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THE OSMOPROTECTIVE EFFECT OF SOME ORGANIC SOLUTES ON *STREPTOMYCES* SP. MADO2 AND *NOCARDIOPSIS* SP. MADO3 GROWTH

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ABSTRACT

The response of two marine actinomycetes such as *Streptomyces* sp. MADO2 and *Nocardiopsis* sp. MADO3 to osmotic stress in minimal medium M63 and in glycerol-asparagine medium (ISP5) was studied. The two strains were moderately halophilic and the behavior of the strain *Streptomyces* sp. MADO2 and *Nocardiopsis* sp. MADO3 towards the salt stress was varied depends on the media composition and the salinity concentration. The strain *Streptomyces* sp. was more sensitive to salt stress than *Nocardiopsis* sp. The growth of both *Streptomyces* sp. and *Nocardiopsis* sp. were inhibited at 1 M NaCl irrespective of the medium used. The *Nocardiopsis* sp. acquired osmoadaptation on ISP5 medium whereas the *Streptomyces* sp. showed poor growth on M63 medium. Glycine betaine (GB), proline and trehalose played a critical role in osmotic adaptation at high osmolarity whereas at low osmolarity they showed an inhibitory effect on the bacterial growth. The present findings confirmed that GB was the powerful osmoprotectant for *Streptomyces* sp. and *Nocardiopsis* sp. grown at 1 M NaCl both in M63 and ISP5 media.

Key words: *Streptomyces* sp., *Nocardiopsis* sp., osmoprotective effect, M63 medium, ISP5 medium.

INTRODUCTION

Actinomycetes are potential producers having immense application in industrial production processes. Their capacity to degrade complex organic compounds and to produce antibiotics has been demonstrated (1). Halophilic or salt tolerant actinomycetes are being developed as model organisms to disclose the mechanism and microbial physiology under extreme environments (3). Microorganisms found in extreme environments have attracted a great attention due to their production of various natural compounds and their specialized mechanisms for adaptation to extreme environments (28). However, relatively little information was available on the osmoregulatory strategy of actinomycetes, even though needs a special attention because of their transitional nature between the simple eubacteria and the fungi (13, 19).

Most microorganisms subjected to water stress accumulate

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organic solutes to control their internal water activity, maintain the appropriate cell volume and turgor pressure, and protect intracellular macromolecules (8). Prokaryotes have developed two strategies to cope with increasing salinities. One is to accumulate and adjust the internal concentration of inorganic ions such as K^+ and Cl^- to values that counteract the external osmolarity (25). A different strategy is the accumulation of osmolytes or compatible solutes including sugars (trehalose), free amino acids (e.g., glutamate and proline), and quaternary ammonium compounds (e.g., glycine betaine, proline betaine, butyrobetaine, and carnitine) that bacteria accumulate from *de novo* synthesis or from externally provided osmoprotectants such as choline, the precursor of glycine betaine (19, 6, 16, 18).

The present study aims to analyze the effect of increasing osmolarity of NaCl (0-1 M) on the growth of *Streptomyces* sp. and *Nocardiopsis* sp. isolated from the marine sponge *Fasciospongia cavernosa* in minimal medium M63 and in glycerol-asparagine medium (ISP5 medium). It was a maiden initiative to use M63 medium to analyze the effect of increased salinity and osmoprotectants on the growth of actinomycetes strains. This minimal medium is generally used for the study of halotolerance of enteric bacteria. The osmoprotective effect of glycine betaine (GB), proline and trehalose was also studied.

MATERIALS AND METHODS

Bacterial strains and antimicrobial screening

The marine actinomycetes such as *Streptomyces* sp. MADO2 and *Nocardiopsis* sp. MADO3 were obtained from marine bioprospecting laboratory, Bharathidasan University, Tiruchirappalli (India). Primary screening of antimicrobial activities was performed on Actinomycetes agar (Himedia) supplemented with 2% NaCl. The screening of the isolates was examined against *C. albicans* PC1, *E. coli* PC2, *P. mirabilis* PC3, *S. haemolytic.* PC4, *P. aeruginosa* PC5, *M. luteus* PC6, *S. epidermidis* PC7, *E. faecalis* PC8, *K. pneumoniae* PC9, *B. subtilis* PC10 and *S. aureus* PC11.

Medium and cultivating conditions

YM medium (19), containing 10 g of malt extract, 4 g of yeast extract, 0.01 g of $CaCl_2$ and 1 ml of trace salts solution (per 100 ml of water: 100 mg of $FeSO_4.7H_2O$, 100 mg of $MnCl_2.4H_2O$, 100 mg of $ZnSO_4.H_2O$ and 100 mg of $CaCl_2$) was used for the preparation of the inoculum of the strains MADO2 and MADO3. Cells were grown aerobically for 72 h at 26°C on a rotary shaking incubator at 250 rpm. After centrifugation and washing with 0.9% NaCl solution, 1 ml of cell suspension served as the inoculum for 50 ml of culture in M63 and in ISP5 medium respectively. The inoculum contained $4.2x10^7$ cfu/ml.

Effect of increased salinity on the bacterial growth

For NaCl endurance experiments, M63 medium [(g/l) KH_2PO_4 13.6 g, KOH 4.2 g, $(NH_4)_2SO_4$ 1.982 g, $MgSO_4.7H_2O$ 0.246 g, $FeSO_4.7H_2O$ 0.0005 g and glucose 1.8 g (4)] and ISP5 [(g/l) asparagine 1 g, glycerol 10 g, K_2HPO_4 1 g and 1 ml of trace salts solution (26)] were used as the basic media. The NaCl concentrations include: 0M, 0.1 M, 0.5 M, 0.8 M and 1 M were enriched in both media.

Osmoprotective effect of GB, proline and trehalose on the bacterial growth

To study the osmoprotective effect of osmoprotectants described above, the media supplemented with GB (1 mM), proline (1 mM) and trehalose (1 mM) were used. MADO2 and MADO3 strains were cultivated in M63 and ISP5 media aerobically at 26°C on a rotary shaking incubator at 100 rpm. Bacterial growth was monitored spectroscopically at 540 nm. The number of cells corresponding to the absorbance was counted using a Petroff-Hausser chamber

RESULTS

Antimicrobial activity

The two isolates were screened for their antimicrobial activities against the indicator strains as mentioned above. *Streptomyces* sp. showed a significant antimicrobial activity

against *C. albicans* PC1 (23 mm), *E. coli* PC2 (16 mm) *P. mirabilis* PC3 (26 mm), *M. luteus* PC6 (27 mm) and *S. aureus* PC11 (26 mm). It was more active than *Nocardiopsis* sp. showed activity against *C. albicans* PC1 (14 mm), *E. coli* PC2 (00 mm), *P. mirabilis* PC3 (12 mm), *M. luteus* PC6 (23 mm) and *S. aureus* PC11 (19 mm) (Table 1).

Table 1. Antimicrobial activity of *Streptomyces* sp. MADO2 and *Nocardiopsis* sp. MADO3 on some clinical pathogens

Clinical pathogens		**Inhibition zone (mm)**	
		***Streptomyces* sp. MADO2**	***Nocardiopsis* sp.MADO3**
C. albicans	PC1	23	14
E. coli	PC2	16	00
P. mirabilis	PC3	26	12
H. streptococcus	PC4	12	08
P. aeruginosa	PC5	13	16
M. luteus	PC6	27	23
S. epidermidis	PC 7	22	14
E. faecalis	PC 8	16	13
K. pneumoniae	PC 9	08	11
B. subtilis	PC 10	23	22
S. aureus	PC 11	26	19

Growth characteristics of strains MADO2 and MADO3 in M63 and ISP5 media

The growth characteristics of *Streptomyces* sp. was compared with those of *Nocardiopsis* sp. using M63 and ISP5 media (Fig.1). The growth of *Streptomyces* sp. was favored irrespective of the media used. However, *Nocardiopsis* sp. growth was better in M63 medium than in ISP5 medium.

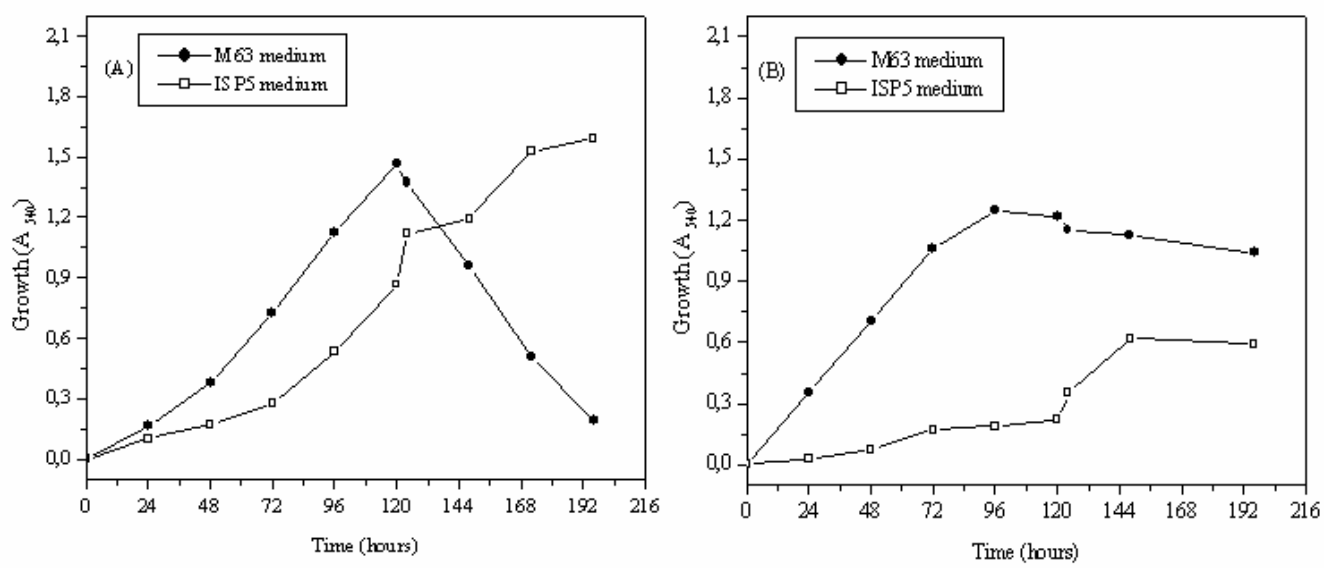

Figure 1. Growth characteristics of *Streptomyces* sp. MADO2 (A) and *Nocardiopsis* sp. MADO3 (B) grown in M63 medium and ISP5 medium.

Effect of increased osmolarity on the bacterial growth

To analyze the response of *Streptomyces* sp. and *Nocardiopsis* sp. to increased osmolarity, cells were cultivated in M63 and ISP5 media containing 0-1 M NaCl. Exposure to elevated osmotic strength reduced the growth of *Streptomyces* sp. At 0.5 M NaCl, the growth rate decreased significantly in both M63 and ISP5 media (Fig. 2). The effect of increased salinity on the growth of *Nocardiopsis* sp. was different to that of *Streptomyces* sp. The increase in growth occurred beyond 0.5 M NaCl, a drastic decline of growth was observed (Fig. 3).

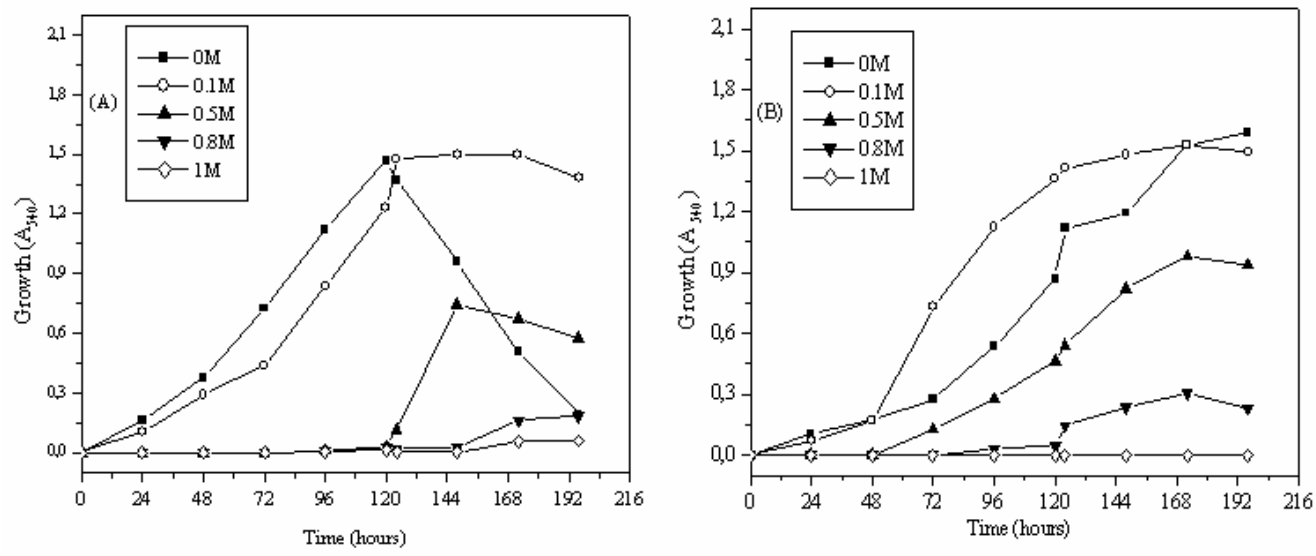

Figure 2. Growth of *Streptomyces* sp. (MADO2) of increased salinity in: M63 medium (A) and in ISP5 medium (B).

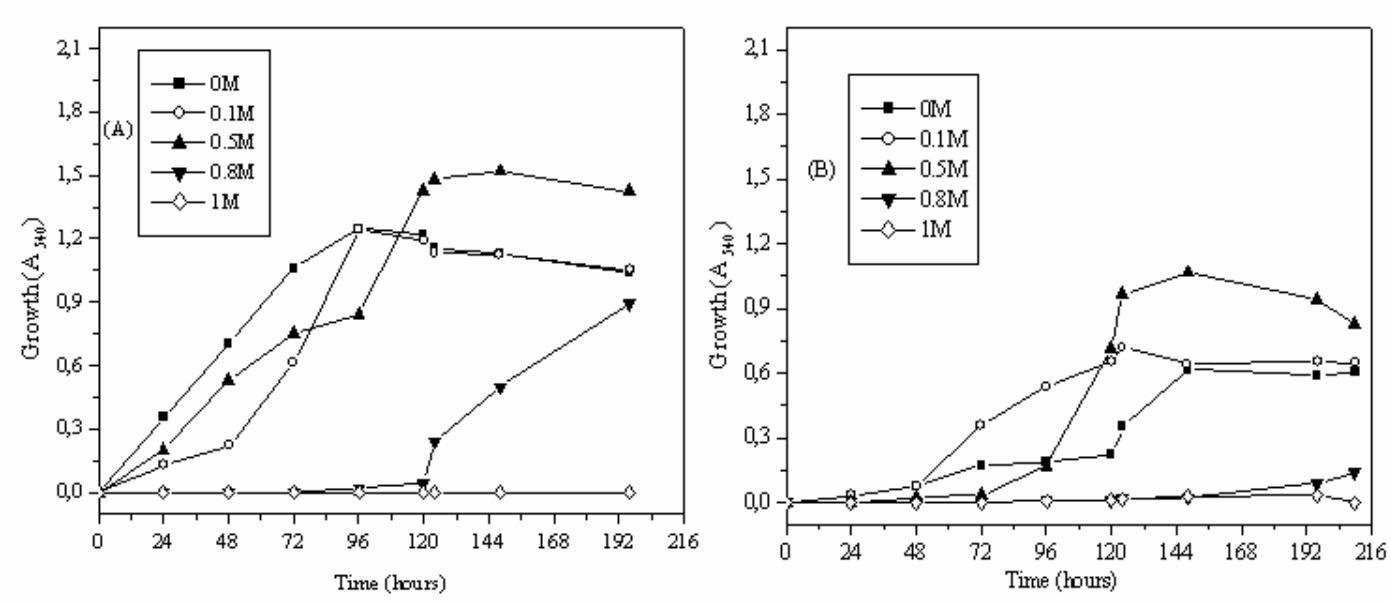

Figure 3. Growth of *Nocardiopsis* sp. (MADO3) of increased salinity in: M63 medium (A) and in ISP5 medium (B).

Effect of GB, proline and trehalose on the bacterial growth at different concentrations of NaCl

At 0.1 M NaCl, the supplementation of trehalose in M63 medium enhanced the growth of *Streptomyces* sp. whereas GB and proline showed an inhibitory effect on the growth. The condition was found to be entirely different in the case of *Nocardiopsis* sp. where the supplement of GB and proline enhanced the growth.

The ISP5 medium supplemented with osmoprotectants inhibited the growth of *Streptomyces* sp. whereas the osmoprotectants trehalose and proline stimulated the growth of *Nocardiopsis* sp. (Fig .4).

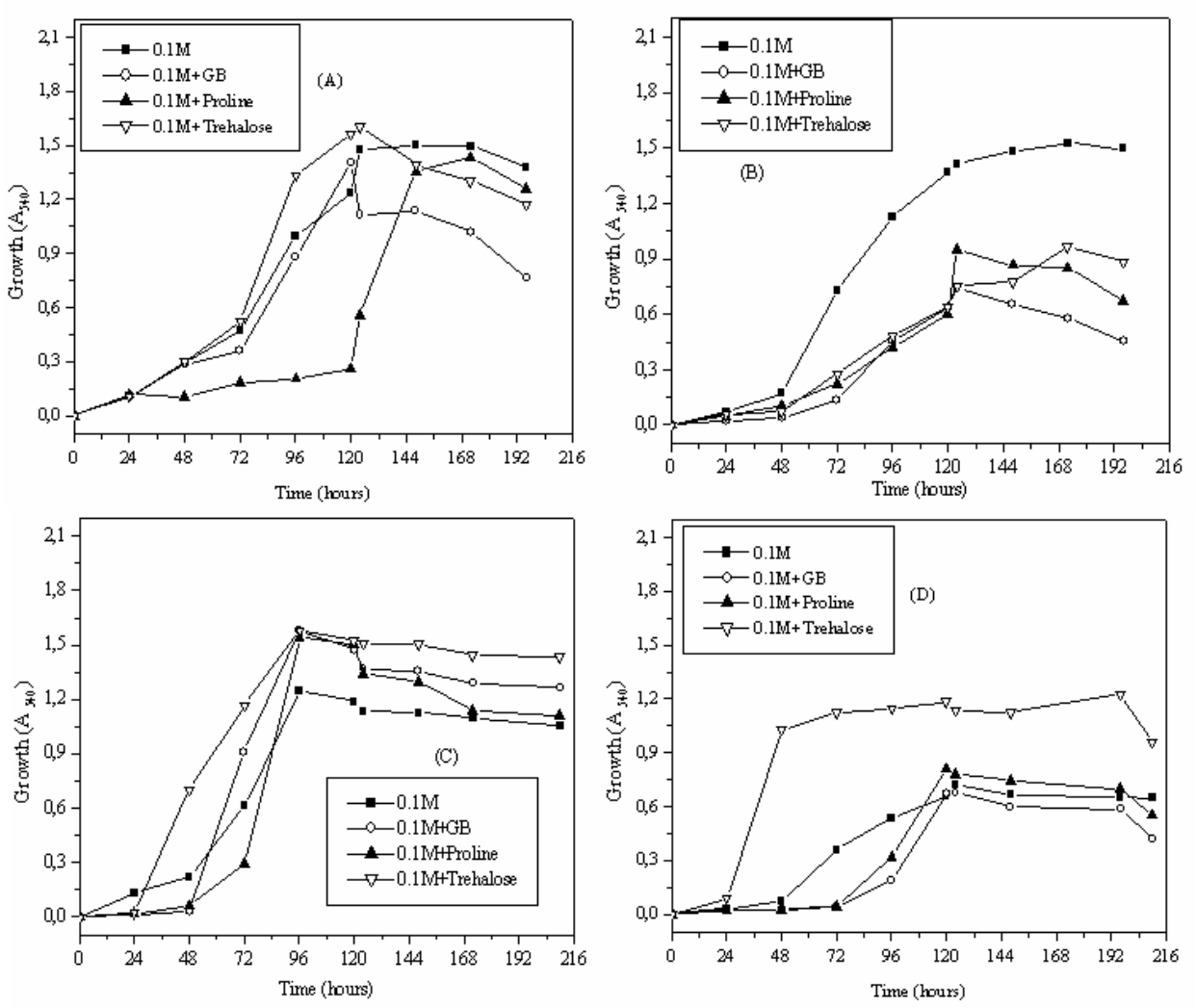

Figure 4. Growth of *Streptomyces* sp. MADO2 in M63 medium (A), in ISP5 medium (B) and *Nocardiopsis* sp. MADO3 in M63 medium (C) and ISP5 medium (D) in presence of 0.1 M NaCl and/or GB, proline, trehalose (1 mM).

In presence of 0.5 M NaCl, both M63 and ISP5 media supplemented with GB, proline and trehalose showed an osmoprotective effect and enhanced the bacterial growth of *Streptomyces* sp. Growth of *Nocardiopsis* sp. was nearly the same in M63 medium with or without GB. Trehalose and proline acted positively on the growth of *Nocardiopsis* sp.

However, the growth was reduced in ISP5 medium supplemented with GB and proline. But trehalose stimulated the growth of *Nocardiopsis* sp. (Fig. 5).

Growth of *Streptomyces* sp. and *Nocardiopsis* sp. were greatly increased at 0.8 M NaCl in M63 medium supplemented with GB, proline and trehalose. However, it is noteworthy that trehalose was more effective than GB and proline. *Nocardiopsis* sp. growth was stimulated significantly in ISP5 medium with GB. Trehalose and proline stimulated the growth of *Streptomyces* sp. Hence, we conclude that trehalose was the potent osomprotectant stimulating the growth of *Streptomyces* sp. besides GB and proline (Fig. 6).

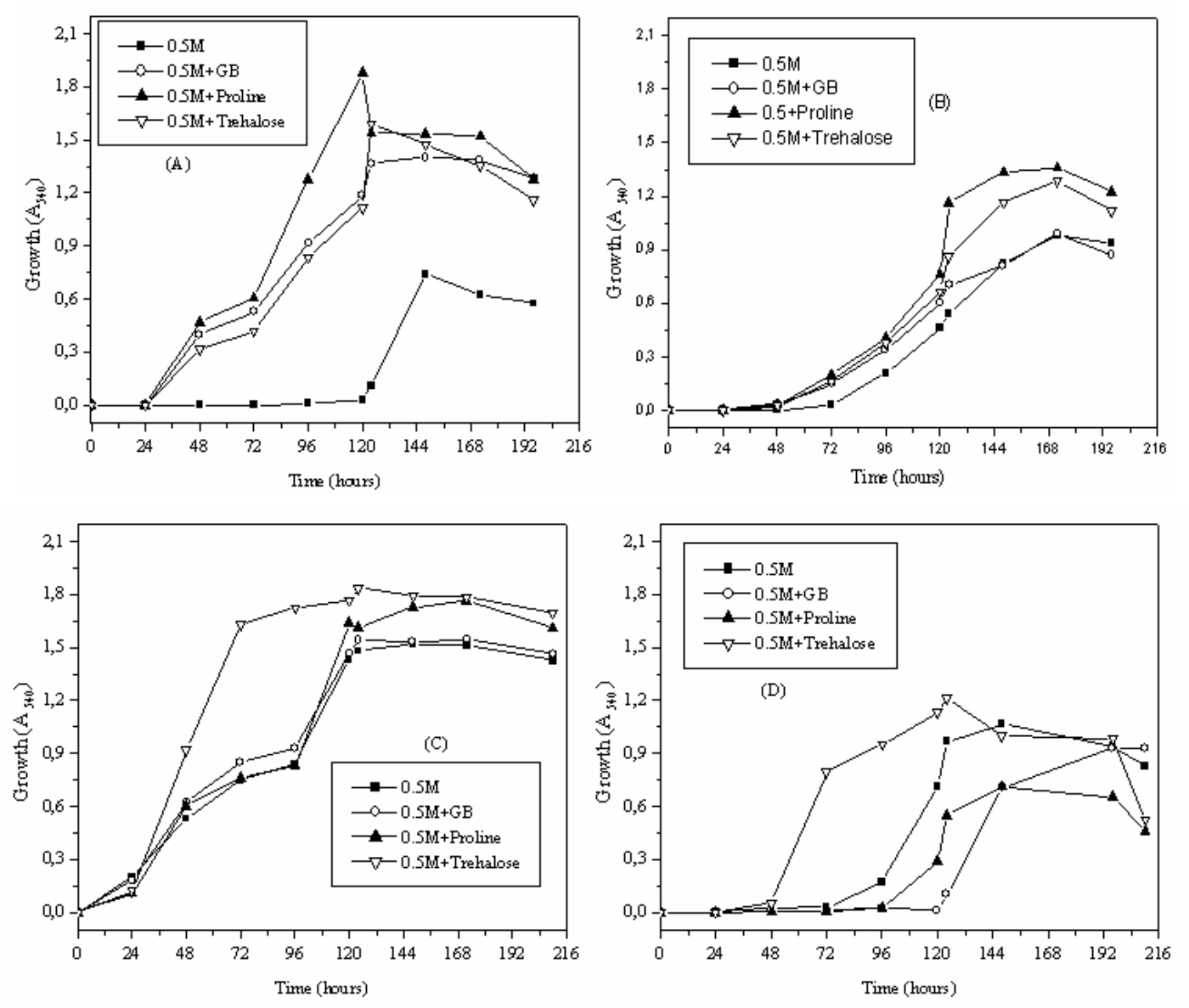

Figure 5. Growth of *Streptomyces* sp. MADO2 in M63 medium (A), in ISP5 medium (B) and *Nocardiopsis* sp. MADO3 in M63 medium (C) and ISP5 medium (D) in presence of 0.5 M NaCl and/or GB, proline, trehalose (1 mM).

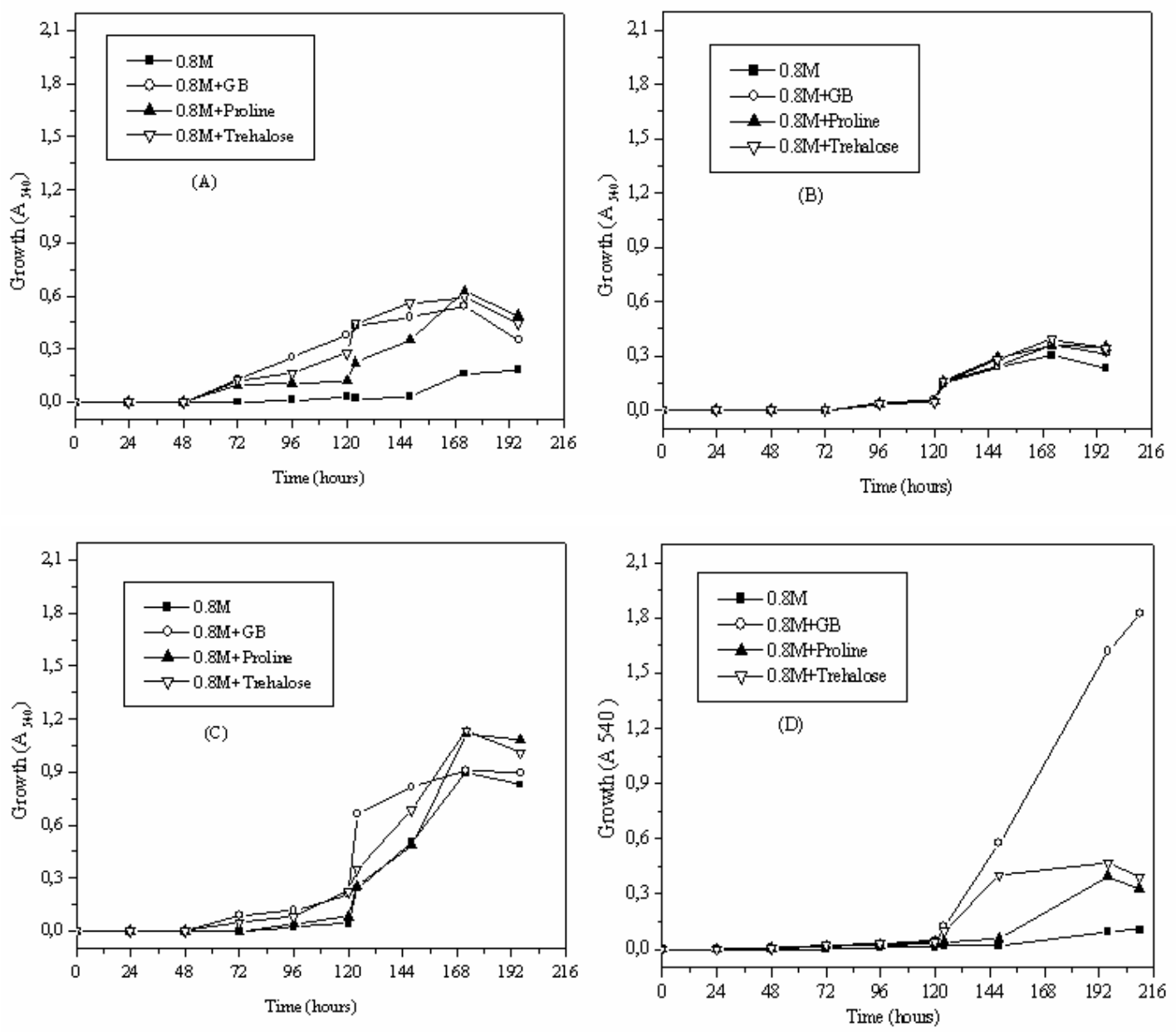

Figure 6. Growth of *Streptomyces* sp. MADO2 in M63 medium (A), in ISP5 medium (B) and *Nocardiopsis* sp. MADO3 in M63 medium (C) and ISP5 medium (D) in presence of 0.8 M NaCl and/or GB, proline, trehalose (1 mM).

In M63 medium supplemented with 1 M NaCl, GB was the most efficient osmoprotectant stimulating the growth of *Streptomyces* sp. and *Nocardiopsis* sp. in comparison with proline and trehalose. Moreover, little or no growth was observed in *Streptomyces* sp. and *Nocardiopsis* sp. respectively without osmoprotectants. In ISP5 medium, GB proved to be a potent osmoprotectant stimulating the growth of *Streptomyces* sp. However, its osmoprotective effect was negligible for *Nocardiopsis* sp. (Fig. 7).

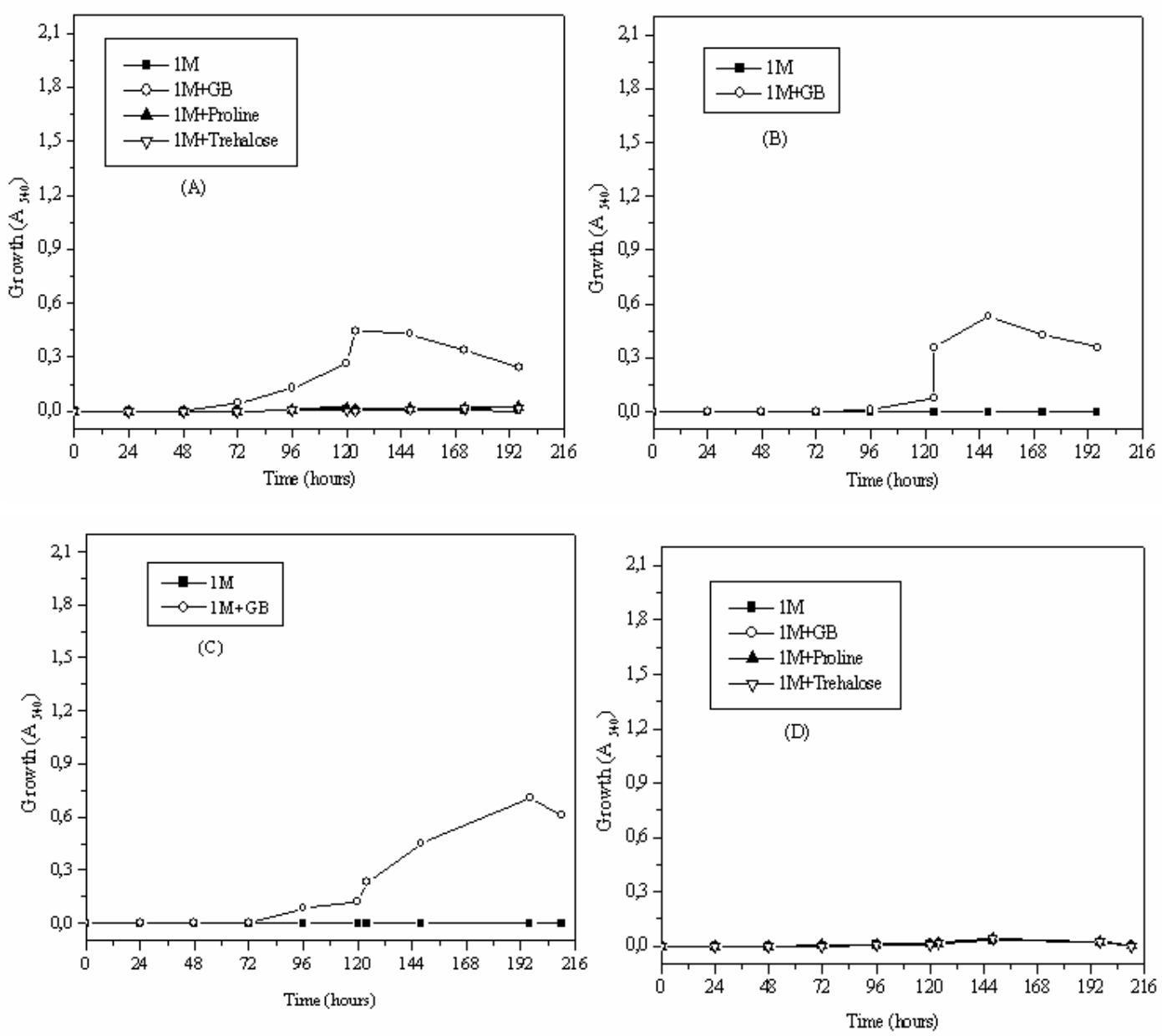

Figure 7. Growth of *Streptomyces* sp. MADO2 in M63 medium (A), in ISP5 medium (B) and *Nocardiopsis* sp. MADO3 in M63 medium (C) and ISP5 medium (D) in presence of 1 M NaCl and/or GB, proline, trehalose (1 mM).

DISCUSSION

Marine organisms produce enormous antibiotics chemicals. They are emerging as an exciting species for the discovery of new classes of therapeutics and it could provide the drugs needed to sustain us for the next 100 years in our battle against drug resistant infectious diseases (30). Our results showed that the two strains had an antimicrobial activity against the clinical pathogens tested. *Nocardiopsis* sp. VITSVK5 (FJ973467) isolated from marine sediment samples collected at the Puducherry coast of India showed a significant antibacterial activity against Gram negative bacteria- *E. coli*

(20 mm), *P. aeruginosa* (18 mm) and *K. pneumonia* (15 mm) and Gram positive bacteria- *E. faecalis* (20 mm), *B. cereus* (13 mm) and *S. aureus* (6 mm). They also showed an antifungal activity against *A. fumigatus* (23 mm), *A. flavus* (15 mm) and *A. niger* (12 mm) (29).

It was found that the behavior of the two strains towards salt stress was different depending on the type of the medium used and the degree of salinity. The absence of NaCl promoted the growth of *Streptomyces* sp. irrespective of the media used. However, *Nocardiopsis* sp. growth was better in M63 medium than in ISP5 medium. It was found that *Streptomyces* sp. utilized both glucose and glycerol as carbon source, while *Nocardiopsis* sp. utilized glucose as sole carbon source. Growth of *Streptomyces clavuligerus* strain Mit-1 grown on different media has been studied (11). The growth was very low in ISP5 medium than in the other media like Actinomycete broth (maximal growth) or in Gelatin casaminoacid broth. Moreover, various sugars were investigated in the same study for their effect on the bacterial growth. Glucose was the best carbon source comparative to lactose, maltose, sucrose and xylose. It should be noted that growth of *Streptomyces* sp. and *Nocardiopsis* sp. decreased in M63 medium. This is can be explained by the poor composition of this minimal medium which contains only glucose as carbon source. In the presence of increased osmolarity, it was obtained that the two strains are moderately halophilic. Halophilic bacteria and actinomycetes have been grouped according to salinity requirements by microbiologists. The method of classification proposed by Kushner (15) appears to be the most popular one.

Streptomyces sp. was more sensitive to salt stress than *Nocardiopsis* sp. while *Nocardiopsis* sp. showed an optimum growth at 0.5 M NaCl. It was observed that *Nocardiopsis* sp. requires 3% of NaCl for its growth while *Streptomyces* sp. requires 0.58% of NaCl. At 0.8 M NaCl, *Streptomyces* sp. growth decreased significantly in M63 medium than in ISP5 medium. It has demonstrated that polyols like glycerol, arabitol and inositol are typical compatible solutes of halophilic/halotolerant fungi, algae, bacteria and plants (7). For *Nocardiopsis* sp., its growth was considerable in M63 medium than in ISP5 medium. This is can be explained by the fact that *Nocardiopsis* sp. synthesizes trehalose from glucose, which is an ingredient of M63 medium. We noted that M63 medium contains 10 mM glucose, which allowed the stressed-cells to accumulate trehalose as an osmoprotectant. According to McBride and Ensign (21), spores of *Streptomyces griseus* grown on DMC medium containing 200 mM glucose contain 21% of their dry weight as trehalose while spores grown on media containing 20 mM glucose contained 1.2% of their dry weight as trehalose. The relationship between glucose concentration in the medium and trehalose content of spores was essentially linear between 5 and 100 mM glucose. It has been mentioned that trehalose is widespread disaccharide occurring in eukaryotic and prokaryotic alike. Because they are often observed in slightly halotolerant of marine organisms growing at 3% NaCl (0.5 mol/l) or just above, a prospective role as osmolytes was assumed (7).

At 1 M NaCl, growth of *Streptomyces* sp. and *Nocardiopsis* sp. was totally inhibited in both ISP5 and M63 media. Poor growth of *Streptomyces* sp. was observed in M63 medium. An osmoadaptation was acquired for *Nocardiopsis* sp. in ISP5 medium. It seems possible that the two strains accumulated or synthesized osmolytes to regulate response to osmotic stress.

Concerning the osmoprotective effect of GB, proline and trehalose, it was observed that the three components play a critical role in osmotic adaptation at high osmolarity whereas at low osmolarity they may have an inhibitory effect on the bacterial growth. Unfortunately, very little is known about the NaCl tolerance, the halophilism and the osmoadaptation of actinomycetes. Further research warranted to explore whether actinomycetes respond to osmotic stress in a way similar to halophilic bacteria or not and their compatible solutes are the same nature as observed in eubacteria (31).

Based on the present findings, the GB has an inhibitory

effect at 0.1 M NaCl on the growth of *Nocardiopsis* sp. in ISP5 medium whereas proline and trehalose stimulated its growth. The inhibitory effect of GB, proline and trehalose was also observed in *Streptomyces* sp. grown in ISP5 medium whereas only GB and proline inhibited the growth of this strain in M63 medium. *Nocardiopsis* sp. growth was also inhibited in ISP5 medium at 0.5 M NaCl by adding GB and proline. However, it should be noted that an osmoprotective effect of GB, proline and trehalose on the growth of *Streptomyces* sp. both in M63 and ISP5 media at 0.5 M NaCl was observed. Therefore, it is noteworthy that the inhibitory effect of GB and proline particularly on the bacterial growth depending not only on the salinity range (low osmolarity) rather the effect also duo to the nature of the culture media. The observations concerning the inhibitory effect of GB, proline and trehalose were similar to that of previous reports (9, 12, 23) which have also demonstrated that GB and other osmoprotectants had an inhibitory effect on growth of *Lactococcus lactis*.

The osmoprotective effect of GB, proline and trehalose on the two strains has shown only at inhibitory salinities. Proline is the main compatible solute in *Streptomyces* sp. at 0.5 M NaCl grown in M63 medium and in ISP5 medium. GB and proline are two major organic osmolytes that accumulate in a variety of plant species (2) and in many microorganisms (5) in response to environmental stresses. In *Salmonella oranienburg*, exogenous proline could alleviate the growth inhibition imposed by osmotic stress (5). It is also often observed that proline is raised in a salt stress situation as in *Streptomyces* sp (7). Furthermore, addition of 12.5 mM proline to the growth medium increased the specific growth yield of *Streptomyces griseus* (14). At 0.8 M NaCl, trehalose confers an osmoadaptation in *Streptomyces* sp. in both M63 and ISP5 media. Trehalose stimulates *Nocardiopsis* sp. growth besides GB and proline. Trehalose is known to stabilize membranes, proteins and whole cell during dehydration and storage. It participates in some way to alleviate osmotic stress and that trehalose is primarily a stress metabolite associated with the onset of unfavorable growth conditions (7). Trehalose is also the major organic osmoprotectant in *Rhodobacter sphaeroides* f. sp. *denitrificans* IL 106 (32). Obtained results confirmed that GB was the powerful osmoprotectant in *Streptomyces* sp. grown at 1 M NaCl in both M63 and ISP5 media and for *Nocardiopsis* sp. grown in M63 medium. Glycine betaine has been generally regarded as a superior protective osmolyte for *E. coli* and has been shown to increase tolerance to sugars, salts and organic acids (5, 22). Glycine betaine is of general importance for osmotic adaptation of most eubacteria (10). It is the most efficient osmoprotectant characterized to date (27). The transport and the accumulation of GB are triggered by raising the osmotic pressure of the external environment (17, 24, 20). The main results proved that the osmoprotective effect of GB, proline and trehalose in *Streptomyces* sp. and *Nocardiopsis* sp. was shown only at inhibitory salinities whereas at low osmolarity they had an inhibitory effect on the bacterial growth.

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