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A FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPIC STUDY ON CELLULAR CHANGES IN THE *MARINOCOCCUS LUTEUS* SSLB 1 UNDER DIFFERENT SALINITY REGIME

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ABSTRACT

Marinococcus luteus SSLB1, isolated from the Sambhar Salt Lake, Rajasthan, India, could tolerate NaCl upto 25%, (w/v) concentration in a complex medium. The overall structural and compositional changes in the bacterial isolate in the presence of variable concentrations of NaCl (0-25%) was analyzed by Fourier transform infrared (FTIR) spectroscopic techniques. Results on FTIR analysis showed NaCl induced changes in the absorption peaks corresponding to different functional groups in the *M. luteus* SSLB1. The wavenumber 1537.6 cm^{-1} (Amide- II), 1538.9 and 1544.5 cm^{-1} N-H (symmetric) bending exhibited band shift at lower NaCl concentration, but they disappeared at the higher concentrations. Similarly FTIR bands observed between 1300-1500 cm^{-1} wave numbers, arising predominately from $>CH_3$ symmetric deformation, COO^{2-} stretching (symmetric) of fatty acids and wavenumber 1400.9 cm^{-1} shifted to higher frequency of wave numbers. These results indicated NaCl induced adaptational and structural changes in *M. luteus*. as evident from increase or decrease or shift in the characteristic FTIR peaks assigned to different functional groups.

KEY WORDS: **FTIR, Sambhar Salt Lake, Sodium Chloride, *Marinococcus luteus* SSLB 1**

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1. INTRODUCTION

A significant portion of the biosphere contains salt e.g. the oceans and seas that cover most of the Earth's surface. Higher salt concentrations are often found in environments such as salt lakes and lagoons.[20] Hypersaline environments created by the evaporation of sea water due to the high temperatures, lower rainfall and low humidity are called thalassohaline environments. Sambhar salt lake, literally called salt pan of the earth is the largest intermittent, saline and alkaline lake situated (Longitude 75° 05' E and Latitude 26 ° 58' N) in the middle of a closed depression in the aravalli schist of Rajasthan. Naturally created hypersaline and alkaline environments called as soda lake and soda desert, represent high pH condition in the environment. Large amount of carbonate minerals present in the lake produced the high pH values 9-11.5.[13] These environments largely distributed throughout the world are in the interior, inaccessible semi-arid or desert areas. Some of the best studied soda lakes include the East African Rift Valley, [13] Central Asian Soda Lakes, [14] and Mono Lake California.[15] Natural aquatic saline lakes are widely distributed throughout the world and cover about 45% of the total aquatic environments, with salinity ranging from 4 - 30%.[25] These extreme habitats have natural bacterial flora, which may play an important role in nutrient cycles and food webs in such ecosystems, in addition to attenuation of pollution in these ecosystems,[24] many strains capable of growing over a wide range of NaCl concentrations (0 to 17% w/v), are termed as haloversatile microorganisms. Moderately halotolerants can grow between 3 to 15% (w/v) salinity and extreme halophiles grow between 15 to 30% (w/v) NaCl. The genus *Marinococcus* includes two species; *Marinococcus halophilus* and *Marinococcus albus*.[16] Two additional species reported so far include *Marinococcus hispanicus,*[18] and *Marinococcus halotolerans*.[19] In this paper, FTIR spectra of bacterial isolate was taken during the growth of *M. luteus* in the presence of varying NaCl concentrations (0-20%) after 48 hours. FTIR spectra of this moderate halotolerant was studied to see the salinity induced adaptational changes in *M. luteus*.

1 MATERIALS AND METHODS

1. Organism: Isolation and Identification

A Gram-positive cocci, motile, aerobic, orange-pigmented bacterium, *M. luteus* SSLB1 was isolated from the saline water of Sambhar salt Lake, Rajasthan, India. The strain was able to grow at pH 6.0-8.0 (optimal growth at pH 7.5), at 20-40^{0}C (optimal growth at 30^{0}C) and in the presence of 0-25 % (w/v) NaCl (optimal growth in the presence of 5-15 % (w/v) NaCl). The bacterium was grown in Complex media used for halophiles.[21] The medium containing (g/L) Yeast extract -10, KCl - 2, $FeCl_3$ - 0.02, Casamino acid - 7.5, tri-sodium citrate -3.0, $MgSO_4.7H_2O$ - 20, NaCl - 100 prepared in Millipore water, The pH was maintained at 7.5 ± 0.02 by using HCl or NaOH. The isolate was considered to be closely related member of the genus *Marinococcus* based on 16S rDNA gene sequence analysis. The sequencing data was submitted in NCBI GenBank and provided accession number for *M. luteus* SSLB1 is KF 250431.

1.2 NaCl treatment of bacterial cultures

Culture was grown overnight in CM Broth to obtain 0.05 optical density. Freshly grown bacterial strains were inoculated in CM broth supplemented with 0% (control), 5%, 10%, 15% and 20% NaCl concentration and incubated at 30˚C at 100 rpm. After 48 hours cells were harvested by centrifugation (6000 × g for 10 min at 4° C). The bacterial pellets obtained from cultures grown at different NaCl concentrations, were washed three times with Millipore water and dried in a hot air oven at 60 °C for overnight.[22]

2.3. Study on FTIR spectra

The dried bacterial cell sample with KBr of spectroscopic quality in the ratio of 1:100 was grinded and pressed by using a manual hydraulic press (150 lb). The discs were then fixed in the FTIR spectrometer for analysis. The spectra of mid- infrared region, 4000-400 cm^{-1}, were obtained with resolution of 5-7 cm^{-1} with the 32 scan number for each spectrum, using Fourier Transform Infra-Red Spectrometer (FTIR) (Thermo- Scientific

Nicole 6700). Background correction for atmospheric air was used for each spectrum.

Figure 1
FTIR spectrum of *Marinococcus luteus* SSLB 1at different NaCl concentrations

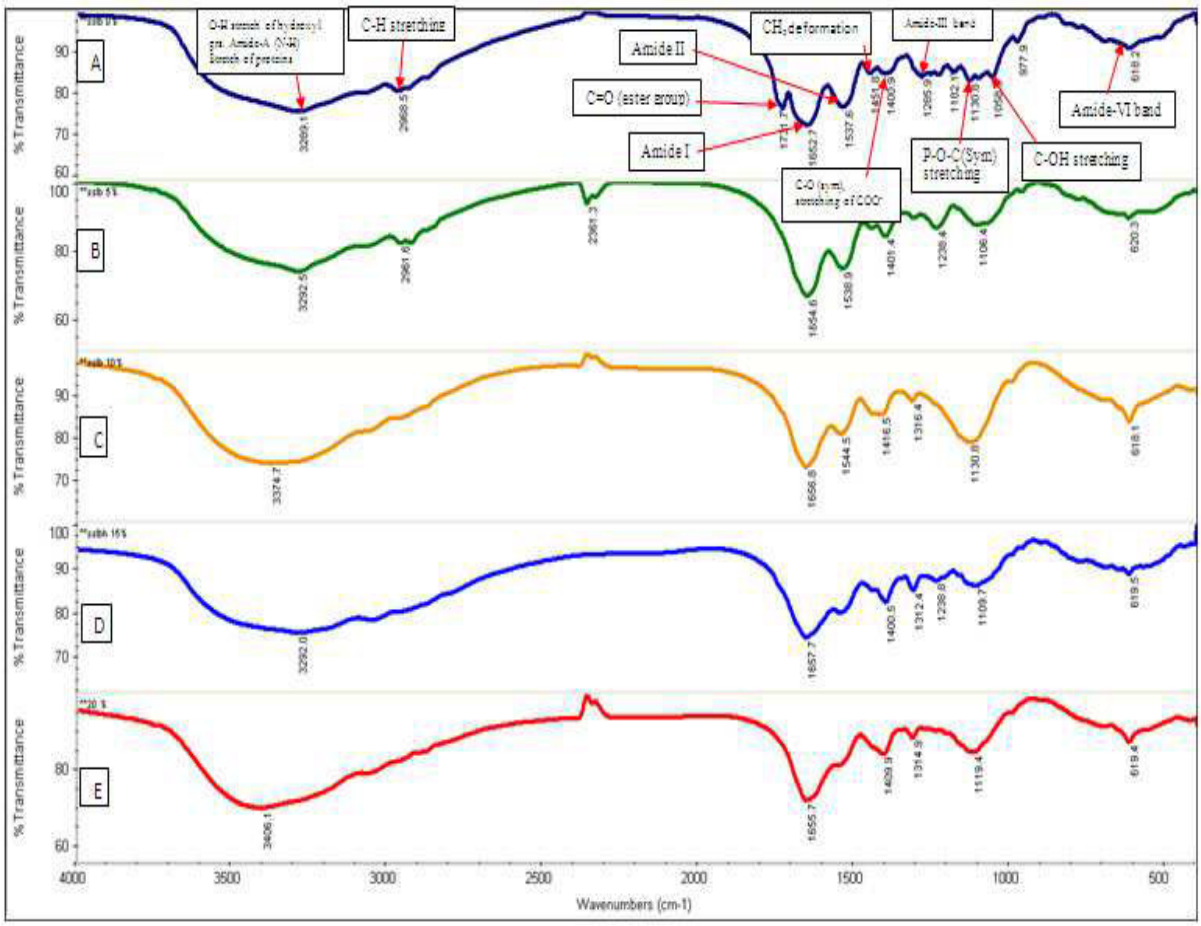

Table -1
FTIR analysis of Marinococcus luteus SSLB 1

Wave numbers denoting NaCl induced changes in different concentration of NaCl (%)						
Frequency range in cm^{-1}	**0**	**5**	**10**	**15**	**20**	**Band Assignments**
3300-3500			3374.7		3406.1	Hydroxyl compounds (O-H stretching free), N-H stretching[9]
3300-3250	3289.1	3292.5		3292		Secondary amide N–H stretching.[1]
3068-2941	2968.5	2961.6				C-H (anti symmetric) stretching. [23]
2200-2400		2361.3				Asymmetric stretching band of CO_2 hydrates[12, 11]
1738-1728	1731.7					Aliphatic aldehyde C=O stretching of ester [10]
1680-1640	1652.7	1654.6	1656.5	1657.7	1655.7	Amide II band, C=O stretching, β-sheet structure of amide- I. [1]
1560-1530	1537.6	1538.9	1544.5			Amide II, N-H (symmetric) bending, C-H (symmetric) stretching. [1]
1450-1400	1451.8					Amide III, C-N stretching and N-H in plane bending [2]
	1400.9	1401.4	1416.5	1400.5	1409.9	CH_3 (symmetric) deformation , COO^{2-} stretching (symmetric) of fatty acids [6, 7]
1360-1310			1316.4	1312.4	1314.9	Amide III band, C-N stretching, N-H (sym.) bending, O=C-N bending[7, 8]
1310-1240	1285.9			1279.4		N-H bending [3]
1250-1220		1238.4		1238.8		P=O asymmetric stretching of PO^{2-} , phospholipids and nucleic acids [4]
1200 -900	1182.1					CH_2- deformation[5]
	1130.8	1106.4	1130.8	1109.7	1119.4	Symmetric stretching P-O-C, [5]
1070-1050	1058.9					C-O-C stretching (nucleic acids and phospholipids) [6]
1000-700	977.9					C-O deoxyribose, DNA, RNA uracil ring stretching; C-H in plane bending, [1, 3]
770-620		620.3				Amide IV band, (OCN deformation) [2]
620-530	618.2		618.1	619.5	619.4	Amide VI band (OCN deformation) Amides, [2]

Wavenumbers of different (0, 5, 10, 15, and 20 %) NaCl concentrations of M. luteus SSLB1 after48hours

2 RESULTS AND DISCUSSION

2.1 Fourier Transform Infrared Analysis

Chemical functional groups present on the bacterial cell wall are important for understanding the stress induced changes in the cell membrane. The most important functional groups are Hydroxyl, Carbonyl, Carboxyl, Sulfhydryl (thiol), Secondary amide and Phosphodiester as given in table 1 and Fig. 1. Many researchers have used FTIR method to determine qualitative and preliminary analysis of the chemical functional groups, denoting cellular changes in response to external environment. For example, changes in the frequency and intensity of the absorption bands between 4000-400 cm^{-1} were analyzed by FTIR spectra of *E.coli, P. aeruginosa, B. subtilis, S. aureus* and *S. epidermidis* [23]. The isolated bacterium *M. luteus* SSLB1 in the presence of NaCl showed changes in the frequencies between wave numbers 3500-3100 cm^{-1} region, resulting from hydroxyl compound and secondary amide groups. The wave number at 3289.1 cm^{-1} observed in the bacterium without NaCl was due to secondary amide, N-H stretching but a shift in wave number (3292.5 cm^{-1}) at lower concentration of NaCl (5-15% w/v) suggested for conformational changes in the secondary amide group[13]. A new FTIR absorption peak in the region of 3500-3300 cm^{-1} observed at high concentration of NaCl (10-20% w/v) was related to O-H stretching of hydroxyl compounds. The region between 3100 and 2800 cm^{-1} exhibiting the CH stretching vibrations of $–CH_3$ and $>CH_2$ functional groups and, is generally considered as fatty acid and amino acid side chains of the membrane.[23] FTIR peak at 2968.5 cm^{1} observed in the absence of sodium chloride, denoting the C-H stretching vibration, shifted to 2961.6 cm^{-1} wave number at 5% NaCl concentration. But at higher concentration, of NaCl there is reduced synthesis of fatty acid and other membrane components. At lower concentration of NaCl, spectral shifts in wave numbers indicated osmotic adjustment within the cells without structural alteration these bands disappeared. With increasing concentration of sodium chloride, no new absorption peak is observed in the region of 1731.7 cm^{-1}, denoting aldehydes[10]. The FTIR peak at 1652.7 cm^{-1} denoting proteins (amide-II) shifted to higher wave number with increasing NaCl concentration.[1] The wavenumber 1537.6 cm^{-1} (Amide- II), 1538.9 and 1544.5 cm^{-1} N-H (symmetric) bending exhibited band shift at lower NaCl concentration but they disappeared at the higher concentrations.[1] Similarly FTIR bands observed between 1300-1500 cm^{-1} arising predominately from $>CH_3$ symmetric deformation, COO^{2-} stretching (symmetric) of fatty acids[6,7] and wavenumber 1400.9 cm^{-1} shifted to higher frequency of wave numbers with increasing concentration of NaCl. Several new peaks appeared in amide- III band regions (1316.4, 1312.4 and 1314.9 cm^{-1}) due to the C-N stretching, N-H (sym.) bending and O=C-N deformations.[7,8] Wavenumber 1285.9 cm^{-1} are produced due to the N-H bending.[3] The new FTIR bands at wavenumber 1238.4 and 1238.8 cm^{-1} were produced due to the breakdown of phospholipids and phosphodiester bonds at high concentration of NaCl indicating changes at membrane level [11]. A FTIR bands at wave number 1182.1 cm^{-1} and 1130.8 cm^{-1} showed reduced absorption with increasing concentration of sodium chloride which are related to symmetric stretching of P-O-C.[5] The region of phospholipids wave number (1058.9 and 977.9 cm^{-1}) present in the spectra taken without sodium chloride was found to be absent in the presence of NaCl.

3 CONCLUSION

In the present study, FTIR spectroscopic analysis of *M. luteus* SSLB 1, after treatment with different NaCl concentration indicated conformational changes at lower concentration of NaCl due to osmotic adjustment. But disappearance of certain FTIR peaks at higher concentration of NaCl suggested inhibitory effect of NaCl on synthesis of cell constituents and overall growth of *M. luteus*.

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