

Original article

Identification and physicochemical characterization of bacterial surface isolated from catering services in health establishment

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Abstract: The initial interaction between microorganisms and substrata is mediated by physicochemical forces, which originate from the physicochemical surface properties of both interacting surfaces. In this context, we have determined the physicochemical properties (hydrophobicity, electron-donor and electron-acceptor) of 37 isolates belonging to three genres of bacteria: *Pseudomonas* spp., *Staphylococcus* spp. and some species of *Enterobacteriaceae* isolated from various surfaces of the equipment and materials used in health establishment catering services. The physicochemical properties of these isolates were determined by contact angles measurements via Sessile Drop Technique. The results revealed that 62% of all bacteria studied exhibit a hydrophilic character ($\Delta G_{\text{wvi}} > 0$) and other strains have a hydrophobic character ($\Delta G_{\text{wvi}} < 0$). Also the results show that all strains have a high electron donor character (high γ^-) (ranging from 22.8 mJ.m^{-2} to 105.4 mJ.m^{-2}). Forty one percent of these strains have a high electron acceptor (γ^+) (ranging from 14.7 mJ.m^{-2} to 34.6 mJ.m^{-2}) and the others express a low electron acceptor character.

Keywords: physicochemical properties, contact angles measurements, *Pseudomonas* spp., *Staphylococcus* spp., *Enterobacteriaceae*

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Introduction

Given the hot climate of Morocco and the lifestyle change of the population, food is increasingly processed and therefore constitutes, once consumed, a risk to consumer health. Food is considered as the first cause of poisoning (22%) [1]. During the period 1999-2008, the Poison Control Center and Pharmacovigilance Morocco (CAPM) have recorded 13 638 cases of food poisoning related to: 11,677 statements provinces received by mail and 1961 statements collected by the Toxicological Information System. The frequency seems much lower than that of other countries such as France where the food poisoning affecting 40,000 people each year [2-3] and the United States where there are approximately 76 million cases of poisoning per year [4]. However, in developing countries, food-borne diarrheal diseases kill 1.9 million people annually [5].

In most countries, bacteria are the leading cause of Food-borne diseases (FBD) and seem to be the causative agents of more than two thirds of the recorded FBD outbreaks [6]. For example, among the predominant bacteria involved in these diseases, *Staphylococcus aureus* is a leading cause of gastroenteritis resulting from the consumption of contaminated food [6].

Biofilm is considered as part of the normal life cycle of bacteria in the environment [7], in which planktonic cells attach to solid

surfaces, proliferating and accumulating in multilayer cell clusters embedded in an organic polymer matrix. This biofilm protects the bacterial community from environmental stresses, from the host immune system and from antibiotic attacks, as opposed to the situation for vulnerable and exposed planktonic cells [8]. This may contribute to the persistence of bacteria in food-processing environments, consequently increasing cross-contamination risks as well as subsequent economic losses due to recalls of contaminated food products. According to literature, food contamination by pathogenic bacteria could be the result of detachment of biofilm bacteria [9-14]. Several studies have reported that bacteria have the capacity to adhere to food contact surfaces such as polystyrene, polypropylene, stainless steel, glass, marble and granite, and also on food products [15-24]. This adhesion is the key step to biofilm formation, and is considered as the result of physico-chemicals interactions. These interactions depend on physicochemical properties of both substratum and cells surfaces. However, the change of these physicochemical properties may affect the biofilm formation, consequently, influence their persistence on food contact surfaces [25-31]. That's why the determination of the physicochemical properties such hydrophobicity and electron donor (γ^-) / electron acceptor (γ^+) character, of the isolates is the key to understanding the bacterial

adhesion and consequently biofilm formation. Several studies have demonstrated the importance of bacteria surface hydrophobicity in the adhesion process [32-36]. The role of electron-donor/electron acceptor in adhesion phenomenon has been also widely studied [23, 37-39].

The first objective of this study was to isolate bacteria from different materials and surfaces commonly used in the catering kitchens as: granite, polypropylene, porcelain and stainless steel. The second objective was to determine the physicochemical properties of these bacteria: hydrophobicity and electron-donor/acceptor character.

Material and Methods

The samplings were conducted from different surfaces of materials and equipment used in the catering kitchens in health establishment: stainless steel, porcelain, polypropylene and granite.

Isolation and identification of bacteria

The isolates sampling protocol was done according to standards and international standards (ISO 16266:2006, NF V 08-014 and NF V08-050) with some additional assays. The surfaces samplings were scraped using a Swabs. The Swabs was suspended in peptone water in test tubes for the stock solution, after serial dilutions, we have seeded 0.1 mL of each dilution solution on selective media depending on the desired germ. The colony forming units (CFU) are discriminated and selected based on their morphology, then inoculated individually on Petri dishes containing specific culture medium to obtain mono-specific microbial cultures.

Isolation and identification of *Pseudomonas* spp.

Isolation and identification of *Pseudomonas* spp. were made using a procedure described in ISO 16266:2006 with some additional assays. We have seeded on selective media (Cetrimide agar plates) as already described, and we have incubated at 37°C for 48h. After 48h we have selected the colonies that show a bluish/greenish or reddish brown pigmentation, or the colonies which were fluorescent when examined under 360±20 nm ultraviolet radiations. These colonies were subcultured on King B plates at 37°C for 24h. The plates were examined under 360±20 nm ultraviolet radiation. The presence of fluorescence during the five days of observation was considered a positive reaction. Additional assays: Gram staining, catalase activities, oxidase test, lipolytic activity, mobility test, antibiogramme. Also two growing temperatures, 4°C and 42°C, were tested on nutrient agar for all the strains, following recommendations in complementary information in ISO 16266:2006. All strains also were subcultured on King A plates for five days. An observation of bluish/greenish pigmentation, caused by pyocyanin production, was considered presumptive evidence of the presence of *P. aeruginosa*.

Thereafter, two commercial biochemical characterization kits were used for the phenotypic identification of isolated strains: API 20 NE (Biomérieux, France) and automated microbiology instruments reference BD-PHOENIX. This later technique was used for some strains only.

Isolation and identification of *Staphylococcus* spp.

Staphylococcus spp. were isolated and identified according to the standard procedure described in NF V 08-014 (1984) with some additional assays. We have seeded on selective media

(Chapman agar plates) and we have incubated at 37°C for 24 h. Then the colonies undergo tests of Gram stain, catalase activity, oxidase tests, mobility tests, coagulase, ADNase and the API Staph (Biomérieux, France) also was used.

Isolation and identification of *Enterobacteriaceae*

Strains of *Enterobacteriaceae* were isolated and identified according to the procedure described in the NF V08-050 with some additional assays. We have seeded on selective media Violet Red Bile Lactose (VRBL) Agar and incubated at 44°C for 24 hours. Colonies were inoculated on the Eosin Methylene Blue Agar (EMB). Subsequently other tested identifications were made such as: Gram stain, catalase activity, oxidase tests, mobility tests, IMViC test and the API 20 E (Biomérieux, France).

Growth and cultures conditions

The Strains identified were cultured in Luria Bertani medium containing the following components (per liter of distilled water): 10g tryptone, 5g yeast extract, 10g NaCl and 15g agar. After incubation at 37°C for 24h, the cells were harvested by centrifugation for 15 min at 8400xg and were washed twice with, and resuspended in, KNO₃ solution with ionic strength (0.1 M).

Contact angle measurements and surface tension components

Contact angle measurements were performed using a goniometer (GB instruments, France) by the sessile drop method. One drop of a liquid was deposited onto dry bacteria surfaces. Contact angles were measured in triplicate with separately cultured bacteria. Three to six contact angle measurements were made on each substratum surface for all probe liquids including formamide (99%), diiodomethane (99%) and distilled water [40].

The method for measuring contact angles on bacterial layers has been described by Busscher et al. [41]. Briefly, a suspension of cells in KNO₃ sterile solution was deposited on a cellulose acetate membrane filter (0.45 µm) (Sartorius) by a first washing of the filter with 10 mL of distilled water for wetting, and then 10 mL of the cell suspension was added to obtain a thick lawn of cells after filtration using a negative pressure. The wet filters were placed carefully on a glass support with double-sided sticky tape and were allowed to air dry until so-called stable "plateau contact angles" could be measured. For each strain, three independently grown cultures were used, from which three filters of each were prepared and measured. Three to six contact angle measurements were made on each filter, for all liquids including water, formamide and diiodomethane.

The cell surface hydrophobicity was evaluated through contact angle measurements and using the approach of Van Oss and co-workers [40-42]. In this approach, the degree of hydrophobicity of a given material (i) is expressed as the free energy of interaction between two entities of that material when immersed in water (w) ΔG_{iwi}. If the interaction between the two entities is stronger than the interaction of each entity with water ΔG_{iwi} < 0 the material is considered hydrophobic. Conversely, if ΔG_{iwi} > 0 the material is hydrophilic. ΔG_{iwi} can be calculated through the surface tension components of the interacting entities, according to:

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

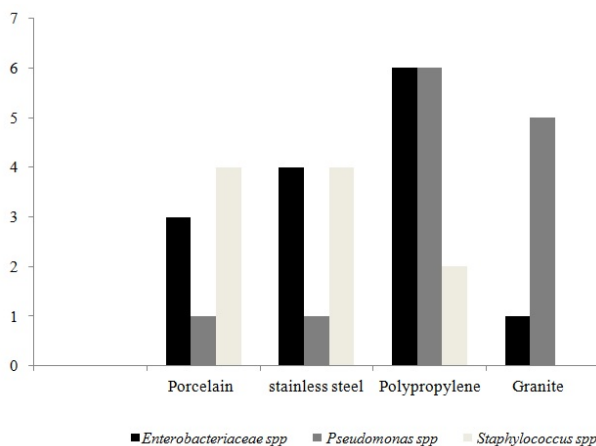


Figure 1. Number of strains samples isolated from different surfaces catering services.

The Lifshitz-Van der Waals (γ^{LW}), electron donor (γ^-) and electron acceptor (γ^+) components of the surface tension of bacteria and for the solid substrates were estimated from the approach proposed by Van Oss et al. [39]. In this approach the contact angles (θ) can be expressed as:

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

θ is measured by contact angle. (S) and (L) denote solid surface and liquid phases respectively.

Lewis acid-base surface tension component is defined by:

$$\gamma_s^{AB} = 2(\gamma_s^- \gamma_s^+)^{1/2}$$

Results

Bacterial identification

Thirty-seven strains were isolated from a catering in a health establishment during this study. 13 of the 37 strains were identified as *Pseudomonas* spp. In which 9 strains were identified as *P. aeruginosa* by the following assays, which are included in the ISO 16266: 2006. The results were confirmed by API 20 NE system that identified 9 of the 13 strains as *P. aeruginosa* too, with a percentage of identification between 83.8% and 99.9% (Table 1).

Also, 10 of 37 strains were identified as *Staphylococcus* spp. according to NF V 08-014: 1984 and one strain was identified as *S. aureus*. These results were confirmed by API Staph system with a percentage of identification between 57.2% and 99.9% (Table 2).

Finally 14 strains were identified as species of *Enterobacteriaceae* by NF V08-015 and API 20 E system with a percentage of identification between 43.0% and 99.1% (Table 3).

The results reported in Figure 1, show the number of bacteria isolated from different surfaces: stainless steel, porcelain, polypropylene and granite. These results show that the bacteria of the species of *Enterobacteriaceae* and *Pseudomonas* are more abundant on polypropylene and granite surfaces and those of *Staphylococcus* are more abundant on the porcelain and stainless steel surfaces. Moreover, if we take into account all individual species, we notice that the polypropylene is the most colonized by these bacteria in the order of (38%), followed by stainless steel (24%) and porcelain (22%) and finally granite (16%).

Typically the polypropylene is the substratum that builds the cutting board used in almost every kitchen that will be collective or domestic. These cutting boards are a synthetic polymer which exhibits an important roughness which makes it the most susceptible material to be colonized by bacteria. On the other hand, the difference in level of physicochemical properties between substrates could explain the high percentage of attached bacteria on polypropylene.

Evaluation of bacterial surface hydrophobicity and electron donor/acceptor character

The surface hydrophobicity ΔG_{iwi} and the electron donor (γ^+) / electron acceptor (γ^-) character of all bacteria were analyzed and listed in: Table 4 for *Pseudomonas* spp., Table 5 for *Staphylococcus* spp. and Table 6 for *Enterobacteriaceae*.

The results presented in Table 4, reported that 46% of *Pseudomonas* spp. exhibit a hydrophobic character ($\Delta G_{iwi} < 0$), and 56% have a hydrophilic character ($\Delta G_{iwi} > 0$) with a marked hydrophobicity for *P. aeruginosa* (P15). Also, the results show that all strains have a high electron donor character and *P. aeruginosa* (P15) expressed the high electron donor ($\gamma^- = 105.4 \text{ mJ.m}^{-2}$). Compared to literature [21, 32], the results show that the cells surfaces for studied bacteria expressed a high electron acceptor character. Some *Pseudomonas* strains have a high electron acceptor character for example (P4, P5 and P12), and the other strains have a medium and low electron acceptor character.

The Table 5 shows that 40% of *Staphylococcus* spp. exhibit a hydrophobic character ($\Delta G_{iwi} < 0$), and the others were hydrophilic ($\Delta G_{iwi} > 0$). Moreover, all strains have a high electron donor character (high γ^-) and the maximum character was noted for *Staphylococcus* spp. (S6). ($\gamma^- = 59.2 \text{ mJ.m}^{-2}$). For electron acceptor character, similar results of *Pseudomonas* spp. were observed for *Staphylococcus* spp. (S8, S9, S17 and S18).

According to the (Table 6), 29% of *Enterobacteriaceae* were hydrophobic and 71% were hydrophilic. We observed that all the strains have a high electron donor character (high γ^-) and the strain E11 have a marked character ($\gamma^- = 63.1 \text{ mJ.m}^{-2}$). As already noted, the electron acceptor character is also marked for *Enterobacteriaceae*. *Enterobacter agglomerans* (E4) and *Enterobacter rcloace* (E10) have important values of the electron acceptor character.

Discussion

If we take account of the origin of bacteria we see that the isolated bacterial cells from porcelain before and after cleaning and disinfecting operations were all hydrophilic ($\Delta G_{iwi} > 0$) but a single strain of *Staphylococcus* spp. (S8) was hydrophobic ($\Delta G_{iwi} < 0$). In contrast, the other bacteria isolated from stainless steel, polypropylene and granite were hydrophilic and hydrophobic. In the light of the obtained results, we can see also that the level of hydrophobicity and electron donor/acceptor character change between same species. Moreover, no clear relation was obtained between origin and hydrophobicity or electron donor/acceptor character of all strains. This fact is corroborated by the results presented by Teixeira et al. [35] when they have determined the hydrophobicity of 10 strains of *P. aeruginosa*, based on contact angle measurements. They observed that each individual strain used had different degrees of hydrophobicity between genera of bacteria and strains of the same species. The same observations have been noted by Van der Mei et al. [32] when studying 142 isolates of various species

among them *P. aeruginosa*, *E. coli*, *Staphylococcus* spp., *Enterococci* and *Streptococci* and they reported that no clear generalizations were noted concerning the physico-chemical surface properties between strains. Also the same results were found by Flint et al. [43] when determining the hydrophobicity of 12 strains of *Streptococci* spp. and they observed that each individual of thermophilic *Streptococci* spp. had different degrees of hydrophobicity.

Conclusion

In this work, we have isolated, identified and determined the physicochemical properties of bacteria isolated from different materials commonly used in the catering kitchens in establishment health. These results emphasize that the level of hydrophobicity and electron donor/acceptor character changes between same species. It was the first time that the electron acceptor character is marked for most of the studied bacteria. Also we have noted that the polypropylene was the most colonized material compared to the other substratum.

Conflict of interest: none declared.

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Table 1. Various biochemical tests for the identification of *Pseudomonas* spp. strains

Code of strains	Automated microbiology instruments Reference BD-PHOENIX		Biochemical tests												
	Identification	Confidence value	Origin	Gram stain	test Oxidase	Catalase tests	Cetrimide agar	King B	King A	Lipase: ; Tween 80 hydrolysis test	Growth at 4 ° C	Growth at 42 ° C	Tests mobility	levan test	Profile API 20 NE
<i>P. aeruginosa</i> NCTC 10332 T								+	+	**	-	+	+	-	Witness (<i>P. Aeruginosa</i> ATCC 27853)
P1	**		Granite before C/D	bacillus -	+	+	+	+	-	-	-	+	+	+	<i>P. stutzeri</i> 83.8%
P3*	<i>P. aeruginosa</i>	99%	Granite before C/D	bacillus -	+	+	+	+	+	+	-	+	+	++	<i>P. aeruginosa</i> 98.5%
P4	**		Granite before C/D	bacillus -	+	-	+	-	-	-	-	-	+	+	<i>P. aureofaciens</i> 95.6%
P5*	**		Granite before C/D	bacillus -	+	+	+	+	+	+	-	+	+	+	<i>P. aeruginosa</i> 99.9%
P6	<i>P. aeruginosa</i>	99%	Granite before C/D	bacillus -	+	+	+	+	+	+	-	+	+	+	<i>P. aeruginosa</i> 99.9%
P7	**		Porcelain before C/D	bacillus -	+	+	+	+	+	+	-	+	+	++	<i>P. aeruginosa</i> 99.9%
P9	**		Stainless steel before C/D	bacillus -	+	+	+	+	+	+	-	-	+	+	<i>P. fluorescens</i> 99.6%
P11*	<i>P. aeruginosa</i>	99%	Polypropylene after C/D	bacillus -	+	+	+	+	+	-	-	+	+	++	<i>P. aeruginosa</i> 98.5%
P12	**		Polypropylene after C/D	bacillus -	+	+	+	+	+	-	-	+	+	+	<i>P. aeruginosa</i> 97.8%
P14	<i>P. aeruginosa</i>	99%	Polypropylene after C/D	bacillus -	+	-	+	+	+	-	-	+	+	-	<i>P. aeruginosa</i> 97.8%
P15	<i>P. aeruginosa</i>	99%	Polypropylene before C/D	bacillus -	+	-	+	+	+	-	-	+	+	-	<i>P. aeruginosa</i> 97.8%
P18	<i>P. aeruginosa</i>	99%	Polypropylene after C/D	bacillus -	+	-	+	+	+	-	-	+	+	++	<i>P. aeruginosa</i> 98.5%
P20	**		Polypropylene before C/D	bacillus -	+	-	+	-	-	-	-	-	+	-	<i>P. aureofaciens</i> 90%

** , tests not made; C/D, cleaning and disinfection.

Table 2. Various biochemical tests for the identification of *Staphylococcus* spp. strains

Code of strains	Origin	Gram stain	Oxidase test	Catalase tests	Tests mobility	DNase test	Coagulas Test	Mannitol degradation (Chapman Agar)	Profile API 20 staph
<i>S.aureus</i> ATCC		+	-	+	-	+	-	+	<i>Staph. aureus</i>
S1	Porcelain before C/D	+	-	+	-	-	-	-	<i>Staph. lentus</i> 96.2 %
S2	Porcelain after C/D	+	-	+	-	+	-	-	<i>Staph. xylosus</i> 89.6%
S6	Porcelain before C/D	+	-	+	-	-	-	-	<i>Staph. xylosus</i> 97.2%
S8	Porcelain before C/D	+	-	+	-	+	+	+	<i>Staph. aureus</i> 85.1%
S9	Polypropylene after C/D	+	-	+	-	-	-	-	<i>Staph. xylosus</i> 57.2%
S10	Polypropylene before C/D	+	-	+	-	-	-	-	<i>Staph. capitis</i> 79.2%
S17	Stainless steel before C/D	+	-	+	-	+	-	-	<i>Staph. xylosus</i> 99.4%
S18	Stainless steel after C/D	+	-	+	-	+	-	+	<i>Staph. xylosus</i> 99.9%
S19	Stainless steel before C/D	+	-	+	-	-	-	-	<i>Staph. sciuri</i> 94.2%
S20	Stainless steel before C/D	+	-	+	-	-	-	-	<i>Staph. xylosus</i> 99.7%

C/D, cleaning and disinfection.

Table 3. The various biochemical tests for the identification of *Enterobacteriaceae* spp. strains

Code of strains	Automated microbiology instruments reference BD-PHONIX		Biochemical tests											
	identification	Confidence value	Origin	Gram stain	Oxidase test	Catalase tests	Tests mobility	IMVIC TEST				Profile API 20 E		lactose fermentation (+) (BCP)
								Indole	Methyl red	Vosges Proskaner	Citrate	Identification	% confidence	
E3	<i>E. coli</i>	0%	porcelain before C/D	-	+	+	-	-	+	-	+	<i>Enterobacter cloace</i>	99.1	+
E4	**		stainless steel before C/D	-	+	+	-	-	+	-	+	<i>enterobacter agglomerans</i>	58.5	+
E5	**		stainless steel before C/D	-	+	+	-	-	+	-	-	<i>Enterobacter cloace</i>	86.6	-
E6	**		Polypropylene after C/D	-	+	+	-	-	+	-	+	<i>Enterobacter agglomerans 1</i>	43.0	-
E7	<i>Citrobacter Freundii</i>	99%	Polypropylene before C/D	-	+	-	-	-	+	-	-	<i>Citrobacter freundii</i>	99.0	+
E8	**		polypropylene after C/D	-	-	+	-	-	+	-	+	<i>Enterobacter cloace</i>	86.6	-
E9	**		polypropylene after C/D	-	+	+	-	-	+	-	+	<i>Enterobacter cloace</i>	86.6	-
E10	**		polypropylene before C/D	-	+	-	-	-	+	-	+	<i>Enterobacter cloace</i>	86.6	-
E11	**		granite after C/D	-	-	+	-	-	+	-	+	<i>Tatumella ptyseos</i>	89.7	-
E14	**		porcelain before C/D	-	-	+	-	-	+	-	+	<i>Chromobacterium.violaceum</i>	95.2	-
E15	**		porcelain before C/D	-	-	+	-	-	+	-	+	<i>chryseomonas luteola</i>	96.2	-
E18	**		polypropylene after C/D	-	+	+	-	-	+	-	+	<i>Enterobacter.annigenus 2</i>	97.7	-
E19	**		stainless steel before C/D	-	+	-	-	-	+	-	+	<i>Enterobacter sakazakii</i>	97.9	-
E20	**		stainless steel before C/D	-	+	+	-	-	+	-	+	<i>Enterobacter cloace</i>	86.6	-
Witness, <i>E.coli</i>	**			-	+	+	-	-	+	-	+	<i>E. coli</i>	97.7	-

** , tests not made; C/D, cleaning and disinfection.

Table 4. Contact angles (in degrees) of water (θ_w), formamide (θ_f), diiodomethane (θ_o), the surface tension of Lifshitz-van der Waals (γ^{LW}), electron-donor (γ^-), electron-acceptor (γ^+) of *Pseudomonas* spp. strains and their free energy of interaction with water (ΔG_{iwi})

Strains	Contact angles			Tension de surface ($mJ \cdot m^{-2}$)			$\Delta G_{iwi} (mj/m^2)$
	θ diiomethane	θ formamide	θ water	γ^{LW}	γ^+	γ^-	
P1 <i>P.stutzeri</i>	98.7(0.4)	54.9(0.3)	39.6(0.2)	9.2(0.1)	6.0(0.1)	56.4(0.4)	20.3
P3 <i>P. aeruginosa</i>	104.6(0.3)	44.1(0.4)	44.9(0.3)	7.1(0.1)	14.7(0.3)	36.3(0.5)	-3.0
P4 <i>P. aureofaciens</i>	114.6(1.1)	36.6(0.5)	31.9(0.3)	4.3(0.2)	21.3(0.8)	46.1(0.1)	-10.2
P5 <i>P.aeuruginosa</i>	131.8(0.3)	31.8(0.2)	35.4(0.2)	1.4(0.1)	34.6(0.2)	37.4(0.2)	-27.6
P6 <i>P.aeuruginosa</i>	98.1(0.4)	61.5(0.2)	34.6(1.7)	9.4(0.1)	2.9(0.2)	74.1(2.6)	42.6
P7 <i>P.aeuruginosa</i>	91.5(0.6)	46.0(1.0)	39.3(0.1)	12.0(0.3)	7.6(0.7)	47.5(1.4)	14.2
P9 <i>P.fluorescens</i>	75.2(0.6)	20.3(0.3)	11.7(0.3)	20(0.3)	7.3(0.2)	55(0.3)	22.2
P11 <i>P. aeruginosa</i>	70.1(0.5)	47.4(0.2)	25.3(0.2)	22.8(0.3)	1.0(0.1)	69.1(0.5)	52.9
P12 <i>P.aeuruginosa</i>	114.8(0.0)	36.0(0.1)	25.0(0.2)	4.3(0.0)	20.6(0.1)	53.2(0.)	-8.7
P14 <i>P. aeruginosa</i>	112.5(0.1)	51.6(0.2)	42.7(0.5)	4.8(0.0)	13.4(0.2)	46.2(0.7)	-2.3
P15 <i>P. aeruginosa</i>	111.5(0.2)	85.8(1.8)	41.8(0.4)	5.1(0.1)	0.1(0.1)	105.4(4.4)	86.3
P18 <i>P.aeuruginosa</i>	115.6(0.2)	41.2(0.4)	27.4(0.2)	4.1(0.1)	18.3(0.4)	55.6(0.3)	-6.4
P20 <i>P.aureofaciens</i>	86.4 (1.3)	59.9 (1.2)	48.7 (0.5)	14.3(0.6)	16.8(15.8)	34.9(16.0)	1.8

Standard deviation was given in parentheses.

Table 5. Contact angles (in degrees) of water (θ_w), formamide (θ_f), diiodomethane (θ_D), the surface tension of Lifshitz-van der Waals (γ^{LW}), electron-donor (γ^-), electron-acceptor (γ^+) of *Staphylococcus* spp. strains and their free energy of interaction with water (ΔG_{iwi}).

Strains	Contact angles			Tension de surface ($mJ \cdot m^{-2}$)			$\Delta G_{iwi} (mj/m^2)$
	θ diiométhane	θ formamide	θ water	γ^{LW}	γ^+	γ^-	
S1 <i>Staph.lentus</i>	96.4 (0.5)	44.5 (0.4)	40.9 (0.3)	10.0(0.2)	10.1(0.4)	42.9(0.7)	6.88
S2 <i>Staph.xylosus</i>	70.8 (0.1)	38.5 (0.3)	31.5 (0.2)	22.5(0.1)	3.2(0.1)	51.7(0.5)	27.91
S6 <i>Staph. xylosus</i>	80.6 (0.1)	58.4 (0.2)	41.7 (0.2)	17.2(0.1)	1.1(0.0)	59.2(0.6)	41.71
S8 <i>Staph. aureus</i>	115.3 (0.2)	33.5 (0.1)	45.7 (1.1)	4.2(0.1)	26.9(0.2)	26.9(1.4)	-13.58
S9 <i>Staph. xylosus</i>	120.2 (1.4)	38.2 (0.9)	51.2 (0.2)	3.2(0.3)	28.4(1.5)	22.8(0.9)	-16.14
S10 <i>Staph.capitis</i>	95.0 (0.2)	36.5 (0.8)	32.7 (0.4)	10.6(0.1)	11.9(0.5)	46.5(1.2)	7.42
S17 <i>Staph. xylosus</i>	114.0 (0.4)	36.6 (0.6)	28.2 (0.4)	4.5(0.1)	20.3(0.2)	50.5(0.6)	-8.33
S18 <i>Staph. xylosus</i>	129.8 (0.2)	36.6 (0.3)	42.6 (0.2)	2(0.0)	32(0.1)	32(0.3)	-24.1
S19 <i>Staph.sciuri</i>	58.8 (0.4)	37.0 (0.2)	31.2 (0.7)	29.2(0.2)	1.5(0.1)	51.4(1.0)	31.4
S20 <i>Staph. xylosus</i>	88.0 (2.6)	46.3 (0.3)	36.1 (0.2)	13.7(1.2)	5.8(0.8)	52.1(0.4)	21.1

Standard deviation was given in parentheses.

Table 6. Contact angles (in degrees) of water (θ_w), formamide (θ_f), diiodomethane (θ_D), the surface tension of Lifshitz-van der Waals (γ^{LW}), electron-donor (γ^-), electron-acceptor (γ^+) of *Enterobacteriaceae* spp. strains and their free energy of interaction with water (ΔG_{iwi}).

Strains	Contact angles			Tension de surface ($mJ \cdot m^{-2}$)			$\Delta G_{iwi} (mj/m^2)$
	θ diiométhane	θ formamide	θ water	γ^{LW}	γ^+	γ^-	
E3 <i>Enterobacter cloace</i>	97.0 (0.4)	1.1	27.1 (0.4)	9.8 (0.2)	17.1 (0.1)	43.6 (0.2)	1.1
E4 <i>Ent.agglomerans</i>	101.3 (0.5)	-3.4	21.0 (0.4)	8.2 (0.2)	21.1 (0.3)	44.8 (0.4)	-3.4
E5 <i>Enterobacter cloace</i>	100.8 (0.4)	-0.8	17.2 (0.4)	8.4 (0.2)	19.1 (0.3)	49.6 (0.2)	-0.8
E6 <i>Ent.agglomerans 1</i>	101.0 (0.4)	-0.8	20.4 (0.3)	8.3 (0.1)	15.4 (0.2)	54.3 (0.5)	-0.8
E7 <i>Citrobacter freundii</i>	100.5(0.6)	12.3	26.0(0.6)	8.5 (0.2)	10.9 (0.2)	59.1 (0.9)	12.3
E8 <i>Enterobacter cloace</i>	59.7(1.6)	23.7	30.2(0.5)	28.8 0.9)	2.7 (0.5)	47.1 (1.3)	23.7
E9 <i>Enterobacter cloace</i>	99.9(0.3)	3.3	25.9(0.5)	8.7 (0.1)	15.3 (0.1)	49.7 (0.6)	3.3
E10 <i>Enterobacter cloace</i>	100.1(0.3)	-3.1	29.6(0.2)	8.7 (0.1)	20.1 (0.2)	39.4 (0.2)	-3.1
E11 <i>Tatumella ptyseos</i>	106.5(0.2)	9.8	25.1(0.4)	6.5 (0.1)	11.8 (0.1)	63.1 (0.4)	9.8
E14 <i>Chnomo.violceum</i>	90.6(0.2)	17.7	32.5(1.0)	12.5 (0.1)	7.8 (0.0)	52.3 (0.7)	17.7
E15 <i>Chryseomonas luteola</i>	90.8(0.3)	6.2	44.7(0.4)	12.3 (0.1)	8.8 (0.1)	37.3 (0.4)	6.2
E18 <i>Enterobacter.annigenus 2</i>	101.4(0.8)	3.9	42.3(1.5)	8.2 (0.2)	11.2 (0.2)	42.9 (1.8)	3.9
E19 <i>Enterobacter sakazakii</i>	90.3(0.1)	4.8	33.7(0.2)	12.6 (0.1)	8.9 (0.1)	35.0 (13.2)	4.8
E20 <i>Enterobacter cloace</i>	92.2(0.3)	22.5	35.8(0.4)	11.7 (0.2)	5.8 (0.1)	55.7 (0.4)	22.5

Standard deviation was given in parentheses.

