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Effect of salinity on the adhesive power actinomycetes in soil

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Abstract

Actinomycetes remain one of the leading sources of microbial-derived natural products. Able to colonize various ecological niches including the most extreme, they are, increasingly, concerned research materials of microbial physiology from adverse circumstance. In this work, actinomycetes were isolated from soils of the region Beni Amir, Morocco, where the salinity is, relatively, high degrees. Glycerol Bacto Agar medium (GBA) and Bennet medium were used for the isolation and growth of actinomycetes. To promote the activities of actinomycetes, by predicting biofilm formation, their characters surface and adhesion on soil was studied. The adhesive behaviour estimation is based on sedimentation of the bacteria adsorbed to substrata power and expressed as the percentage of cells adhered to substrata. Physico-chemical characters of surface cells were estimated by measurement of the water and different solvents contact angle on a lawn of cells. The percentage of cell adhered on soil increase by adding salt in growth medium or in bacterial suspension. Physicochemical characterization of our strains revealed a hydrophilic and electron donor character. Nevertheless, cell surface hydrophobicity increases with salinity, while electron donor character seems not be affected by salt.

Keywords: Actinomycetes; Sol; Salinity; Bioadhesion; Cell surface physicochemical properties; Contact angle measurement.

Introduction

The majority of microorganisms in natural ecosystems are attached to solid supports [1].Indeed, adhesion of bacteria to surfaces, earliest stages of biofilm formation, is a general phenomenon in natural environments with important ecological implications [2]. This state usually induced physiological changes in microorganisms, making them more resistant, and enabling them to ensure their multiplication in hostile or stressful environments [3]. So, biofilm are often more resistant to adverse environmental conditions, such as desiccation [4] and extreme temperatures [5], than planktonic (free-living) cells.

Adhesion is mediated by physicochemical interactions (electrostatic, van der waals, and acid-base) between the substrate and the surface of microorganisms. These interactions, depends on the physicochemical properties such as electrostatic charges, hydrophobicity and electron donor/ electron acceptor [6-12].Surface functional groups' compositions are implicated [6-10, 13, 14]. Irreversible adhesion to surfaces is often associated with the expression of extracellular material and the formation of biofilms [15, 16]. The degree of irreversible binding ofmay be related to the degree of cell surface hydrophobicity [17].

Cell surface polymers such as proteins and components of certain gram-positive bacteria (mycolic acids) appear to dominate attachment to hydrophobic substrata [18].

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Environmental conditions influence, also, adhesive bio behavior [2, 19, 20].Actinomycetes, important part of the telluric microflora [21], are micro-organisms that show important
characteristics of both fungi and prokaryotes such as bacteria [22]. Made of their abilities to degrade xenobiotic, environmental pollutants, or otherwise slowly biodegradable natural polymers as well as to transform or synthesize possibly useful compounds, they are the most economically and biotechnologically valuable prokaryotes [23, 24].
They are helpful in biological buffering of soils; they contribute to its fertility by nitrogen fixation [25], solubilizing phosphate [26, 27], decomposing organic and recalcitrant compounds, especially cellulose and chitin as a food source, they, also, break down bark, paper, and plant stems, they have an important role in composting conducive to crop production [28, 29]. They will provide a valuable resource for bio-active metabolites that have been commercialized for agricultural uses [30],thus, study of actinomycetes and identification of their metabolic properties are most important tasks in biotechnology [31].
The formation and stability of the biofilm of actinomycetes in soil would have practical consequences on the physiology of these bacteria and, therefore, their agronomic and environmental activities. Biofilm actinomycetes can, also, be used to ensure the protection of the environment, such as cleaners of nature and humus producers, decomposition of the most recalcitrant substances namely cellulose and lignin.
The study of the adhesive behavior of actinomycetes on soil is a preliminary work to understand their biofilms ecology; which will allow promoting the benefic activities of these bacteria in soil.Limited data concerning this subject have been published previously. Hence, this work is an initiative study of the physico-chemical properties of actinomycetes surfaces, and the effect of various degrees of salinity on their adhesive behavior on soil.

2. **Experimental**

2.1. Isolation and culture of bacteria:

The actinomycete strain is isolated from soils of the region Beni Amir–Morocco where the salinity is, relatively, high degrees.
Samples (2g, wet weight) were diluted 10 times in sterile physiological water (NaCl, 9g/l), homogenised by vortexing and sonicated for 10-15min according to Ouhdouch et al. [32].
Isolation was carried out onGlycerol Bacto Agar medium (GBA)[33] (20g/l glycerol, 20g/l amidon, 10g/l peptone, 5g/l Extrait de viande, 3g/l $CaCO_3$, 15g/l agar,pH 7. Plates were incubated at 25 °C for 21 days. Actinomycetes were recognized on the basis of their characteristic morphology of colony. Air mycelium filaments, observed under light microscopy, confirm the diagnosis. All observed colonies were isolated, purified and conserved.
Actinomycete, often, congregate during the liquid culture,this makes them difficult to manipulate in this work. One strain, that has a homogeneous liquid culture, is selected for further manipulation.

2.2.Preparation of microbial suspension

The bacterium is passaged on 30 ml of liquid medium Bennett [33] (1g/l Meatextract,2g/l Yeast extract, 2g/l Peptone10g/l Glucose 1.0%, pH7.2), incubated for three days at 25 °C. To remove the residues of culture medium, the cells are washed by a series of three centrifugation steps (15 min at 8400g) and placed in suspension in $KNO_3 10^{-1}$M.

2.3.Contact angle measurements and estimation of bacterial surface tension components:

Measurements were performed on a cell using the sessile drop technique according to the method described by Busscher et al. [34]. Briefly, bacteria were deposited on membrane filters (0.45 µm Sartorius) after filtration by means of negative pressure. The filters were left during about 30 min to air dry at room temperature. Contact angle were measured with a goniometer (GBX instruments, France). According to the approach of Good, van Oss and Chaudhury (acid–base theory) [35], the surface energy components of a surface (γ_S^+, γ_S^- and γ_S^{LW}) were determined by performing contact angle measurements using three probes liquids (one apolar and two polar) withknown surface tension parameters (γ_L^+, γ_L^- and γ_L^{LW}) and employing Young's Eq :

$$\cos\theta = -1 + 2(\gamma_S^{LW}\gamma_L^{LW})^{1/2}/\gamma_L + 2(\gamma_S^{+}\gamma_L^{-})^{1/2}/\gamma_L + 2(\gamma_S^{-}\gamma_L^{+})^{1/2}/\gamma_L$$

Where θ is the measured contact angle, γ^{LW} is the van der Waals free energy component, γ^{+} is the electron acceptor component, γ^{-} is the electron donor component and the subscripts (S) and (L) denote solid surface andliquid phases respectively.The surface free energy is expressed as:

$\gamma_S=\gamma_s^{LW}+\gamma_s^{AB}$ where$\gamma_s^{AB}=2(\gamma_S^{+}\gamma_S^{-})^{1/2}$is the acid–base free energy component.

Three liquids with different polarities were used: water, formamide, and diiodomethane (Table1).

Table 1: Energy characteristics (mj m^{-2}) of pure liquid used to measure contact angles [12].

Liquids	γ^{tot}	γ^{LW}	$\gamma+$	$\gamma-$
Water	72,8	21,8	25,5	25,5
Formamide	58,0	39,0	2,3	39,6
Diiodomethane	50,8	50,8	0	0

Where$\gamma^{tot}$is surface total energy, γ^{LW} is the van der Waals free energy component, γ^{+} is the electron acceptor component, $\gamma^{-}$is the electron donor component.
Experiments were carried out in triplicate with separately cultured bacteria.

2.4. Adhesion on soil:
Substrates selected for this study is soil ground and sieved. Sterile soil is suspended in sterile saline at 20%.This technique is inspired from the method of joining cellulose [36].The adhesive behavior estimation is based on sedimentation of the bacteria adsorbed to substrata power (soil) and expressed as the percentage of cells adhered to the soil.
1 ml of bacterial suspension, 1ml of KNO_3 $10^{-1}$and 1ml of sterile soil suspension is placed in a test tube, vortexed for 20 seconds and allowed to settle for 1 hour, and the optical density DO of the aqueous phase is again measured at 405 nm (DO_f), we prepare, in the same way, another tube without soil suspension (DO_i).The percentage of cells having adhered to the soil is given by the relation:
% of adhesion = 1- (DO_f /DO_i) × 100

2.5.Effect of soil salinity on the adhesion of actinomycetes to the soil:
Study of adhesion is achieved in the same manner as previously, adding 0,5MNaCl(Sodium Chloride: Analytical Quality), directly, into the culture medium of the strain, or indirectly, to the tube containing bacterial suspension.

3. Results and discussion

3.1. *Effect of salinity on actinomycetes hydrophobicity*
Microbial cell surface hydrophobicity is recognized as one of the determinant factors in microbial adhesion to surface [37, 38]. Figure 1 shows the effect of different salt levels on cell surface hydrophobicity.
Actinomycete was very hydrophilic at 0M and relatively hydrophobic at 0,5M. Our results were in accordance with works which reported that cell surface hydrophobicity increase with ionic strength[6] reported that increase in ionic strength causes a sharp increase of hydrophobicity of*S.aureus*.

3.2. *Effectof NaCl on acidebase character of actinomecytes*
Additionally, intervention of the electrondonor / electron acceptor character may be important in the explanation of the phenomenon of adhesion [35]. Figure 2 shows the evolution of this character with salinity. We observed that no significant variation with salinity.

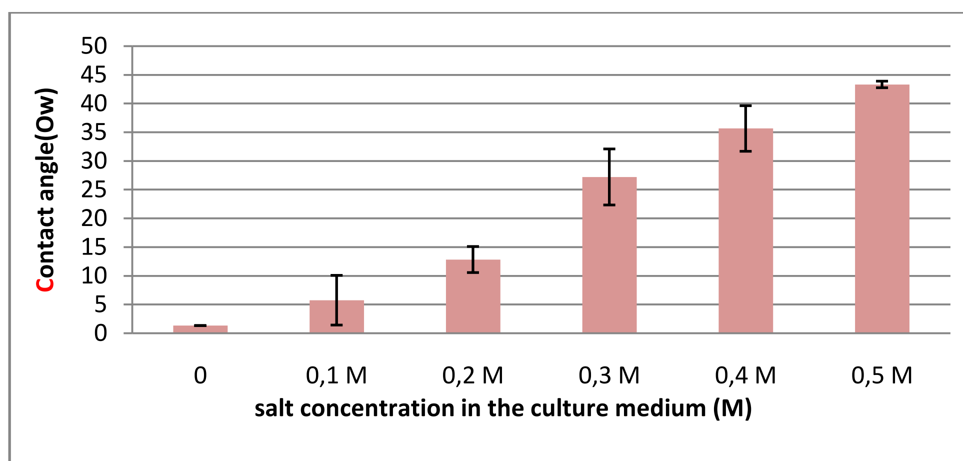

Figure 1: Effect of salinity on hydrophobicity

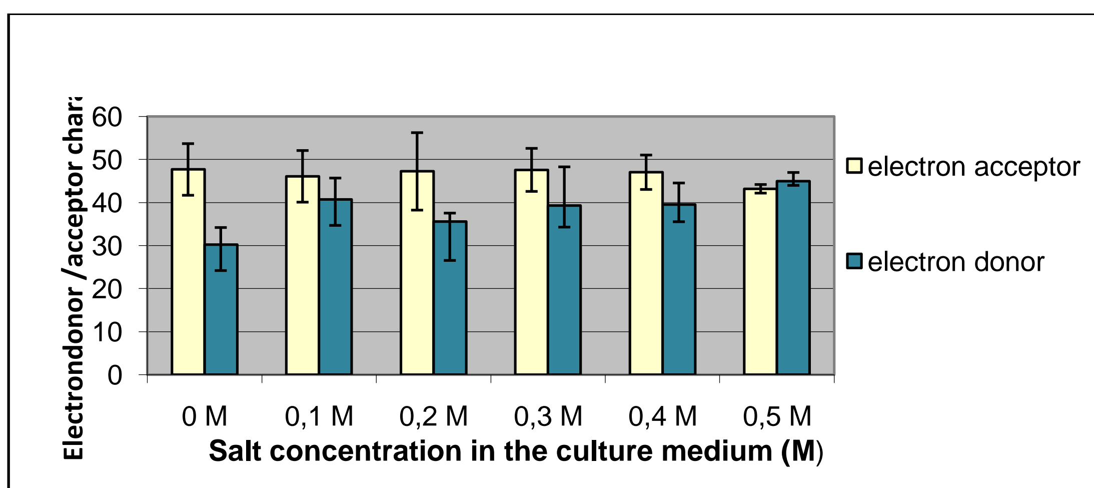

Figure 2: Effect of salinity on electron donor / electron acceptor character.

Our results were in accordance with works which reported that whatever the ionic strength and pH, *Staphylococcus aureus*, *streptococcus thermophilus*and *Leuconostocmesenteroides*, has electron donor character [39, 40]. The electron donor character can be attributed to the presence of basic groups, such as carboxyl group (COO^-), phosphate (PO_4^{3-}) and amine (NH_2), on the cell surface [11, 40].
Furthermore, high temperature and desiccation induced a direct phenotypically change in microbial membranes composition [41], that may be partially attributed to rapid physiological adjustments of cells, allowing bacteria to cope with stress [39,42,43].
The observed changes suggest an autoecological response at adverse environments. It seems that increase in surface cell hydrophobicity is an optimization of bacterial surface towards water, to fight against osmotic stress.Indeed, a drop of water takes up less space on a hydrophobic surface than on a hydrophilic surface. So, decrease of passage of salt in cytoplasm.
We suggest that actinomycetes, to acclimate to salt stress, would create an intermediate microenvironment between bacterial surface and external environment. This microenvironment is a film of water, a fairly largeformethickness to protect the cell against osmosis, the magnitude increases with salt concentration. Also, it may be that there is a passage of salt in this microenvironment, creating a concentration gradient, which softening the immediate entourage of cell surface. This is agreeing with studies reporting that fibrils and polymers may form strong links between the cell and a solid surface and encourage film development. A film provides the largest surface area

available for rewetting, and a film with a clay envelope, especially monmorillonite, may protect bacteria from excessive desiccation [44].Bacterial scavenging of surface-localized nutrients is related to the degree of irreversible binding of dwarf and starved bacteria, related to the degree of cell surface hydrophobicity [17]. Increase of hydrophobicity with salinity can be explained by reduction of repulsive negative electrostatic interactions.Many workers have described the effects of environmental parameters on hydrophobicity and charges, and, subsequently, on the adhesion process [11, 14, 19, 45].

3.3. *Effect of NaClonactinomecytes adhesion on soil*

Figure 3 presents the percentage of actinomycetes adhesion on soil. We observed that actinomycetes have capacity to adhere to soil (35%), level of adhesion increases with the presence of NaCl.

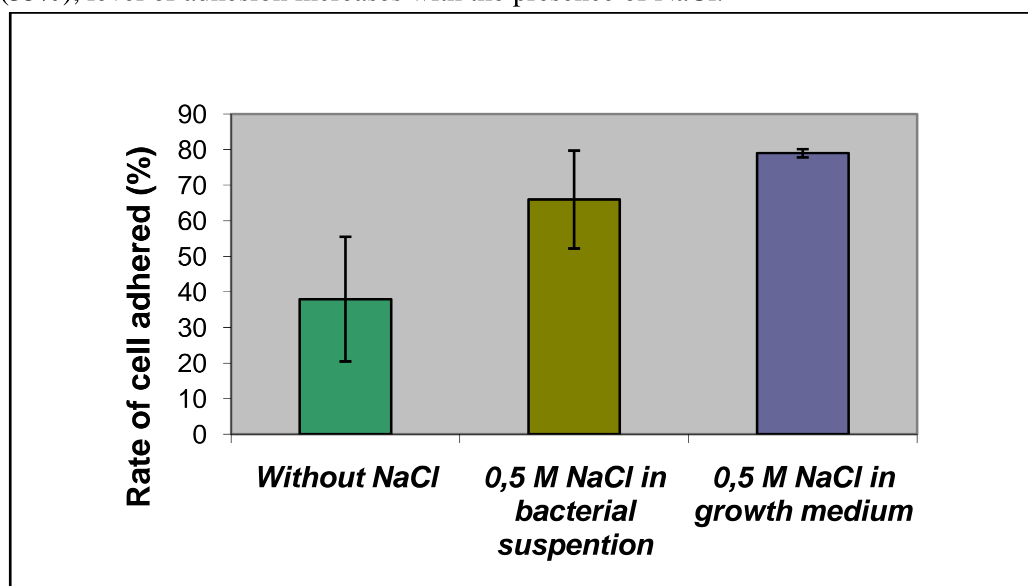

Figure 3: Adhesion rate of the strain to soil in absence and presence of NaCl.

This is in accordance whit many studies reported the effects of various environmental parameters, such as adding NaCl in medium growth, on bacteria growth and adhesion [46, 47].

It is known that bacterial adhesion is dictated by long and short range forces between bacteria and substrate [12, 48].Liefshitz van der Waals and electric double layer forces are long range forces (several nanometers), where the former is attractive and the latter can be both attractive and repulsive. In contrast, Lewis acid-base interactions operate at short range [49].

The physico-chemistry of surfaces defines the extent of these forces, and, thereby, decides the interaction between approaching surfaces. Interactions are also influenced by properties of the surrounding liquid. Ionic strength affects thickness of the electric double layer [50], and, thereby, the electrostatic interactions, promoting cell adhesion on the surface.

Role of membrane fluidity and composition on survival of bacteria at extremes temperature and salinity was, previously, described [51]. Most adaptive mechanisms are concerned with the maintenance of bilayer gel phase of the membrane, ensuring its proper function [52].

Carpenter and Crowe reported that maintenance of membrane integrity, in anhydrobiotic organisms, represents a central mechanism of desiccation tolerance [53, 54]. So, membrane structure is modified phenotypically in response to changes in environmental stress. Adaptations may include adhesion to the substrate [55, 56].

Furthermore, actinomycetes, Gram positive bacteria, are known to be tolerant to desiccation [57], but may be sensitive to osmotic up shock, and high temperatures [40].Then, increase of bacterial adhesion with salinity could be explained by the fact that salt affect surface cell structures. Several studies have reported that cell surface

physicochemical properties can be modified depending on surface cell structures [3, 13, 14, 50] and environmental factors such as temperature, medium composition, ionic strength and pH [2, 11, 19, 20, 37].

Conclusions

In conclusion, actinomycetes isolated from, relatively, saline soils of the region BeniAmir, Morocco, have arelative hydrophilic character, and can adhered to soil. Assay adhesion of actinomycetes to soil, at different concentrationsof salt in growth medium, shows a good correlation between the number of adhering bacteria and cell surfacehydrophobicity, determined by contact angle measuring.

Hydrophilic character is variable depending on environmental conditions; precisely, hydrophobicity increases with different salt levels, resulting increased rate of adhesion to soil. This very marked adhesive character to soil indicates autecological responses, leading colonization of this group of microorganisms in very diverse environments, and even hostile.

Evolution of cell surface hydrophobicity, with different salt levels, could be explained by an increase in the rate of amine at wall bacteria, linked to synthesis of protein components, and thereafter the physicochemical changes.Thus, this adhesive behavior, in presence of salt, says affection of surface cell structures by salt, what is a rapid physiological adjustment of cells, allowing them to cope with stress.

Results presented here could contribute to understand actinomycetes adhesion to inert surface.

This will allow good control of this and a master’s biofilm training, with the aim of optimizing their secondary metabolism very valuable and very diversified.

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