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Study of the Physicochemical Properties of Streptomyces Cell Surface and their Relationship with Soil Salinity of Origin

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ABSTRACT

Physicochemical properties of the bacterial surface are involved in several interfacial phenomena, such as microbial adhesion. Ecology Soil salinity is a crucial parameter for the distribution of *Streptomyces*. The objective of this study was to investigate the impact of NaCl on the hydrophobicity and electron donor/acceptor characteristics of the cell surface of fourteen *Streptomyces* strains isolated from soils of the Beni Amir region (Morocco) with different salinities. The physicochemical properties of the surface were evaluated using the MATS (microbial adhesion to solvents) method at two concentrations of NaCl (0.1 M and 1.2 M). The results obtained show a significant change from hydrophilic to hydrophobic character. In particular, the *Streptomyces lilaceus* A53 strain showed the lowest variation (4.21%). On the other hand, the *Streptomyces albogriseolus* A65 strain presented the greatest variation (86.15%). These changes were observed when the salt concentration increased significantly from 0.1 M to 1.2 M NaCl. The electron donor/acceptor character systematically decreases and even becomes null for the majority of strains. Furthermore, a strong correlation between cell surface hydrophobicity and salinity of the original soil was observed with MATS at 1.2 M NaCl. This study highlighted the crucial importance of the NaCl concentration in the modulation of the physicochemical properties of the surface of *Streptomyces bacteria*.

Keywords: *Streptomyces*, hydrophobicity, electrons donor/electrons acceptor properties, soil salinity, MATS method, NaCl concentration.

INTRODUCTION

Streptomyces, a significant genus within the actinomycetes, are Gram-positive bacteria renowned for their capacity to synthesize a diverse array of bioactive compounds. These include antibiotics, antitumor agents, and immunosuppressants (Procópio *et al*., 2012; Zabala *et al*., 2013; Bolourian *et al*., 2018). Their economic and biotechnological importance is immense, particularly in the pharmaceutical industry, where they are used to develop new drugs (Solanki et al., 2008). *Streptomyces* are widely distributed in various environments, including soils, composts and decomposing materials, where they play a crucial role in the degradation of organic matter (Buzón-Durán *et al*., 2020; Bhatti *et al*., 2017). These microorganisms have also demonstrated a remarkable ability to adapt to extreme environmental conditions (Sivalingam *et al.,* 2019*)*. They thrive in acidophilic, alkaliphilic, psychrophilic, thermophilic and xerophilic environments, and notably in saline environments (Yaradoddi *et al*., 2021). In natural saline environments, halophilic and halotolerant *Streptomyces*, represent a particularly promising area of research for the discovery of new bioactive substances (Abdelshafy *et al.,* 2018).

Like all bacteria, *Streptomyces* can be found in the state of biofilms and therefore play a crucial role that enables them to create interactions with the soil, influencing the decomposition of organic matter and the release of essential nutrients (Van Elsas *et al*., 2019 ; Costa *et al*., 2018). These biofilms, which adhere to soil particles, stabilize soil aggregates, improving its structure and water-holding capacity (Costa *et al*., 2018). Outside, biofilm formation begins with the adhesion stage, an essential process that initiates surface colonization (Song *et al*., 2015). This adhesion is governed by the physicochemical properties of the cell surface, which determine their ability to attach to abiotic supports. Electrostatic interactions, Van der Waals forces and Lewis acid-base interactions are the physicochemical forces that play a crucial role in this process (Bellon-Fontaine *et al.,* 1996; Briandet *et al.,* 1999; Krepsky *et al.,* 2003, *Pagedar* et al.*,* 2010; Xu *et al.,* 2010). These cell surface properties can be influenced by various environmental factors, such as salinity, pH, ionic strength, medium composition, and temperature (Zahir *et al*., 2016; El Othmany *et al*., 2021; Assaidi *et al*., 2018; Elgoulli *et al*., 2021; Hamadi *et al*., 2011). In addition, studies have examined the physicochemical characteristics of the surface of actinomycetes and the effect of culture medium composition on these physicochemical parameters (Zanane *et al*., 2023; El Othmany *et al*., 2021; Zahir *et al*., 2016; Maataoui *et al*., 2014).

The physicochemical characteristics of *Streptomyces* and the specific environmental conditions of the soil, determine their ability to adhere and form stable biofilms, also facilitating their beneficial interactions with the soil ecosystem. However, little in-depth research has been carried out on the physicochemical characteristics of *Streptomyces* and especially on the relationship between these characteristics and their ecology, in terms of interactions as a function of salinity. For this reason, the authors believe that it would be extremely beneficial to determine how salinity influences the surface properties of these bacteria in order to better understand their ability to form biofilms and interact with their environment. This could open up new avenues for the discovery of bioactive substances and the improvement of agricultural practices through better management of soil quality. In this work, the influence of two NaCl concentrations, 0.1 M and 1.2 M, on the hydrophobicity and electron donor/acceptor characteristics of the cell surface of fourteen *Streptomyces* strains isolated from soil in the Beni Amir region of Morocco was investigated. These strains were selected for their tolerance to high concentrations of NaCl, assessed by the MATS (Microbial Adhesion to Solvents) method.

MATERIALS AND METHODS

Bacterial strains and salt tolerance

The 80 actinomycetes bacteria utilized in this research were isolated from soils of the Beni Amir region (Morocco) with different salinity levels and identified (Zanane *et al*., 2018a; Zanane *et al*., 2018b). These strains were purified and inoculated into Petri dishes containing the BENNET medium, with NaCl concentrations of 0, 50 and 70 g/L. The dishes were then incubated at 28 °C for 21 days, and the development of the strains in the different media was observed.

Physicochemical surface proprieties

Choice of strains and concentrations

After testing NaCl tolerance at different concentrations, only 14 *Streptomyces* out of the 80 isolated actinomycete strains were selected for their ability to withstand a high concentration of NaCl, i.e. 70 g/L. Thus, the choice of the two concentrations of NaCl is based on the results of the sodium chloride tolerance test. The 0.1 M concentration corresponds to the optimal concentration for growth, while the 1.2 M concentration represents the maximum tolerance of NaCl. The fourteen selected strains will be the subject of a physicochemical surface characterization study.

Growth conditions

The strains were cultivated on BENNET medium, composed of 10 g D-glucose, 1 g yeast extract, 1 g meat extract, 2 g peptone, 15 g agar, and 1 liter of distilled water. The pH of the medium was approximately adjusted to 7.5. After incubation at 28 °C for 21 days, spores were collected by scraping the surface of sporulated cultures in liquid BENNET medium supplemented with two different concentrations of NaCl (0.1 M or 1.2 M) and incubated under agitation for 7 days at 28 °C.

Liquid cultures of *Streptomyces* were washed with 0.1 M and 1.2 M NaCl twice by centrifugation (8600 g for 15 min). The suspension was then subjected to a Stomacher laboratory homogenizer for 20 min to ensure dispersion of the *Streptomyces* mass and prevent aggregation. The concentration of each bacterial suspension was adjusted by measuring the optical density (OD) at 405 nm between 0.7 and 0.8 corresponding to 10^8 CFU/mL with an ELISA spectrophotometer (Multiskan EX, Labsystems).

Microbial adhesion to solvents

Each bacterial suspension (2.4 mL) was agitated using a vortex for 90 seconds alongside 0.4 mL of solvent. Afterwards, the mixture was left undisturbed for 15 min to ensure thorough phase separation. Subsequently, the optical density of the aqueous phase was determined using a spectrophotometer. Finally, the percentage of cells bound to each solvent, representing percent adhesion, was computed using the provided equation.

$$\%\,Adhesion = \left(1 - \frac{A}{A_0}\right) \times 100 \qquad (1)$$

where: A_0 – represents the absorbance measured at 405 nm for the bacterial suspension before mixing, and A is the absorbance after mixing.

Measurement of electrical conductivity

Twenty grams (20 g) of soil sieved through a 2 mm sieve was suspended in 100 mL of distilled water. After stirring for an hour on a rotary shaker, followed by half an hour rest, the suspension was decanted into a beaker and the conductivity measured using a conductivity meter.

Statistical analysis

The data underwent statistical analysis employing one-way analysis of variance (ANOVA) using SPSS (Statistical Program for Social Sciences) version 20.0 for Windows. Each analysis was conducted in triplicate (n = 3), and the results were presented as means with standard deviation (SD), allowing for comparison of mean values across different strains.

RESULTS

Salt tolerance of actinomycetes

Table 1 shows the results of the growth test for actinomycetes strains on Bennet medium at different NaCl concentrations. On the basis of the results obtained, divided the actinomycetes strains isolated from Beni Amir soil were divided into 2 groups. The first group is made up of 61 strains that grew on the medium containing a concentration of 50 g/L NaCl, corresponding to 76.25% of the actinomycetes studied. The second group consisted of 14 strains capable of growing on the BENNET medium with a NaCl concentration of 70 g/L, representing 17.5% of all the actinomycetes. These results suggest that these actinomycetes strains are halotolerant actinomycetes, capable of adapting to high concentrations of NaCl.

Physicochemical surface proprieties

As it was previously described in the materials and methods section, the cultivation and washing of *Streptomyces* strains in the presence of two concentrations of NaCl aimed to determine the influence of high NaCl concentration on the adhesive behavior of *Streptomyces* by the MATS method. In particular the hydrophobicity and the electron donor/acceptor characteristics, while approaching the natural conditions of their environment of origin.

Effect of salt on hydrophobicity

The surface hydrophobicity of microbial cells plays a crucial role in the adhesion phenomenon (Oliveira *et al*., 2001; Krasowsk *et al*., 2014). This hydrophobic or hydrophilic character is determined by the percentage of cells that adhere to hexadecane (or hexane). When this percentage exceeds 50%, the surface is considered relatively hydrophobic; otherwise, it is deemed relatively hydrophilic (Bellon-Fontaine *et al*., 1996). Figure 1 illustrates the percentage of *Streptomyces* cells adhering to hexadecane.

At the optimal concentration of 0.1 M NaCl, it appears that strain *S. rochei* A58 (53.1%) has a percentage of adhesion to hexadecane greater than 50% and thus exhibits hydrophobic character. Strains *S. lilaceus* A30, *S. bellus* A44, *S. azereus* A50 have an affinity for hexadecane of 48%, 47% and 46% respectively, showing that they are moderately hydrophilic, while the remaining strains *S.griseorubens* A79 (43%), *S.albogriseolus* A76 (41.8%), *S.griseorubens* A64 (40.5%), *S.griseorubens* A63 (39.6%), *S. bellus* A49 (36.6%), *S. bellus* A46 (33%), *S.albogriseolus* A65 (31.1%), *S.labedae* A60 (27.8%) and *S. albogriseolus* A57 (20.3%) are strongly hydrophilic (low affinity for hexadecane). Strain *S. lilaceus* A53 (4.21%) has the lowest affinity for hexadecane is therefore the most hydrophilic.

For the high concentration of NaCl (1.2 M), the affinity of *Streptomyces* strains for hexadecane (thus hydrophobicity) increases for all strains, except *S.labedae* A60 and *S.griseorubens* A63. Strains *S.albogriseolus* A65, *S.albogriseolus* A76, *S.griseorubens* A64, *S. azereus* A50, *S.griseorubens* A79, *S. bellus* A46, *S. bellus* A44, *S. bellus* A49,

Table 1. Growth test for actinomycetes strains on BENNET medium at different NaCl concentrations

Actinomycete isolates	NaCl concentration			Actinomycete isolates	NaCl concentration		
	0 g/L	50 g/L	70 g/L		0 g/L	50 g/L	70 g/L
A1	+++	-	-	A41	+++	+	-
A2	+++	-	-	A42	+++	-	-
A3	+++	-	-	A43	+++	--	-
A4	+++	+	-	A44	+++	+++	+++
A5	+++	-	-	A45	+++	+	-
A6	+++	-	-	A46	+++	+	+
A7	+++	++	-	A47	+++	+	-
A8	+++	++	-	A48	+++	+++	-
A9	+++	++	-	A49	+++	++	++
A10	+++	++	-	A50	+++	-	+
A11	+++	++	-	A51	+++	-	-
A12	+++	++	-	A52	+++	++	-
A13	+++	++	-	A53	+++	+++	++
A14	+++	-	-	A54	+++	-	-
A15	+++	+	-	A55	+++	+++	-
A16	+++	++	-	A56	+++	+	-
A17	+++	++	-	A57	+++	+	+
A18	+++	++	-	A58	+++	+++	++
A19	+++	++	-	A59	+++	+	-
A20	+++	++	-	A60	+++	+++	+++
A21	+++	++	-	A61	+++	-	-
A22	+++	++	-	A62	+++	+	-
A23	+++	+	-	A63	+++	++	++
A24	+++	++	-	A64	+++	+	+
A25	+++	+	-	A65	+++	+++	+++
A26	+++	++	-	A766	+++	+	-
A27	+++	++	-	A67	+++	-	-
A28	+++	++	-	A68	+++	+	-
A29	+++	++	-	A69	+++	-	-
A30	+++	+++	++	A70	+++	+	-
A31	+++	++	-	A71	+++	-	-
A32	+++	++	-	A72	+++	+	-
A33	+++	+++	-	A73	+++	-	-
A34	+++	+	-	A74	+++	+	-
A35	+++	++	-	A75	+++	+	-
A36	+++	+++	-	A76	+++	+++	++
A37	+++	+	-	A77	+++	-	-
A38	+++	+	-	A78	+++	-	-
A39	+++	-	-	A79	+++	+++	+++
A40	+++	++	-	A80	+++	+	-

Note: (-) No growth, (+) slow growth, (++) moderate growth (+++) abundant growth

S. rochei A58, and *S. lilaceus* A30 have an affinity for hexadecane greater than 50% (86%, 83%, 83%, 82%, 81%, 81%, 78%, 76%, 71%, and 63%, respectively), so they are highly hydrophobic. Meanwhile, strains A57 (36.5%), A53 (25.2%), A63 (24.7%) and A60 (0%) are hydrophilic since their percentage of binding to hexadecane does not exceed 50%. It can therefore be concluded that the hydrophobicity increases significantly when the concentration of sodium chloride increases from 0.1 M to 1.2 M NaCl.

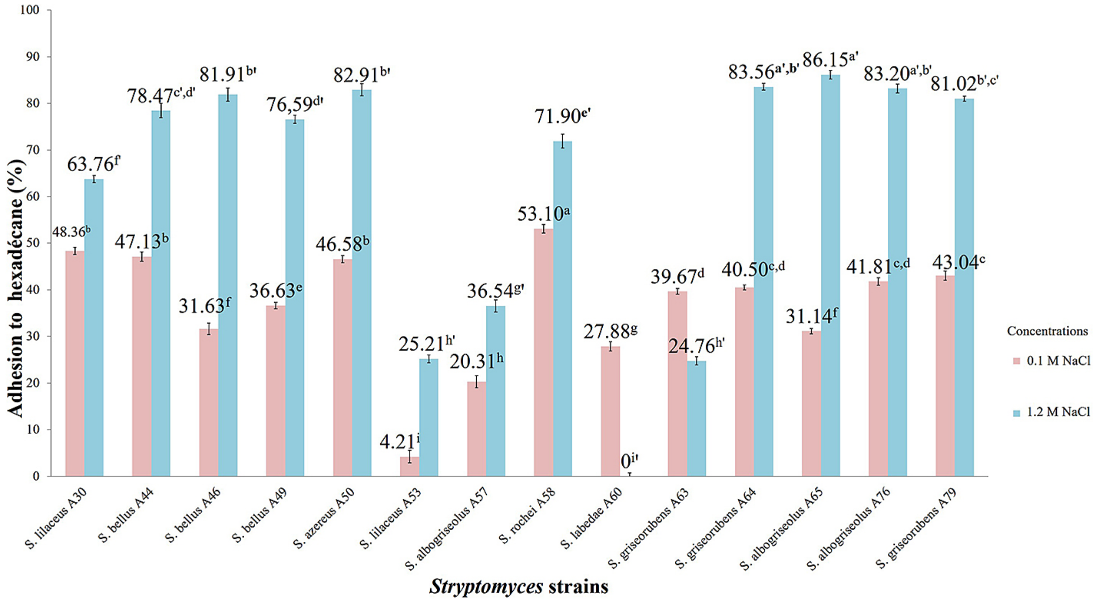

Figure 1. Hydrophobic character of the 14 strains of Streptomyces to hexadecane at two concentrations: 0.1 M and 1.2 M of NaCl. The letters a, b, c, d, e, f, g,h,i used to indicate statistical groups at the concentration 0.1 M of of NaCl and the letters a', b', c',d',e',f',g',h', i' used to indicate statistical groups at the concentration 1.2 M of NaCl. Data were presented as mean ± standard deviation (SD)

Effect of salt on the electron donor/acceptor character of the surfaces of *Streptomyces* strains

Electron donor character

The electron donor character is assessed by determining the disparity in cellular affinity between chloroform and hexadecane (Bellon-Fontaine *et al*., 1996). The results of the cell surface electron donor properties of *Streptomyces* strains are shown in Figure 2.

At the concentration of 0.1 M NaCl, the results reflect a well-expressed electron donating character for all strains, except for *S. albogriseolus*

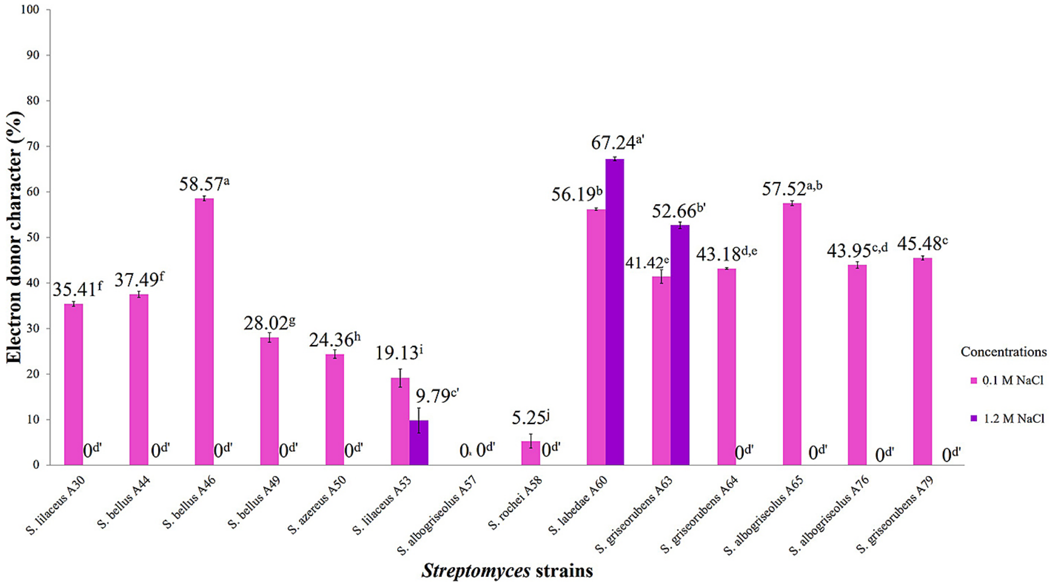

Figure 2. Electron donor character of the 14 strains of Streptomyces at two concentrations: 0.1 M and 1.2 M of NaCl. The letters a, b, c, d, e, f, g ,h, I, j, k, used to indicate statistical groups at the concentration 0.1 M of of NaCl and the letters a', b', c', d', used to indicate statistical groups at the concentration 1.2 M of NaCl. Data were presented as mean ± standard deviation (SD)

A57, which shows a null electron donating character. The results show that the electron donating character of strains *S. labedae* A60 (67.2%) and *S. griseorubens* A63 (52.7%) increases with salinity, while it decreases for strain *S. lilaceus* A53 (9.79%) or becomes null for the other strains.

Electron acceptor character

The electron acceptor character of a microbial cell is estimated by calculating the difference between its affinity to diethyl ether and to hexane. According to Bellon-Fontaine *et al.,* (1996), only positive values indicate an electron acceptor character. The results of the cell surface electron acceptor properties of *Streptomyces* strains are shown in Figure 3.

The electron acceptor character is important for the eight strains *S. bellus* A46 (36.6%), *S. bellus* A49 (31.6%), *S. azereus* A50 (27.9%), *S. labedae* A60 (18.1%), S63 (29.6%), *S. griseorubens* A64 (38.2%), *S. albogriseolus* A76 (25.5%) and *S. griseorubens* A79 (42.4%). For the rest of the studied strains *S. lilaceus* A30, *S. bellus* A44, *S. lilaceus* A53, *S. albogriseolus* A57, *S. rochei* A58 and *S. albogriseolus* A65, their electron acceptor character is null. By increasing the concentration of NaCl to 1.2M, the electron acceptor character decreases for *S. griseorubens* A64 (8.04%) and *S. griseorubens* A79 (7.48%)) or becomes null, except for the two strains *S. lilaceus* A53 (20.6%) and *S. rochei* A58 (57.1%) for which this character increases (Figure 3). It can be concluded that by increasing the concentration of NaCl to 1.2 M, the electron acceptor character becomes null for most of the *Streptomyces* studied.

Soil electrical conductivity

Table 2 shows the electrical conductivities of the soils from which the strains were isolated. The results presented enable to classify soils S7; S9; S11 with electrical conductivities between 2.02 and 2.27 dS/m as highly saline, while the other soils S1; S2; S3; S4; S8 are considered saline (Richards, 1969).

DISCUSSION

Streptomyces are recognized as among the most economically and biotechnologically valuable prokaryotes (Dharmaraj, 2010). Conversely, significant metabolic diversity and biotechnological potential have been uncovered in halophilic and halotolerant microorganisms. Both these groups present valuable resources ripe for exploitation in ecological applications.

In the conducted study, this result is in line with that found by (Cai *et al*., 2009) who showed that 10 strains could grow on 70 g of NaCl/L

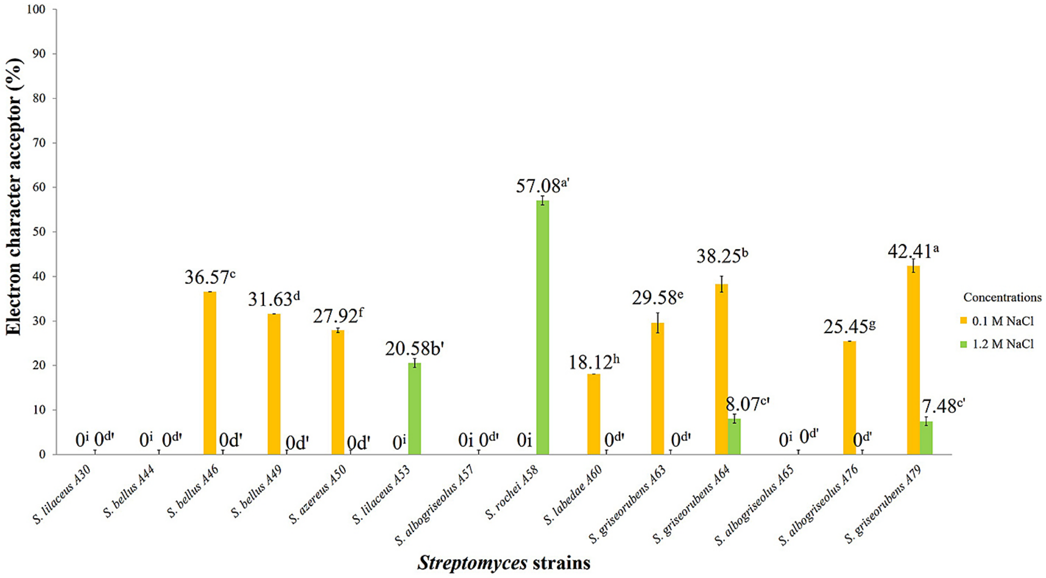

Figure 3. Electron acceptor character of the 14 strains of Streptomyces at two concentrations: 0.1 M and 1.2 M of NaCl. The letters a, b, c, d, e, f, g, h, i used to indicate statistical groups at the concentration 0.1 M of of NaCl and the letters a', b', c', d' used to indicate statistical groups at the concentration 1.2 M of NaCl. Data were presented as mean ± standard deviation (SD)

Table 2. Electrical conductivity of Beni Ami soil samples

Strains	Soils	Electrical conductivity (ds/m)
S. lilaceus A30	SOL8	1.521
S. bellus A44	SOL4	1.464
S. bellus A46	SOL3	1.29
S. bellus A49	SOL2	1.25
S. azereus A50	SOL1	1.225
S. lilaceus A53	SOL7	2.3
S. albogriseolus A57	SOL11	2.029
S. rochei A58	SOL 8	1.52
S. labedae A60	SOL9	2.23
S. griseorubens A63	SOL7	2.3
S. griseorubens A64	SOL3	1.29
S. albogriseolus A65	SOL2	1.25
S. albogriseolus A76	SOL8	1.52
S. griseorubens A79	SOL3	1.29

medium. The obtained results showed salinity tolerance and adaptation to adverse conditions, in line with studies on *Bacillus okuhidensis* and *Bacillus halodurans* (Li *et al*., 2002).

The studies conducted by Petrovicˇ *et al*., (2002) and Kis-Papo *et al*., (2003) reported that varieties of fungi were isolated from the Dead Sea growing in a medium at (340 g/L NaCl). To adapt to these conditions, halophiles generally accumulate high concentrations of dissolved substances or osmolytes (Roberts, 2005) and cryoprotectants in their cytoplasm. The osmolytes that accumulate in the halophilic cell are generally amino acids and polyols such as betaine, glycine, acetoin, trehalose and glycerol, which do not interfere with the metabolic process and have no charge except for their effect on pH (Sarma, 2001, Kis-Papo *et al*., 2003). The bacterial strains tested display halotolerance, possessing the endogenous capacity to balance their turgor pressure and resist osmotic stress without any external osmoprotection. Therefore, they carry the genetic information and enzymatic equipment required for osmotolerance (Kerbab, 2018). Halophilic actinomycetes can also serve as useful models for the production of metabolites and enzymes essential for stress response (Abdelshafy *et al*., 2018). Salle (1948) observed that small amounts of metal ions or salts promote the growth of microorganisms, while higher concentrations have an inhibitory effect. Furthermore, Tresner *et al*., (1968) reported that *Streptomyces* bacteria can tolerate NaCl levels ranging from 4% to 13%.

The literature reports a multitude of studies on the physicochemical characteristics of bacterial surfaces (Zanane *et al*., 2023; Elgoulli *et al*., 2021; Koubali *et al*., 2021; Elfazazi *et al*., 2021; El othmany *et al*., 2021; Maataoui *et al*., 2014). Several studies have shown the influence of salinity on these characteristics (Naïtali *et al*., 2009; Boutaleb *et al*., 2008; Bereksi *et al*., 2002). The majority of studies on actinomycetes have reported biotechnology, metabolism and tolerance to salts. However. few studies have been carried out on their physicochemical properties (Zahir *et al*., 2016). In this sense, a first characterization of *Streptomyces* strains by the MATS method is being published. The present work aimed to study the effect of NaCl on the physicochemical properties of the surface of *Streptomyces* by the MATS method.

Several works have investigated the effect of environmental factors such as temperature, media composition, ionic strength, pH and salinity on hydrophobicity and charge. The influence of these factors on surface properties can lead to changes in wall composition and can also be interpreted in terms of Lifshitz-van der Waals (LW) and acid-base (AB) forces. (Beck *et al.,* 1988; Latrache *et al.,* 1994; Latrache*,* 2001; Gallardo-Moreno *et al.,* 2002; Habimana *et al.,* 2014; Zahir *et al.,* 2016).

The hydrophobicity of the cell surface plays a critical role in adhesion because hydrophobic interactions are typically enhanced when one or both of the surfaces involved are nonpolar in nature, such as the microbial cell surface and the substrate surface (Donlan, 2002).-

The results of the present study showed that all the *Streptomyces* strains studied showed a hydrophilic character at the optimal concentration (0.1 M). Increasing the concentration of NaCl strongly influenced this character, by changing the hydrophilic character to hydrophobic character for the majority of strains. This suggests that external factors, such as the concentration of NaCl in the culture medium of these strains, could induce alterations in the surface physicochemical characters.

Zahir *et al*., (2016) proposed that *Streptomyces*, when adapting to salt stress, form an intermediate microenvironment between the bacterial surface and the external surroundings. This microenvironment consists of a water film, sufficiently thick to shield the cell from osmotic pressure, with its thickness increasing in correlation with salt concentration. Furthermore, it is possible that salt diffuses into this microenvironment, generating a concentration gradient, thereby modulating the immediate vicinity of the cell surface.

These results are in agreement with Zahir *et al*., (2016) who measured the contact angle and reported that the hydrophobicity of actinomycetes strains isolated from soil increases by increasing the concentration of NaCl. In addition, increasing KNO_3 concentration for *Staphylococcus aureus* and *Escherichia coli* (Hamadi *et al*., 2004) and NaCl concentration *Listeria monocytogenes* and *Pseudomonas fluorescens* (Bereksi *et al*., 2002; Abu Quba *et al*., 2023) directly influences the bacterial surface parameters. In the same sense, the hydrophobicity of the bacterial surface of *Campylobacter jejuni*, according to DyKes (2003), changes with the change of NaCl concentration. For example, Xue *et al*., (2010) demonstrated that *S. aureus*, *L. monocitogenes*, and *S. typhimirium* exhibited increased hydrophobicity when exposed to elevated concentrations of NaCl. Bacterial hydrophobicity can be modified by changes in fatty acid (phospholipid) and protein composition that occur in response to salt stress conditions (Yasuhiro *et al*., 1972).

Indeed, from the results above, it can be inferred that the increase in hydrophobicity of the *Streptomyces* cell surface in the presence of high NaCl concentration could be due to the effect of NaCl on the change in membrane composition.

Streptomyces have a unique life cycle marked by the passage of cells from a vegetative state to an aerial state by forming aerial hyphae. These are characterized by the presence of rodelets composed of two classes of proteins: chaplins and rodlines (Claessen *et al*., 2004). These chaplins are at the origin of the hydrophobic character of the hyphae due to the orientation of their hydrophobic part towards the outside of the cell (Elliot MA *et al*., 2003).Thus, the change in hydrophobic could be due to a change in surface composition quantity of chaplin proteins or in their nature.

The relationship between *Streptomyces* hydrophobicity and soil salinity could be complex and dependent on the specific environmental context. *Streptomyces*, an important group of actinomycetes widely distributed in soils, are known for their ability to produce a wide variety of secondary metabolites, some of which may be hydrophobic. Under the conditions of environmental stress, such as high salinity, *Streptomyces* can develop defense mechanisms, including the production of hydrophobic compounds, to protect their cells from desiccation and water loss. Consequently, in some cases, a positive correlation between *Streptomyces* hydrophobicity and soil salinity is observed, with the most salinity-resistant strains possibly being those that also exhibit increased hydrophobicity. However, it should be noted that this relationship could be influenced by a multitude of factors, such as soil chemistry, nutrient availability and the presence of other microorganisms.

To study the relationship between the surface physicochemical properties of *Streptomyces* and environmental salinity, the correlation between the hydrophobicity behavior and salinity of *Streptomyces* isolation soils was evaluated. The results showed a strong linear correlation $R^2 = 0.9037$ between hydrophobicity and soil salinity at high ionic strength (1.2 M of NaCl) Figure 4.

This suggests that *Streptomyces* change their cell surface composition to become more hydrophobic in response to an intensely saline environment. This change could be explained by an increase in the abundance of lipids or other hydrophobic molecules on the cell surface. Increased hydrophobicity may help *Streptomyces* to protect their internal cellular components from osmotic stress and other adverse effects of a saline environment. However, the correlation of these two parameters seems less pronounced ($R^2 = 0.3361$) at low ionic strength (0.1 M of NaCl), Figure 5. This suggests that factors other than salinity influence the surface hydrophobicity of *Streptomyces* at lower salt concentrations. These factors could include soil composition, temperature, nutrient availability, and the presence of other microorganisms. At lower salinities, the effect of these other factors may be greater than the effect of salinity on hydrophobicity.

According to the results obtained, the electron donor character becomes zero for the majority of the strains by increasing the NaCl concentration. The increase of the salt concentration probably induces a decrease of the electrostatic charge of the bacterial surface. The reduction in electrostatic charge may be attributed to the considerable adsorption of cations, leading to the neutralization of charged groups present on the cell surface (Van der Wal *et al*., 1997; Hamadi *et al*., 2004; Gaboriaud *et al*., 2006).

Hamadi *et al*., (2008) studied a range of Gram-negative and Gram-positive bacterial strains and found much lower acceptor values at 0.1 M. However, in this study, strains that expressed an acceptor character at 0.1 M NaCl showed a significant decrease in this character at 1.2 M NaCl.

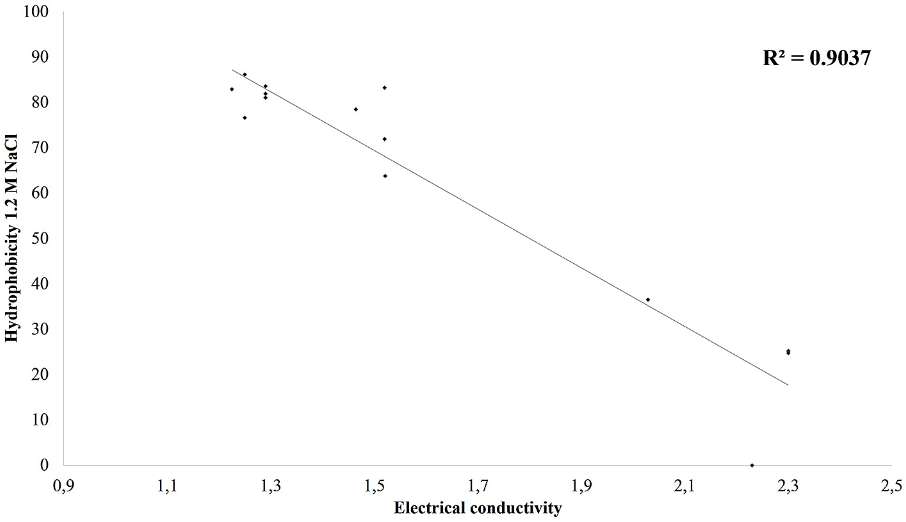

Figure 4. Correlation between hydrophobicity of Streptomyces strains and the soil salinity ionic strength 1.2 M of NaCl

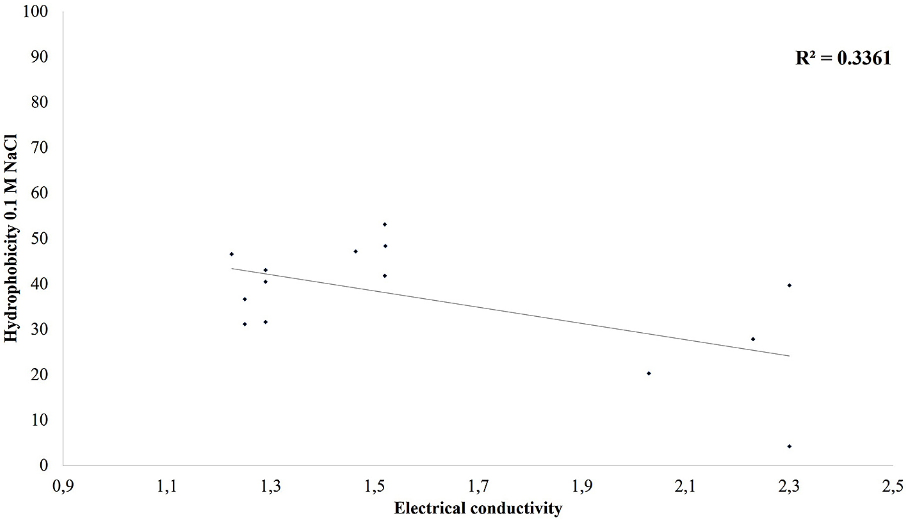

Figure 5. Correlation between hydrophobicity of Streptomyces strains and the soil salinity ionic strength 0.1 M of NaCl

For each character, a decrease was obtained with increasing NaCl concentration, except for two strains, which were not the same for the two characters. Several mechanisms can explain the observed changes in the physicochemical properties of *Streptomyces* in response to salinity. Adaptation to osmotic stress can lead to changes in cell wall composition, including alterations in peptidoglycan structure and the production of osmoprotectants (Bremer and Krämer, 2019). These modifications can affect the hydrophobicity and electron donor/acceptor properties of the cell surface, thereby enhancing adhesion and biofilm formation. In addition, salinity can affect membrane fluidity by altering the lipid composition of the cell membrane (De Carvalho *et al.*,2010), which can influence surface interactions and the ability of the cell to form stable biofilms. The expression of surface proteins, such as extracellular polysaccharides (EPS) (Han *et al.,* 2021)*,* can also be increased under high salinity, strengthening the biofilm matrix and increasing surface hydrophobicity.

The physicochemical characteristics of *Streptomyces* and their responses to salinity are of vital importance in improving ecological restoration strategies. These microorganisms are key players in ecosystems, supporting critical processes such as the decomposition of organic matter, nitrogen fixation and the degradation of pollutants (Beroigui, O., Errachidi, F. 2023). A better understanding of their interactions with salinity conditions can not only enhance microbial biodiversity, ensuring the resilience of ecosystems to environmental perturbations, but also serve to monitor ecosystem health as valuable bioindicators. In addition, this knowledge can guide the development of suitable biofertilizers and biopesticides, promoting sustainable agricultural practices. In biotechnology, the characterization of the surface properties of Streptomyces can optimize bioprocesses, improve microbial engineering for the efficient production of high-value bioproducts, as well as support the design of advanced biocatalysts and biosensors for innovative environmental and industrial applications (Zerva *et al*., 2019). This integrated approach promises significant advances in the sustainable management of ecosystems and in the industrial exploitation of microbial capacities.

CONCLUSIONS

In this work, the direct effect of high NaCl concentration on the surface physicochemical properties of *Streptomyces* was examined for the first time. The physicochemical properties of the cell surface differ from strain to strain, which must be taken into account in their adhesive behavior. Moreover, the effect of increasing the concentration of NaCl from 0.1 M to 1.2 M on these properties is very marked. The hydrophilic character becomes hydrophobic, and the electron donor and acceptor characters become null for the majority of the *Streptomyces* strains. The relationship between salinity and the physicochemical properties of the *Streptomyces* cell surface could serve as a basis for investigating the interactions of these bacteria with different surfaces.

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