

# Influence of Consistency and Composition of Growth Medium on Surface Physicochemical Properties of *Streptomyces*

Rabha EL Othmany , Hafida Zahir\* , Chorouk Zanane , Mostafa El louali   
and Hassan Latrache 

Laboratory of Bioprocess and Biointerfaces, Faculty of Sciences and Technics, Sultan Moulay Slimane University, B.P. 523, 23000, Beni Mellal, Beni-Mellal 23000, Morocco.

## Abstract

*Streptomyces* are known for their ability to produce various secondary metabolites used in biotechnology, human medicine and agriculture. Understanding of surface properties is very interesting in the control of interfacial phenomena. The objective of this study was to investigate the effect of consistency and composition of growth medium on the physicochemical properties of the surface of *Streptomyces* strains. To achieve this objective, Six *Streptomyces* strains belonging to bioprocess and bio-interfaces laboratory are cultivated in two media Bennett (rich) and GBA (minimum). Both media are tested in solid (agar) and liquid (broth) mode. The wettability  $\theta_w$ , electron donor character  $\chi$  (-), electron acceptor character  $\chi$  (+) and Surface free energy  $\Delta G_{iwi}$  are determined using contact angle measurements. On the two solid media Bennett and GBA, *Streptomyces* strains develop a hydrophobic surface ( $96.9^\circ < \theta_w < 167.9^\circ$ ) with a weak electron donor character ( $0.3 \text{ mJm}^{-2} < \chi (-) < 12.14 \text{ mJm}^{-2}$ ) and a strong electron acceptor character ( $0.26 \text{ mJm}^{-2} < \chi (+) < 17.8 \text{ mJm}^{-2}$ ) and a negative surface free energy ( $-11.8 \text{ mJm}^{-2} < \Delta G_{iwi} < -110 \text{ mJm}^{-2}$ ). Whereas on both Bennett and GBA liquid media, the surfaces of *Streptomyces* strains are generally hydrophilic ( $1.3^\circ < \theta_w < 9.33^\circ$ ) with a strong electron donor character ( $13.76 \text{ mJm}^{-2} < \chi (-) < 70.06 \text{ mJm}^{-2}$ ) and a positive surface free energy. By changing the composition of the culture medium, only a slight change in the degree of hydrophobicity and surface free energy of *Streptomyces* is observed. Regarding the effect of medium composition on the surface properties of *Streptomyces*, the degree of wettability and the values of surface free energy are no longer the same when the composition of the medium changes. These results could be applied in further studies interested in interfacial phenomena and microbial adhesion in biotechnological fields.

**Keywords:** Surface physico-chemical properties, Growth medium, agar, liquid, *Streptomyces*, contact angle measurement

\*Correspondence: hafidazahir@yahoo.com

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## INTRODUCTION

*Streptomyces* is a bacterial group belonging to the phylum *Actinobacteria*. They contribute to the production of many useful molecules in biotechnology. *Streptomyces* are characterized by a complex and special development cycle<sup>1,2</sup>. This complexity of their growth explains the limited number of studies on adhesion, surface tensions components in this important genus.

Currently, *Streptomyces* are used in several biotechnological processes<sup>1,3,4</sup>. However, deep studies on the surface properties and the influencing parameters are still limited<sup>5-7</sup>. The examination of the surface properties could be used for the control of the cell attachment processes in the reactors and subsequently the control of the stability<sup>8</sup> and the metabolism of the Strain<sup>9</sup>.

Early investigations on the physico-chemistry of the microbial surface have shown the relationships between hydrophobicity and acid-base character and the implication of these parameters in the interaction of microbes with supports<sup>10</sup>. Then, the extended theory Derjaguin–Landau–Verwey–Overbeek XDLVO<sup>11</sup> has allowed the determination of microbial surface tensions.

In very recent studies the surface properties have been used in the control and management of adhesion in different fields<sup>12-16</sup>. It has been shown for other bacteria such as *E.coli*<sup>17</sup> and *Lactobacillus*<sup>18</sup> that the culture conditions affect the properties of the bacterial surface. However, for *Streptomyces* few reports are available in this issue<sup>5,7</sup>. To our knowledge, No studies have been performed to test the effect of the composition and consistency of culture medium on the surface properties of *Streptomyces*.

The objective of this research is to analyze the surface properties of *Streptomyces* in order to extend the knowledge about this important genus of bacteria and to provide useful data for the control of adhesion/attachment in biotechnology. We tested the effect of consistency and composition of the culture medium on the hydrophobicity, electron donor/acceptor character and surface free energy of six *Streptomyces* strains.

## MATERIALS AND METHODS

### Bacterial strains, culture conditions and preparation of microbial suspension

*Streptomyces* strains are belonging to bioprocess and bio-interface laboratory, they are isolated from Moroccan saline soil and identified in a previous study<sup>19</sup>.

*Streptomyces* strains are cultivated in two Liquid mediums which were different in the composition were chosen in this study, liquid Bennett (Glucose 10g/l; yeast extract 2g/l; meat extract 1g/l; peptone 2g/l, 1 liter of distilled water) and liquid GBA (Glycerol 20g/l; soluble starch 10g/l; CaCO<sub>3</sub> 3g/l; peptone 10g/l; meat extract 5g/l). Strains were grown 7 days at 28°C under shaking. Then, cells were harvested by centrifugation for 15 min at 84000 × g, washed twice and suspended in potassium nitrate solution (KNO<sub>3</sub>, 0, 1 M).

Two agar medium used for this study agar Bennett (Glucose 10g/l; yeast extract 2g/l; meat extract 1g/l; peptone 2g/l, 1 liter of distilled water and bacteriological agar 18g/l). Agar GBA Glycerol 20g/l; soluble starch 10g/l; CaCO<sub>3</sub> 3g/l; peptone 10g/l; meat extract 5g/l and bacteriological agar 18g/l). After 7 days at 28°C, pieces of agar containing a bacterial layer has been cut for later contact angle measurements.

### Contact angle measurements and estimation of bacterial surface tension components

Briefly, bacterial cells suspended in KNO<sub>3</sub> 0.1M were filtered through nitrocellulose filter with 0.45 μm pore size using a negative pressure. The filters were left during about 30 min to air dry at room temperature until contact angle measurements. Experiments were carried out in triplicate for each strain. Contact angle measurements were accomplished using a goniometer (GBX Instruments, France) by sessile drop method. A drop of 10 μl of the liquid test (water, formamide or diiodomethane) was dispensed on the surface of the filter. Wettability was obtained directly by measuring the contact angle with water. The surface tension components are determined based on Young-Dupre equation which is related to the surfaces tension components in equation (1)

Surface free energy ( $\Delta G^{wi}$ ), Lifshitz-van der Waals components ( $\gamma^{LW}$ ), electron donor

character or Lewis base ( $\gamma^-$ ) and acceptor character of electron or Lewis acid ( $\gamma^+$ ) were estimated from the approach proposed by Van Oss<sup>20</sup>. In this approach the pure liquid (L) contact angles ( $\theta$ ) can be expressed as:

$$\cos \theta = -1 + 2 \frac{\sqrt{\gamma_S^{LW} \gamma_L^{LW}} + \sqrt{\gamma_S^+ \gamma_L^-} + \sqrt{\gamma_S^- \gamma_L^+}}{\gamma_L} \quad \dots(1)$$

As (S) and (L) mean solid phase and liquid phase respectively. The Lewis acid-base component has been obtained by:

$$\gamma^{AB} = 2\sqrt{\gamma^- \gamma^+} \quad \dots(2)$$

Cell surface hydrophobicity also called surface free energy is an expression of the Lifshitz–can der Waals  $\gamma^{LW}$  and acid–base  $\gamma^{AB}$  surface tensions<sup>21</sup>, it is usually assessed by contact angles of water, formamide and diiodomethane (purity  $\geq 99\%$ ). Energies characteristics of these solvents is presented in Table 1<sup>22</sup>.

The degree of hydrophobicity also called surface free energy was calculated by measuring the contact angle based on the Van Oss approach<sup>24</sup>. According to this approach the degree of hydrophobicity for a given material (i) is expressed as the interaction energy between two entities of a material (i) when it is immersed in water (w). The latter has been evaluated through surface tensions by the following equation (3):

$$\Delta G_{iwi} = -2\gamma_{iw} = -2 \left[ \frac{(\gamma_i^{LW})^{\frac{1}{2}} - (\gamma_w^{LW})^{\frac{1}{2}}}{2((\gamma_i^+ \gamma_i^-)^{\frac{1}{2}} + (\gamma_w^+ \gamma_w^-)^{\frac{1}{2}})} - (\gamma_i^+ \gamma_w^-)^{\frac{1}{2}} - (\gamma_w^+ \gamma_i^-)^{\frac{1}{2}} \right] \quad \dots(3)$$

Contact angle measurements report the aptitude of a liquid to extent on a surface

depending on its wettability. The principle of this method is to measure the angle  $\theta$  of the tangent with the studied surface of the profile of a drop with defined dimensions, of a probe liquid deposited on the substrate to be analyzed.

Using water as a drop application liquid lead to conclude about surface wettability also called qualitative hydrophobicity. The use of different references liquids lead to evaluate the total surface free energy and its components based on Young-Dupre equation – presented in earlier paragraph- and also the energy components characterizing the analyzed substrate thermodynamically.

Using the approach of Van Oss<sup>20,24</sup>, it is possible to evaluate the absolute degree of hydrophobicity of surfaces. Based on the XDLVO approach,  $\Delta G_{iwi}$  (surface free energy) can be calculated. Therefore, surfaces with positive surface free energy ( $\Delta G_{iwi} > 0$ ) are classified as hydrophilic and surface with negative surface free energy ( $\Delta G_{iwi} < 0$ ) are considered as hydrophobic.

#### Statistical analysis

We used SPSS (IBM, version 20 for Windows) for statistical analyses and considered  $p < 0.05$  as statistically significant.

## RESULTS

### Physicochemical properties of *Streptomyces* strains in liquid GBA, agar GBA, liquid Bennett and agar Bennett media

#### Wettability

Cell wettability or qualitative hydrophobicity ( $\theta_w$ ), According to Vogler a surface is called hydrophobic if ( $\theta_w < 65^\circ$ )<sup>25</sup> while it is hydrophilic when  $\theta_w > 65^\circ$ <sup>25</sup>. Wettability of *Streptomyces* surfaces on solid and broth culture media is presented in the Fig. 2. GBA and Bennett are used in the present study. All *Streptomyces* grown on agar media are relatively hydrophobic

**Table 1.** Energy characteristics of solvents used for contact angle measurements<sup>23</sup>:  $\gamma^{LW}$  : Van der Waals component of surface free energy Solvents;  $\gamma^+$  : Electron acceptor component of free surface energy of solvents;  $\gamma^-$  : Electron donor component of free surface energy of solvents:

Solvent	Formule	$\gamma^{LW}$ (MJ/ m <sup>2</sup> )	$\gamma^{ab}$ (MJ/ m <sup>2</sup> )	$\gamma^+$ (MJ/ m <sup>2</sup> )	$\gamma^-$ (MJ/ m <sup>2</sup> )
Water	H <sub>2</sub> O	21,8	51,0	25,5	25,5
Diiodomethane	CH <sub>2</sub> I <sub>2</sub>	50,8	~0	~0	~0
Formamide	CH <sub>3</sub> NO	39,0	19	2,3	39,6

( $96.9^\circ < \theta_w < 167.9^\circ$ ). Except *Streptomyces griseorubens* A23 which was hydrophilic on agar GBA ( $\theta_w = 17.3^\circ$ ). However, on liquid mediums *streptomyces* strains showed a hydrophilic behavior ( $1.3 < \theta_w < 59.33$ ). Our results showed a significant change in qualitative hydrophobicity between solid and liquid media. From GBA to Bennett media, cell surface properties exhibits slight changes. Wilcoxon text performed on the data significance 0.05.

**Electron donor- Electron acceptor characters**

From figure 2 and figure 3 we observe that *Streptomyces* surface is highly electron donor ( $13.76 \text{ mJ m}^{-2}$  ;  $70.06 \text{ mJ m}^{-2}$ )/weakly electron acceptor ( $0.43 \text{ mJ m}^{-2}$ ;  $14.96 \text{ mJ m}^{-2}$ ) when

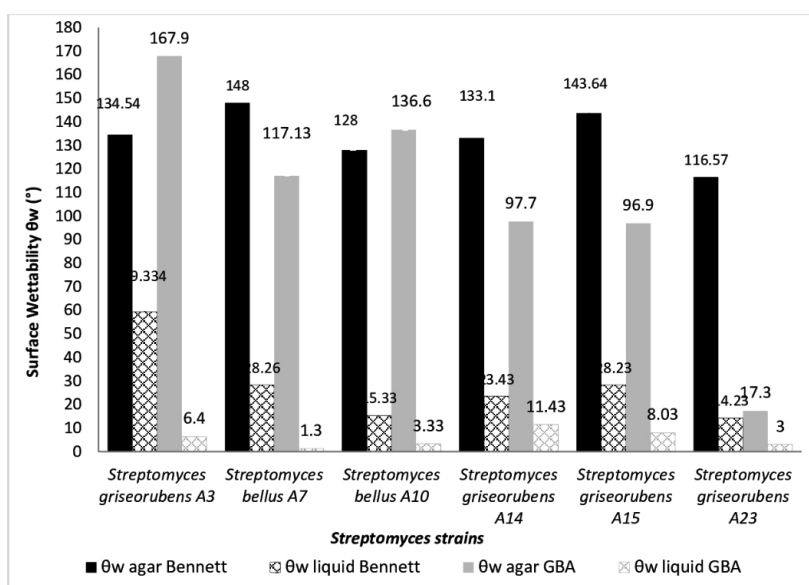
cultivated on liquid media. However, on solid media *Streptomyces* cell surfaces are considered as bipolar, acid components and base components are slightly similar. Electron donor values vary from  $0.57 \text{ mJ m}^{-2}$  to  $13.75 \text{ mJ m}^{-2}$  and electron acceptor vary from  $0.04 \text{ mJ m}^{-2}$  to  $17.8 \text{ mJ m}^{-2}$  (data presented in Fig. 2 and 3). Wilcoxon test confirm at 0.95 that the composition impact significantly the electron donor/ electron acceptor. Whereas, non-significant fluctuation was observed when the composition of growth medium was substituted.

**Surface free energy**

Surface free energy ( $\Delta G_{\text{wi}}$ ) indicates the quantitative hydrophobicity. The surface is called hydrophobic if  $\Delta G_{\text{wi}} < 0$  negative and hydrophilic

**Table 2.** Wettability of Six *Streptomyces* strains (in degree),grown in Bennett liquid, agar Bennett, GBA liquid, and agar GBA media

	Wettability ( $^\circ$ ) of <i>Streptomyces</i> cell cultivated on GBA and Bennett medium			
	$\theta_w$ agar Bennett	$\theta_w$ liquid Bennett	$\theta_w$ agar BA GBA	$\theta_w$ liquid GBA
<i>Streptomyces griseorubens</i> A3	134.54± 6.824	59.334±7.617	167.9±9.753	6.4±1.153
<i>Streptomyces bellus</i> A7	148±2.86	28.267±0.416	117.1334±8.295	1.3±0.45
<i>Streptomyces bellus</i> A10	128±6.06	15.334±5.48	136.6±6.547	3.33±1.154
<i>Streptomyces griseorubens</i> A14	133.1±9.12	23.434±5.11	97.7±5.183	11.433±0.28
<i>Streptomyces griseorubens</i> A15	143.64±3.05	28.234±5.346	96.9±0.346	8.03±0.75
<i>Streptomyces griseorubens</i> A23	116.57±5.79	14.234±4.186	17.3±0.5	3.0±2.64



**Fig. 1.** Surface wettability ( $\theta_w$ ) of Six *Streptomyces* strains cultivated on Bennett liquid, agar Bennett, GBA liquid and agar GBA media.

when  $\Delta G_{iwi} > 0$  positive. Surface free energy of *Streptomyces* cells grown on Bennett and GBA media are mentioned in the Fig. 4. Bennett and GBA are used as agar and broth mode. Surface free energy of *Streptomyces* cultivated on agar Bennett and agar GBA are negative (from  $-11.86 \text{ mJm}^{-2}$ ) to  $-110 \text{ mJm}^{-2}$ ):  $(-11.86 \text{ mJm}^{-2} < \Delta G_{iwi} < -110 \text{ mJm}^{-2})$ . *Streptomyces griseorubens* A15 show a positive value of surface free energy  $(+8.86 \text{ mJm}^{-2})$ .

On liquid media, surface free energy values are positives and included in this interval  $(+12.9 \text{ mJm}^{-2} < \Delta G_{iwi} < +33.7 \text{ mJm}^{-2})$ .

The average values of the surface free energy obtained after growth on Agar Bennett  $(-110 \text{ mJm}^{-2} < \Delta G_{iwi} < -11.86 \text{ mJm}^{-2})$ , Agar GBA  $(-67.36 \text{ mJm}^{-2} < \Delta G_{iwi} < +8.67 \text{ mJm}^{-2})$ , Liquid bennet  $(-8 \text{ mJm}^{-2} < \Delta G_{iwi} < +33.7 \text{ mJm}^{-2})$ , and Liquid GBA  $(+23 \text{ mJm}^{-2} < \Delta G_{iwi} < +34.9 \text{ mJm}^{-2})$ . (See Fig. 4)

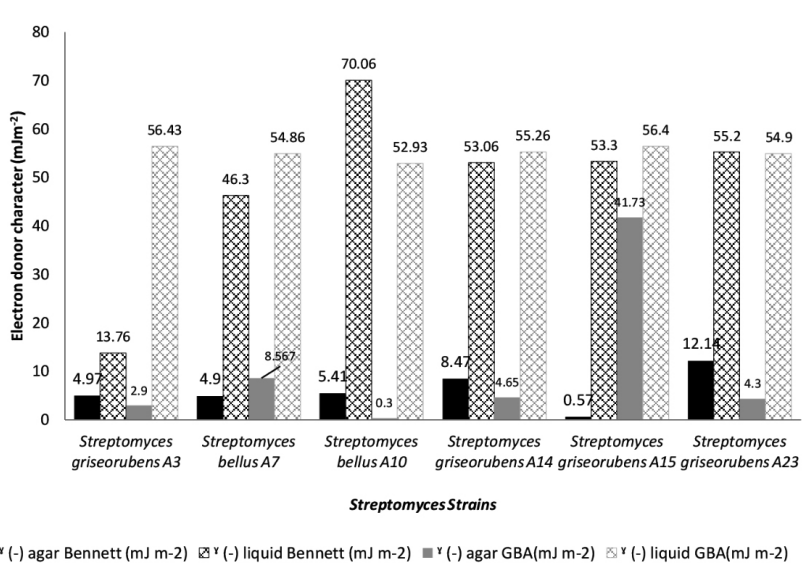
## DISCUSSION

### *Streptomyces* cells modify their wettability behavior

The measurement of the surface wettability of *Streptomyces* strains has shown

**Table 3.** Electron donor of Six *Streptomyces* strains (in  $\text{mJ m}^{-2}$ ),grown in Bennett liquid, agar Bennett, GBA liquid, and agar GBA media

	Electron Donor ( $\text{mJ m}^{-2}$ ) of <i>Streptomyces</i> cell cultivated on GBA and Bennett medium			
	$\gamma$ (-) agar Bennett ( $\text{mJ m}^{-2}$ )	$\gamma$ (-) liquid Bennett ( $\text{mJ m}^{-2}$ )	$\gamma$ (-) agar GBA ( $\text{mJ m}^{-2}$ )	$\gamma$ (-) liquid GBA ( $\text{mJ m}^{-2}$ )
<i>Streptomyces griseorubens</i> A3	4.97±3.8	13.76±7.60	2.9±3.36	56.43±0.46
<i>Streptomyces bellus</i> A7	4.9±1.55	46.3±7.16	8.567±3.55	54.867±0.15
<i>Streptomyces bellus</i> A10	5.44±5.4	70.06±7.96	0.3±0.36	52.93±0.152
<i>Streptomyces griseorubens</i> A14	8.47±12.9	53.06±3.81	4.65±1.9	55.267±2.884
<i>Streptomyces griseorubens</i> A15	0.57±0.05	53.3±5	41.73±3.94	56.4±0.3
<i>Streptomyces griseorubens</i> A23	12.14±3.83	55.2±1.47	4.3±0.1	54.9±0.264



**Fig. 2.** Electron donor character ( $\gamma$  (-) in  $\text{mJm}^{-2}$ ) of Six *Streptomyces* strains cultivated on Bennett liquid, agar Bennett, GBA liquid and agar GBA media.

that the surfaces of these strains are generally hydrophobic on solid medium (agar) and hydrophilic on liquid medium.

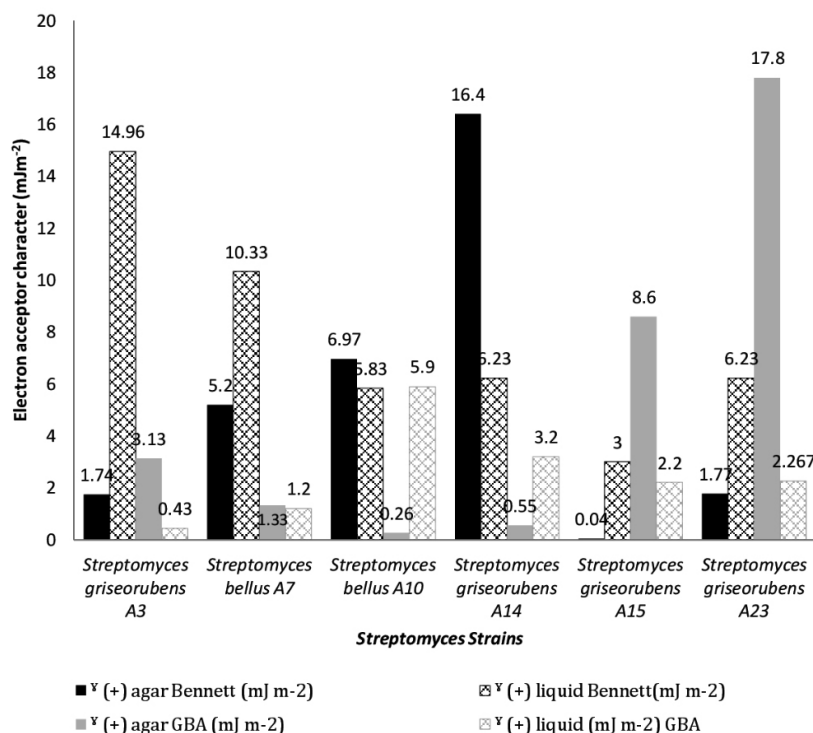
The development cycle of *Streptomyces* is characterized by its complexity. Spores undergo germination then vegetative hyphae develop into aerial hyphae. Aerial hyphae and spores are described in the work of Claessen and Elliot by specific proteins on the surface which allows

the cells to rise from the liquid medium. We have characterized the surface after 7 days of incubation<sup>26,27</sup>.

Moreover, certain types of proteins expressed on the aerial hyphae called Chaplin. The latter are characterized by their amphipatic nature and anchored in the cell wall. These proteins undergo an assembly in such a way that the hydrophobic part is oriented towards the outside

**Table 4.** Electron acceptor of Six *Streptomyces* strains (in mJ m<sup>-2</sup>),grown in Bennett liquid, agar Bennett, GBA liquid, and agar GBA media

	Electron acceptor (mJ m <sup>-2</sup> ) of <i>Streptomyces</i> cell cultivated on GBA and Bennett medium			
	<sup>γ</sup> (+) agar Bennett (mJ m <sup>-2</sup> )	<sup>γ</sup> (+) liquid Bennett (mJ m <sup>-2</sup> )	<sup>γ</sup> (+) agar GBA (mJ m <sup>-2</sup> )	<sup>γ</sup> (+) liquid GBA (mJ m <sup>-2</sup> )
<i>Streptomyces griseorubens</i> A3	1.74±0.08	14.96±1.93	3.13±0.73	0.43±0.115
<i>Streptomyces bellus</i> A7	5.2±0.98	10.33±4.5	1.33±0.98	1.2±0.26
<i>Streptomyces bellus</i> A10	6.97±1.68	5.83±1.95	0.26±0.25	5.9±0.36
<i>Streptomyces griseorubens</i> A14	16.4±8.05	6.23±2.34	0.55±0.07	3.2±0.6
<i>Streptomyces griseorubens</i> A15	0.04±0.05	3±0.78	8.6±7.44	2.2±0.1
<i>Streptomyces griseorubens</i> A23	1.77±1.15	6.23±2.34	17.8±0.1	2.267±0.152



**Fig. 3.** Electron acceptor character (<sup>γ</sup> (+) in mJm<sup>-2</sup>) of Six *Streptomyces* strains cultivated on Bennett liquid, agar Bennett, GBA liquid and agar GBA media.

of the cell thus conferring hydrophobicity to the aerial mycelium which contains them. Chaplins can be used to modify a variety of hydrophilic and hydrophobic surfaces in vitro thereby changing their nature. Assembly on glass leads to a protein coating that makes the surface hydrophobic<sup>28</sup>. On liquid medium the expression of surface proteins is neither as a solid medium where the cells undergo a transition from the vegetative phase to the aerial phase<sup>29,30</sup>.

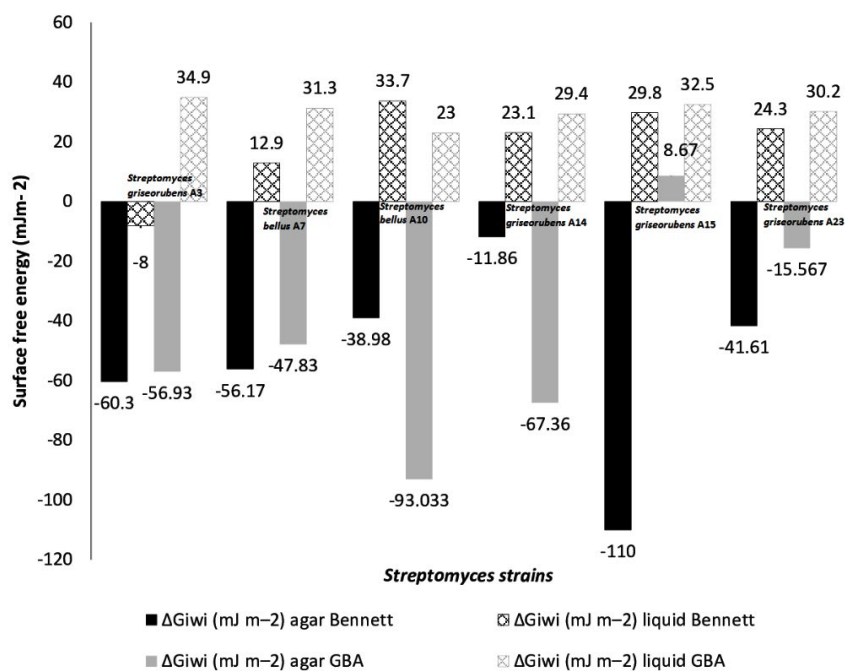
In addition, it was demonstrated that the availability of glucides and yeast extract in

the fermentation medium impact the surface hydrophobicity of *Lactobacillus acidophilus*. Cell wall hydrophobicity, which is low for cultures grown in complete medium and in the absence of carbohydrates, becomes quite high for cultures grown in medium without peptones and medium without yeast extract<sup>18</sup>.

Kim et al. explained that *Streptomyces* growth in liquid media is characterized by the expression some specific molecules on the surface and the existence of DNA extracellular which make these pellets hydrophilic<sup>31</sup>.

**Table 5.** Surface free energy of Six *Streptomyces* strains (in  $\text{mJ m}^{-2}$ ),grown in Bennett liquid, agar Bennett, GBA liquid, and agar GBA media

	Surface free energy of Streptomyces cell cultivated on GBA and Bennett medium			
	$\Delta G_{\text{iwi}}$ ( $\text{mJ m}^{-2}$ ) agar Bennett	$\Delta G_{\text{iwi}}$ ( $\text{mJ m}^{-2}$ ) liquid Bennett	$\Delta G_{\text{iwi}}$ ( $\text{mJ m}^{-2}$ ) agar GBA	$\Delta G_{\text{iwi}}$ ( $\text{mJ m}^{-2}$ ) liquid GBA
<i>Streptomyces griseorubens</i> A3	(-) 60.3±3.8	(-) 8±3.40	(-) 56.933±11.73	34.9±0.6
<i>Streptomyces bellus</i> A7	(-) 56.17±1.11	12.9±8.62	(-) 47.833±2.74	31.3±0.5
<i>Streptomyces bellus</i> A10	(-) 38.98±5.4	33.7±11.08	(-) 93.033±2.02	23.0±0.7
<i>Streptomyces griseorubens</i> A14	(-) 11.86±12.9	23.1±7.37	(-) 67.367±9.58	29.4±1.7
<i>Streptomyces griseorubens</i> A15	(-) 110±0.05	29.8±5.05	8.67±7.90	32.5±0.5
<i>Streptomyces griseorubens</i> A23	(-) 41.61±5.02	24.3±5.64	(-) 15.567±7.13	30.2±0.5



**Fig. 4.** The surface free energy (SFE) (in  $\text{mJm}^{-2}$ ) of Six *Streptomyces* strains cultivated on Bennett liquid, agar Bennett, GBA liquid and agar GBA media.

### Electron donor / electron acceptor of *Streptomyces* surfaces

The consistency of the culture medium affects significantly the electron donor/acceptor character, whereas the effect of the composition is not very pronounced. This change in the electron donor/acceptor character is probably due to the surface proteins. Various works concerning this issue are carried out on other bacteria. In fact, Briandet showed that cell surface charge, hydrophobicity and electron donor/electron acceptor character of *Listeria monocytogenes* ScottA strains change when glucose or lactic acid are added to growth medium<sup>23</sup>. In fact proteins structures are influenced by the amino-acid source available in the medium. In the same manner, other scientists showed the impact of growth conditions on physicochemical properties of bacteria like *Enterococcus faecalis*<sup>21</sup>, *Lactobacilli* strains<sup>32</sup>, *Enterocci* strains<sup>33</sup>.

### Surface free energy (SFE) of *Streptomyces* variations

Surface free energy (SEF) is from the important parameters required in interfacial phenomena. The obtained results from contact angle measurements and calculations of surface free energy of the surface of *Streptomyces* grown on broth Bennett, agar Bennett, liquid GBA and agar GBA showed a significant impact of the consistency and composition of surface free energy. These results lead to suggest that *Streptomyces* may use their advanced quorum sensing in detecting the environmental conditions. In fact, Jones et al. have highlighted that *Streptomyces* could exist as an exploratory growth. This bacterial group could detect organic volatil compounds coming from fungi and changes the morphology and the cells organization<sup>34</sup>.

Many interesting studies are carried on other bacteria in order to understand the factors affecting the Physico-chemistry of the bacterial surface. Latrache et al. demonstrated that Surface free energy and other surface tensions of *Escherichia coli* are not equivalent in solid LB medium as in liquid LB medium<sup>17</sup>. Also the environmental conditions such as pH, ionic strength or presence of antimicrobial substances<sup>35</sup> affect surface physicochemical properties. The surface tensions of *Staphylococcus aureus*

and *Escherichia coli* are influenced by pH and ionic strength, their influence seemed to be significant on experimental adhesion assessed by scanning electron microscopy<sup>36</sup>. A deep analysis of the molecules of cell wall leads to explain the relationship between chemical composition of the cell wall and the electron donor/electron acceptor. Phosphate groups have a key role in evaluating the property of the electron donor (base). Amine groups decrease electron acceptor property (acid)<sup>37</sup>. Further Electron donor character electron acceptor character of bacterial surface are related to its composition on functional groups<sup>38</sup>. Contact angle measurements, xylene adsorption and cell partition in a polyethylene glycol/dextran (PEG/DEX) biphasic system are used to determine cell surface hydrophobicity of *Escherichia coli*. Chemical composition of bacterial surface obtained by XPS lead to determine C/O and C/N ratios also it can provide molecular content of the bacterial surface. A correlation obtained between these two parameters lead to conclude physicochemical properties from chemical analysis of cell surface<sup>39</sup>.

The conclusions and rules established derived from other bacteria could not be valuable for *Streptomyces*, because they have specific amino-acids in their cell wall<sup>40,41</sup>. However, Del sol reported that characterization of cell surface of *Streptomyces* using atomic force microscopy (AFM) indicated that vegetative hyphae are hydrophilic while aerial hyphae are hydrophobic. Vegetative hyphae have a relatively smooth surface and are attached to an inert silica surface by means of a secreted extracellular matrix. Aerial hyphae, which appear in aerial growth are poorly decorated with fibers<sup>42</sup>. In addition, analysis of extracted and identified extracellular proteins from vegetative and aerial hyphae showed different expression of proteome<sup>43</sup>.

From analysis of the cited literature above, The difference in surface free energy could be explained by the variation of the expression of proteins in each medium, since it is known that the culture medium in which bacterial survive plays a key role in cellular metabolism, as well as in the control of gene expression. *Streptomyces* wall depends on the composition of the medium and the availability of nutrients.



## CONCLUSION

Physicochemical properties of cell surface *Streptomyces* changes significantly depending on the consistency and composition of culture medium on which *Streptomyces* were grown. In fact, on both agar media the surfaces of *Streptomyces* were qualitatively and quantitatively hydrophobic, weak electron acceptor, weak electron donor (bipolar surface). However, on the two liquid mediums, the surfaces showed a qualitatively and quantitatively hydrophilic character, high electron donor character, weak electron acceptor character (monopolar surface). In addition, the levels of the qualitative hydrophobicity, the electron donor / electron acceptor characters and the quantitative hydrophobicity (surface free energy) differ according to the composition of growth medium. Thus, the characterization of surfaces properties of *Streptomyces* could be a key factor in the control of interfacial phenomenon.

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None.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## AUTHORS' CONTRIBUTION

LH, HZ, ME conceived and designed the experiments; REO and CZ performed the experiments and analyzed the data; REO and HZ wrote the paper. All authors read and approved the manuscript.

## FUNDING

None.

## DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

## ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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