390.
25. Oren, A. (2002). Adaptation of halophilic archaea to life at high salt concentrations. *Salinity: Environment-Plants-Molecules .Springer*, Nether-lands: 81-96.
26. Oren, A. (2011). Ecology of halophiles. *Extremophiles Handbook*. *Springer,* Japan: 343-361.
27. Oren, A., and Gunde- Cimerman, N. (2012). Fungal life in the Dead Sea. *Biology of Marine Fungi. Springer*, Berlin Heidelberg: 115-132.
28. Rampelotto, P.H. (2010).Resistance of microorganisms to extreme environmental conditions and its contribution to astrobiology. *Sustainability*, Vol. 2(6): 1602-1623.
29. Raghavan, T., and Furtado, I. (2004). Occurrence of extremely halophilic Archaea in sediments from the continental shelf of west coast of India. *Current Science*, Vol. 86(8): 1065-1067.
30. Satbhai, A., Kasodekar, A., Pachuau, L., and Bharambe, N. (2015). Isolation of halophiles from the Dead Sea and exploring their potential biotechnological applications. *Int J Curr Microbiol Appl Sci,* Vol. 2: 1-17.
31. Shivanand, P., and Mugeraya, G. (2011). Halophilic bacteria and their compatible solutes-osmoregulation and potential applications. *Current Science (Bangalore),* Vol. 100(10): 1516-1521.
32. Ventosa, A., de la Haba, R. R., Sánchez- Porro, C., and Papke, R. T. (2015). Microbial diversity of hypersaline environments: a metagenomic approach. *Current Opinion in Microbiology*, Vol. 25: 80-87.
33. Ventosa, A., Nieto, J. J., and Oren, A. (1998). Biology of moderately halophilic aerobic bacteria. *Microbiology and molecular biology reviews*, Vol. 62 (2): 504-544.
34. Waditee- Sirisattha, R., Kageyama, H., and Takabe, T. (2016). Halophilic microorganism resources and their applications in industrial and environmental biotechnology. *AIMS Microbiology*, Vol. 2(1): 42-54.

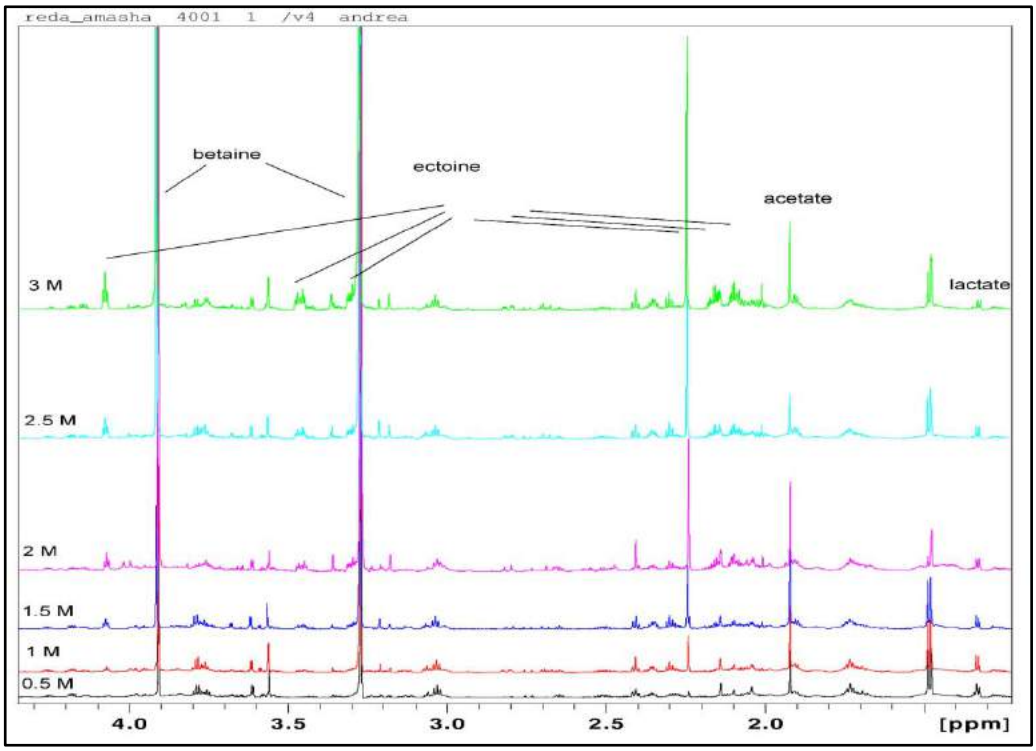

Figure 1: NMR ^{1}H spectra of cell extracts from *Halomonas aquamarina* at 0.5, 1.0, 1.5, 2. And 3.0 Na Cl (M) in saline nutrient medium spectra (Source Red Sea Sedimen

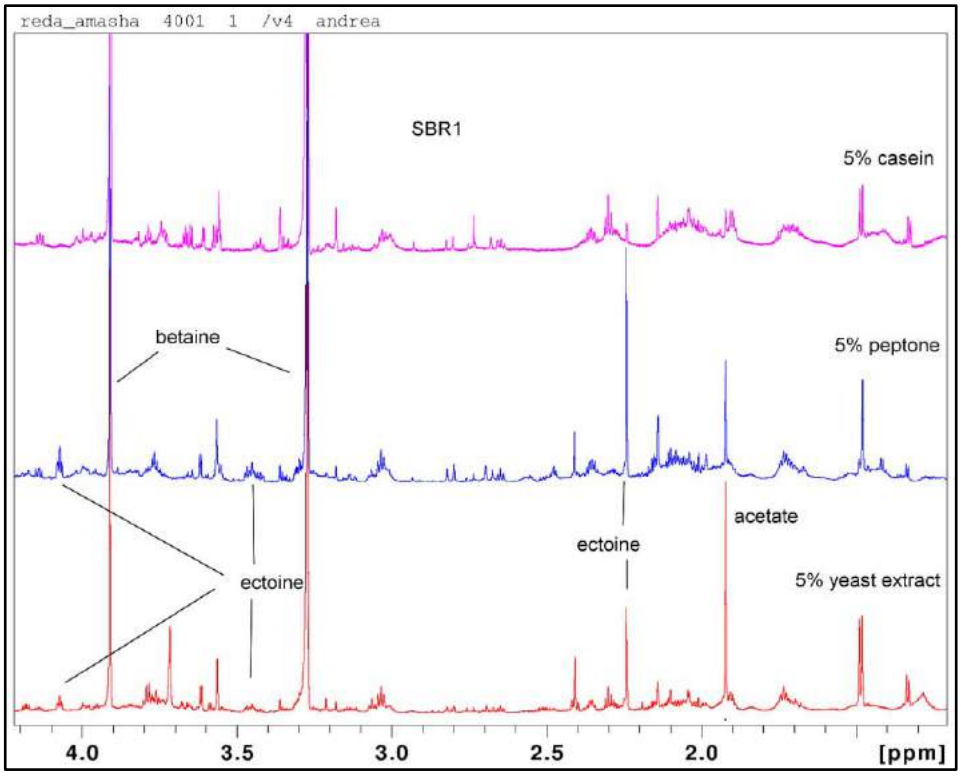

Figure 2: NMR ^{1}H spectra of cell extracts from *Halomonas aquamarina* using yeast extract peptone and casein in saline nutrient medium spectra (Source Red Sea Sediment).

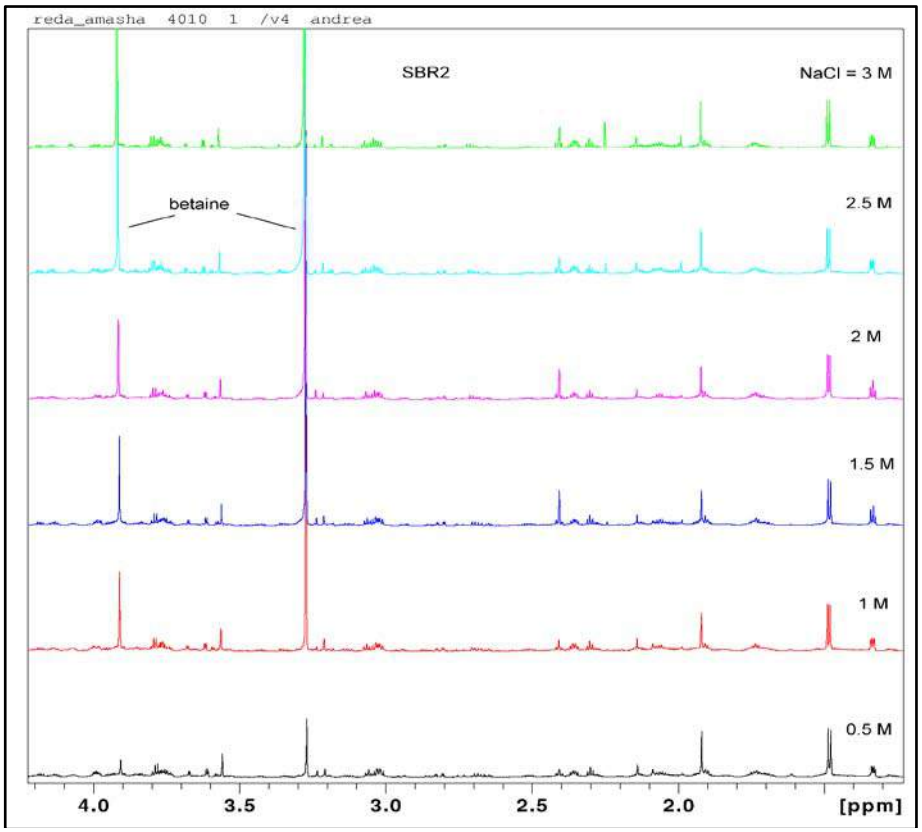

Figure 3: NMR ^{1}H spectra of cell extracts from *Sediminibacillus* sp. at 0.5, 1.0,1.5, 2.0, 2.5 and 3.0 Na Cl (M) in saline nutrient medium spectra spectra(Source Red Sea Sediment

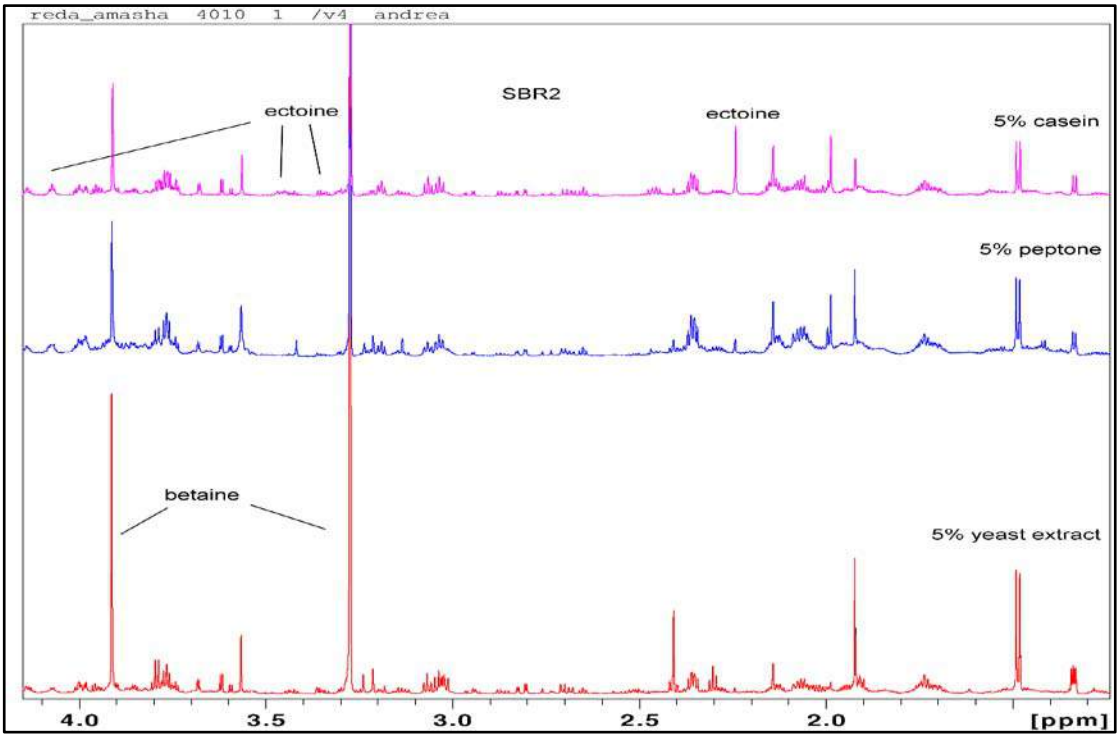

Figure 4: NMR ^{1}H spectra of cell extracts from *Sediminibacillus* sp. using yeast extract, peptone and casein in saline nutrient medium spectra spectra (Source Red Sea Sediment).

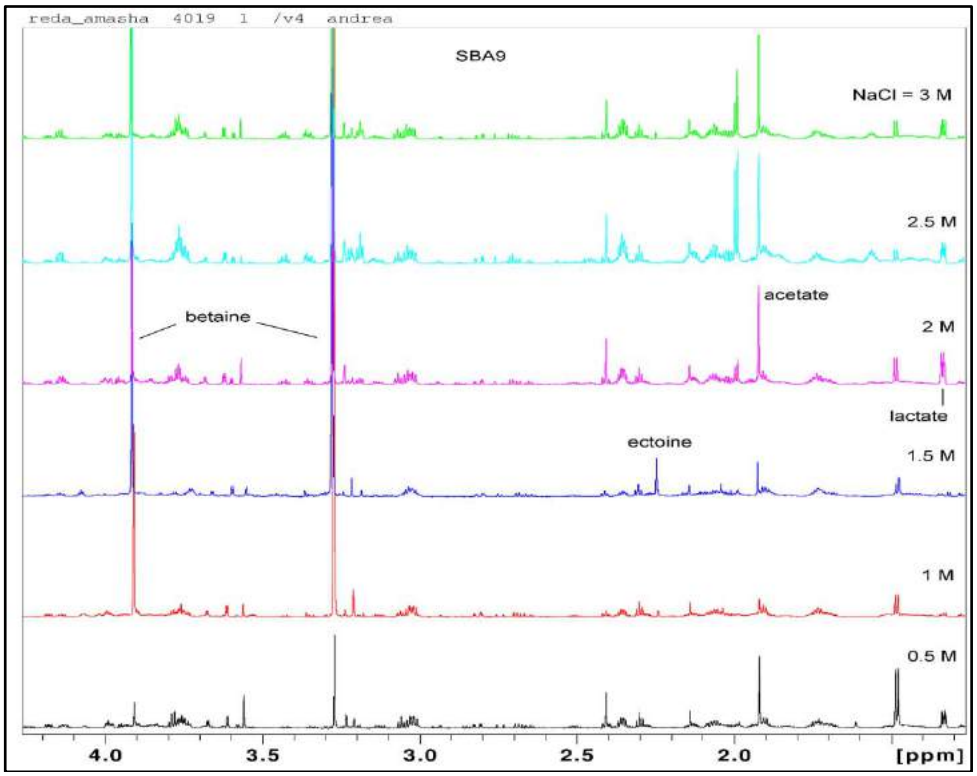

Figure 5: NMR ^{1}H spectra of cell extracts from *Halobacillus* sp. at 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 NaCl (M) in saline nutrient medium spectra (Source Arabian Sea water)

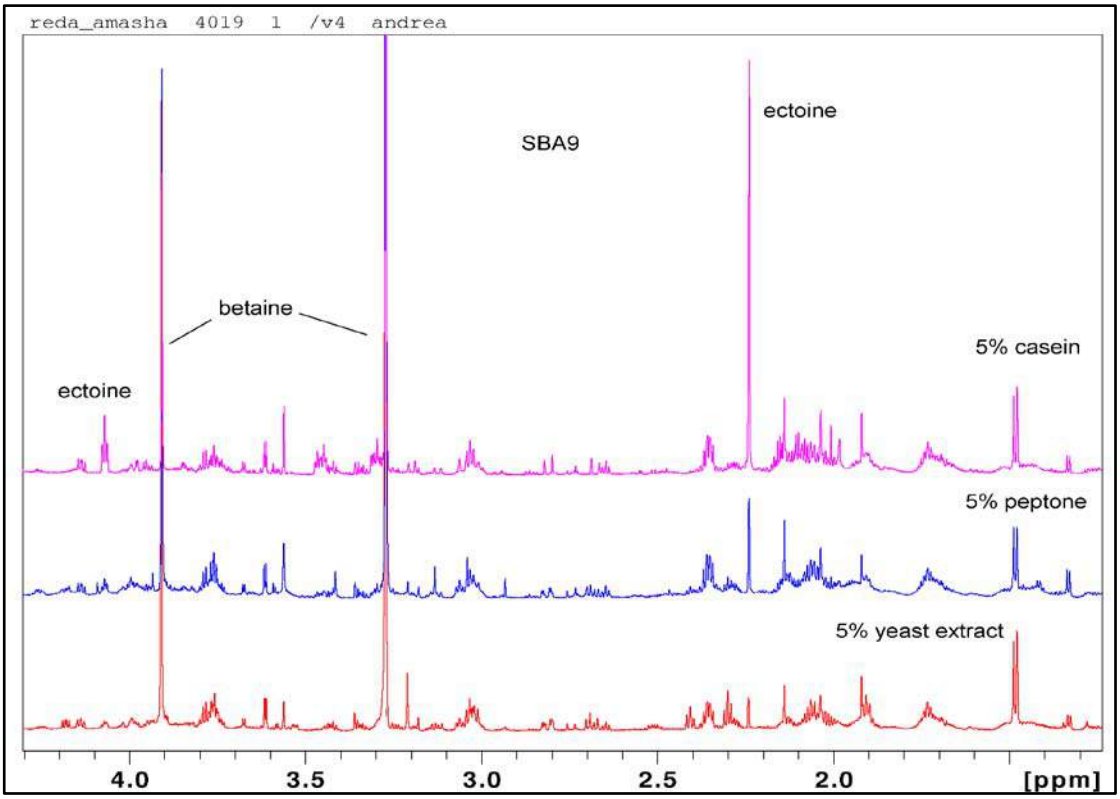

Figure 6: NMR ^{1}H spectra of cell extracts from *Halobacillus*sp. using yeast extract, peptone and casein in saline nutrient medium spectra (Source Arabian Sea Water)

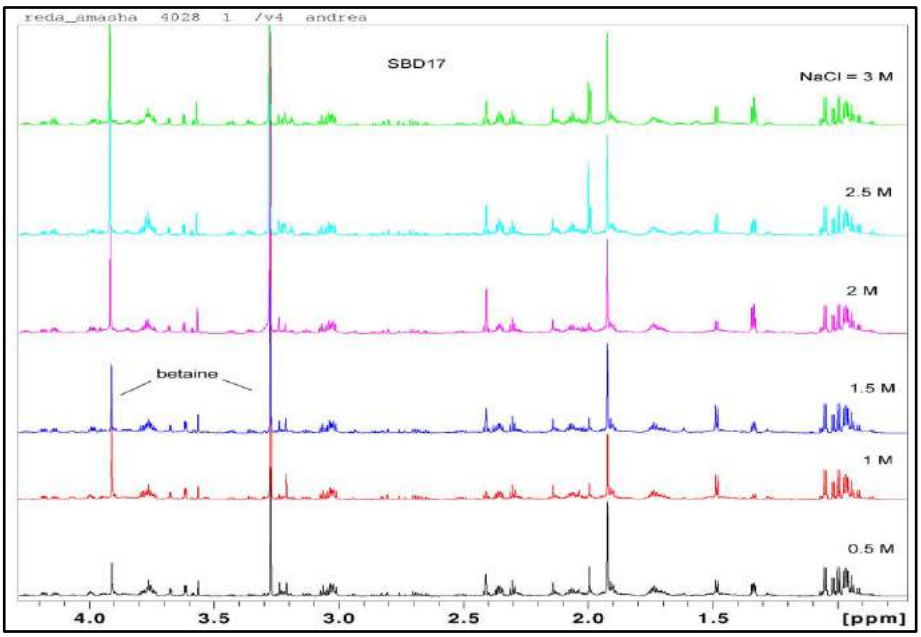

Figure 7: NMR ^{1}H spectra of cell extracts from *H. dabanensis* at 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 Na Cl (M) in saline nutrient medium (Source Red Sea mud)

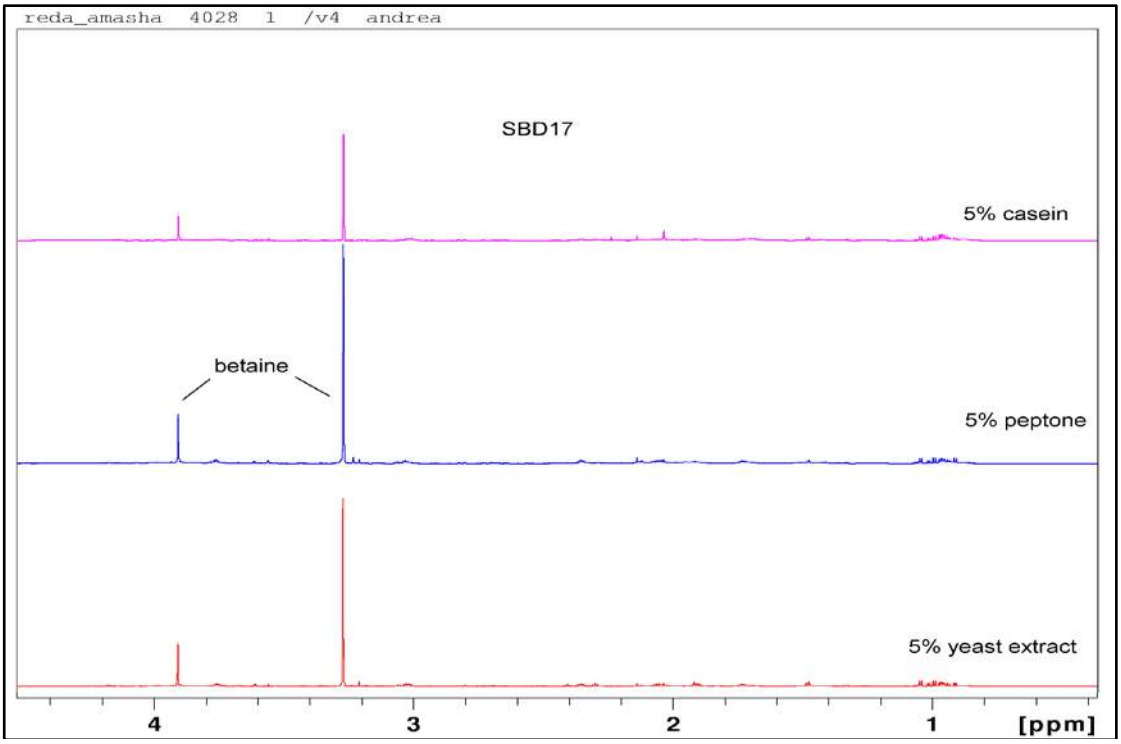

Figure 8: NMR ^{1}H spectra of cell extracts from *H. dabanensis* using yeast extract, peptone and casein in saline nutrient medium spectra (Source Red Sea mud)