

Surface properties and behaviour on abiotic surfaces of *Staphylococcus carnosus*, a genetically homogeneous species

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Received 3 December 2005; received in revised form 23 March 2006; accepted 23 March 2006

Available online 25 April 2006

Abstract

This work aimed to characterize the surface properties of *Staphylococcus carnosus* and the influence of different media on their ability to adhere and grow on industrial supports. As their colonization could be dependant of the strain, the genetic diversity of the strains was studied. The diversity of 13 strains analysed by pulsed-field gel electrophoresis revealed that the *S. carnosus* strains formed a homogeneous genetic group. Their surface properties, characterized by studying their affinity to solvents, were hydrophilic with a strong negative surface charge. The *S. carnosus* strain CIT 833 hardly adhered to polytetrafluoroethylene (PTFE) and stainless steel chips. Tryptic soy broth (TSB) was the most favourable medium for growth on stainless steel support while TSB/NaCl was better for growth on PTFE. Scanning electron microscopy (SEM) showed that this strain weakly colonized both supports and did not form cell aggregates. Indeed, the strain did not synthesize polysaccharides. These results showed that *S. carnosus* adhered on different abiotic surfaces which are used in food factories but was not able to accumulate on these surfaces. The inability of *S. carnosus* to form biofilm could explain why *S. carnosus* is rarely isolated in meat processing environment.

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Keywords: Genetic diversity; Surface properties; Bacterial colonization; Scanning electron microscopy; Polysaccharides

1. Introduction

The name of *Staphylococcus carnosus* is derived from its occurrence in meat products (Schleifer and Fischer, 1982) but its natural habitat is not determined although it might be expected to be in relation with animals and their products. It is used as a starter culture in combination with lactic acid bacteria for sausage manufacturing in which it contributes to the development of sensorial qualities. In sausage, it reduces nitrate to nitrite, which plays a role in the colour of the products. It produces anti-oxidant enzymes, catalase and superoxide dismutase, that prevent rancidity in food products (Barrière et al., 2001). It contributes to the characteristic flavour of sausage by

degrading branched chain amino acids into aromatic compounds: methyl aldehydes, methyl alcohols and methyl acids. It also synthesizes methyl ketones involved in the flavour of sausage (Talon et al., 2002).

The survival of staphylococci in food processing environments is frequently mentioned. Their survival in a food environment could be associated to their capacity to colonize abiotic surfaces (Gerke et al., 1998; Cramton et al., 1999; Moretto et al., 2003). In food industry, biofilm formation may be undesirable for hygienic and safety reasons because of attachment of food spoilage or pathogenic or opportunistic pathogenic micro-organisms to food or food surfaces. Nevertheless, in food systems, colonization of surface by bacteria used as starters, such as *S. carnosus*, *Staphylococcus xylosus* and *Staphylococcus equorum* or by the saprophytic species *Staphylococcus sciuri*, could be desirable to inhibit the colonization by

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spoilage or pathogenic bacteria. Among these species, *S. sciuri* and *S. xylosus* were described as biofilm positive in abiotic surface usually found in food environment (Leriche and Carpentier, 2000; Norwood and Gilmour, 2001). Furthermore, *S. sciuri* biofilm was able to prevent adhesion and growth of *Listeria monocytogenes* to stainless steel surfaces (Briandet, 1999; Leriche and Carpentier, 2000). Concerning *S. carnosus*, only one strain, the UT TM300, has been studied and described as “non-biofilm forming” on plastic and glass supports (Gross et al., 2001; Götz, 2002). But the behaviour of this species is not known in industrial supports such as stainless steel and polytetrafluoroethylene (PTFE, teflon). Moreover, it is well established that the formation of a biofilm is often dependant of the strain inside the same species (Moretro et al., 2003).

Colonization starts by bacterial adhesion to solid surfaces. This adhesion depends partly on the nature of the surfaces and partly on the surface properties of the bacteria such as Van der Waals forces, polar or Lewis acid-base and electrostatic interactions (Bellon-Fontaine et al., 1996). The physiochemical surface properties of bacteria are influenced by the culture conditions (Donlan, 2002). In particular the ionic strength of the media modified either by adding sodium chloride or divalent cations played a role in the rate of biofilm formation in *Staphylococcus aureus* or *Staphylococcus epidermidis* (Dunne and Burd, 1992). Furthermore, these cations enhanced the production of polysaccharides by *S. epidermidis* (Ozerdem Akpolat et al., 2003). These exopolysaccharides are often associated to bacteria growing in biofilm in which they play a key role in the architecture (Donlan, 2002).

The aim of this work was to characterize the surface properties of *S. carnosus* and the influence of different media on their ability to adhere and grow on industrial supports. As their colonization could be dependant of the strain, the genetic diversity of the strains was also studied.

2. Materials and methods

2.1. Macrorestriction typing of the strains

Thirteen strains of *S. carnosus*, all isolated from sausages but from different collections and from different sampling periods, were studied (Table 1). Among these strains, the “non-biofilm forming” UT TM300 strain was included (Götz, 2002). The strains were grown at 37 °C in Brain Heart Infusion broth (Difco, Le pont de Claix, France). A suspension of Staphylococcal culture at optical density of 1 at 600 nm was centrifuged and resuspended in 0.5 volume of TEE buffer (Tris 10 mmol l⁻¹ pH 9, EDTA 100 mmol l⁻¹, EGTA 10 mmol l⁻¹). The suspension of bacteria was mixed in a ratio of 1:1 with 1.6% low-melting-point agarose (GIBCO BRL, Cergy Pontoise, France). Agarose plugs were incubated with 40 µg ml⁻¹ of lysostaphin (Sigma, St. Quentin, France), 0.05% sarkosyl (Fluka) and 5 mg ml⁻¹ of lysozyme (Eurobio, les Ullis, France) at 37 °C for 2 h. Then, they were lysed overnight in TEE buffer containing 1% of sodium dodecyl sulfate and 1 mg ml⁻¹ of proteinase K (Eurobio) at 55 °C. Plugs were washed 3 times for 1 h each, once in TE buffer (Tris 10 mmol l⁻¹ pH 8, EDTA 1 mmol l⁻¹) containing 20 mmol l⁻¹ of phenylmethylsulfonyl fluoride (Sigma) and twice in TE buffer. They were stored at 4 °C until restriction. DNA within half a plug was digested by 15 U of *Apa*I or *Sma*I (Promega, Lyon Charbonnières, France) for 16 h at 37 °C or 25 °C, respectively. Pulse field gel electrophoresis was performed in 1% agarose using a CHEF-DRIII apparatus (Bio-Rad, Ivry, France) in 0.5 × Tris-borate-EDTA buffer (TBE) at 6 V cm⁻¹ at 14 °C. Pulse times increased from 10 to 30 s over 23 h. Lambda ladder (Promega) was used as molecular weight marker. Gels were stained with ethidium bromide and digitalized with the Gel Doc 2000 apparatus (Bio-Rad).

Table 1
Percentage of affinity to the solvents of *S. carnosus* strains

<i>S. carnosus</i>	Sampling date	Chloroform (%)	Hexadecane (%)	Ethyl acetate (%)	Decane (%)
DSM 20501 ^a	1982	95 ± 4	25 ± 4	8 ± 3	33 ± 6
UT TM 300 ^b	1983	94 ± 3	18 ± 5	7 ± 4	32 ± 2
CIT 833 ^c	1997	95 ± 3	25 ± 3	12 ± 3	33 ± 5
CIT 836	1997	95 ± 2	17 ± 1	5 ± 2	30 ± 3
CIT S01014	2002	90 ± 8	20 ± 4	9 ± 3	24 ± 3
CIT S01015	2002	99 ± 1	32 ± 5	10 ± 2	41 ± 4
CIT S01016	2002	98 ± 2	21 ± 4	17 ± 5	43 ± 4
CIT S01017	2002	94 ± 2	27 ± 6	12 ± 4	36 ± 3
CIT S01018	2002	92 ± 6	23 ± 3	8 ± 4	34 ± 6
UB M427 ^d	1995	96 ± 2	20 ± 4	11 ± 1	49 ± 14
UB M429	1995	97 ± 3	17 ± 4	7 ± 5	47 ± 13
UB M431	1995	98 ± 1	17 ± 4	8 ± 3	50 ± 10
UB M433	1995	99 ± 0	19 ± 2	13 ± 4	51 ± 6

Values are means ($n = 6$), ± standard deviations.

^aDSM: Deutsche Sammlung von Mikroorganismen.

^bUT: University of Tübingen.

^cCIT: Collection of INRA Theix.

^dUB: University of Bath.

2.2. Affinity of the strains for the solvents

The Microbial Adhesion To Solvents (MATS) method which compares the microbial cell affinities for monopolar and non-polar solvents was used according to Bellon-Fontaine et al. (1996). Two pairs of solvents were used as follows: (i) chloroform (acidic solvent and electron-acceptor) and hexadecane (non-polar *n*-alkane solvent); (ii) ethyl acetate (basic solvent and electron-donor) and decane (non-polar *n*-alkane solvent). The first pair determined the Lewis basic character of the strain and the second the Lewis acid character of the strain.

Three successive subcultures were performed for all strains in the same conditions: orbital shaking (150 rpm) at 30 °C for 16 h in Tryptic Soy Broth (TSB) supplemented with 6 g l⁻¹ of yeast extract (TSB, Difco, Le pont de Claix, France). The third subculture was collected in stationary phase, washed and resuspended in saline water (SW, 8.5 g l⁻¹ NaCl) to give an OD = 0.6–0.7 at 400 nm (*A*₀). In all, 2.4 ml of this suspension were vortexed for 60 s with 0.4 ml of solvent. The mixture was stored 15 min at room temperature to allow the separation of two phases. OD_{400 nm} was measured in 1 ml of aqueous phase (*A*). The percentage of cells adherent to each solvent was calculated by % Affinity = $[1 - (A/A_0)] \times 100$. Experiments were performed in triplicate with two repetitions for each solvent.

2.3. Electrophoretic mobility

The surface charge was determined by the electrophoretic mobility. Bacterial cells of the third subculture of *S. carnosus* strain CIT 833 were washed in SW and then suspended at a final concentration of 10⁷ colony forming unit (cfu) per ml in a 1.5 mmol l⁻¹ NaCl solution (pH 5.7). The pH was adjusted within the range of 2–8 by adding nitric acid (0.01 mol l⁻¹) and potassium bromide (0.01 mol l⁻¹). The electrophoretic mobility was measured in a 50-V electric field using a laser Zetameter (CAD Instrumentation, Limours, France) coupled to a BX 40 microscope (Olympus) and to an image analysis system. Experiments were performed in duplicate with two repetitions.

2.4. Adhesion and growth on stainless steel and PTFE chips by the *S. carnosus* strain CIT 833

The study was carried out on two supports, stainless steel chip (AISI 304, hydrophilic, 4.5 cm²) and PTFE chip (hydrophobic, 3.0 cm²). These two supports are commonly used in food plants.

The third bacterial subculture was diluted in the culture medium to obtain an OD₆₀₀ of 0.6–0.7. Seven millilitres of this bacterial suspension were poured into a small Petri dish containing one chip of PTFE or stainless steel. After a 2 h-incubation at 30 °C, the bacterial suspension was removed and replaced by 7 ml of sterile culture medium.

Incubation lasted for 48, 72 and 120 h at 30 °C, with renewing the medium every 24 h.

Cell adhesion after 2 h-incubation and growth after 48, 72, and 120 h-incubation at 30 °C were evaluated by cell numeration on the chip. After incubation, the chips were washed twice with 35 ml of sterile tryptone salt (TS: 1 g l⁻¹ bactotryptone and 8.5 g l⁻¹ NaCl) on an orbital shaking table (Belly Dancer, Greensboro, USA) to remove non-adherent cells. Adherent cells were detached in 10 ml of TS in a sonication bath (Deltasonic, Meaux, France) for 3 min at 50 Hz. Serial dilutions were plated on tryptic soy agar (TSA, Difco, Le pont de Claix, France) and incubated for 24 h at 37 °C. Cell adhesion was evaluated by cell numeration and expressed by the number of cfu by square centimeter of chip (cfu/cm²).

Adhesion and growth were studied in the complex medium TSB, or TSB supplemented with CaCl₂ 0.03% (w/v) and MgSO₄ 0.05% (w/v) (Prolabo, Paris, France) TSB/Ca–Mg, or TSB supplemented with NaCl 2% (w/v) TSB/NaCl, and in the chemical defined media: MCDB medium (Molecular Cellular Development of Biology 202, CryoBiosystem, L'Aigle, France) supplemented with 1% yeast nitrogen base without amino acids (Difco, Le pont de Claix, France) and Hussain medium (Hussain et al., 1991) with or without addition of NaCl 1% (w/v).

For one experiment, adhesion was done in SW during 2 h and then the bacterial suspension was removed and replaced by TSB for 48 h or 120 h growth at 30 °C. This last condition was noted TSB/SW.

Experiments were performed in duplicate, with three stainless steel chips and three PTFE chips per incubation time for each experiment.

2.5. Polysaccharide assay

The *S. carnosus* strain CIT 833 was grown in TSB for 48 h or 120 h on stainless steel strips as described above. The adhered cells were detached from strips by sonication (4 min at 30 °C) in SW and population was numerated on TSA. The sonicated suspension was centrifuged at 20 000g for 30 min at 4 °C and the supernatant was filtered on a 0.45 µm membrane. Then the samples were concentrated by centrifugation (3000g, 40 min) on a 10 kDa ultrafiltration membrane (Centricon, Millipore). The quantity of polysaccharides was assayed on the concentrated samples by the method of Dubois et al. (1956). Results were expressed in µg of polysaccharides per log 10⁸ cfu, and represented the mean of six measures from three independent cultures.

2.6. Observations of the colonization by scanning electron microscopy (SEM)

The *S. carnosus* strain CIT 833 was grown on PTFE and stainless steel chips, in TSB during 48 h at 30 °C. Non-adherent cells were removed from the surfaces by washing in TS. Adherent cells were fixed on the strips with a solution of 3% glutaraldehyde in 0.2 mol l⁻¹ sodium

cacodylate buffer (pH 7.4) for 1 h and washed in this buffer three times for 10 min each. After post-fixation for 30 min with osmic acid vapours, strips were rinsed in cacodylate buffer three times for 10 min each. Cells were dehydrated using a graded ethanol series (70%, 95% and 100%), three baths of 10 min each and subjected to an acetone dehydration series of 30%, 50%, 70% acetone, three baths of 15 min each and three baths in 100% acetone for 10 min each. Strips were coated with gold and observed with a scanning electron microscope (Philips SEM 505) at 25 keV.

3. Results

3.1. Macrorestriction analysis of the *S. carnosus* strains

Macrorestriction analysis by pulsed-field gel electrophoresis showed that the 13 strains of *S. carnosus* had identical patterns either after *ApaI* or *SmaI* restriction (Fig. 1A and B). The 13 strains isolated of diverse geographical origins by different laboratories over a period of several years as mentioned in Table 1 were genetically very homogeneous.

3.2. Physicochemical surface properties

The 13 strains shared similar affinity for the polar and apolar solvents used (Table 1). They had a strong affinity to the chloroform, an acidic solvent and an electron acceptor. Their adhesion to chloroform was higher than to hexadecane, two solvents with similar Van der Waals properties. Moreover, the strains had a low affinity for ethyl acetate, which is a strong basic solvent and an electron donor. These data showed that the *S. carnosus* strains had hydrophilic properties with a marked basic character surface, i.e. an electron donor character.

All the *S. carnosus* strains, including the UT TM300 strain, were genetically homogeneous and showed identical surfaces properties. Consequently, one strain, the *S. carnosus* strain CIT 833 isolated by our laboratory was selected to be representative of the whole *S. carnosus* group.

The electrophoretic mobility was measured at various pHs ranging from 2 to 8 for *S. carnosus* strain CIT 833 to determine the cell surface net charge (Fig. 2). This strain presented a maximum electronegativity at pH 4, reaching value of $-3.17 \mu\text{m V}^{-1} \text{s}^{-1} \text{cm}^{-1}$. This negative surface charge progressively decreased with more acidic pH to reach a neutral surface charge at pH 2. The electrophoretic mobility was checked for two other strains at pH 5.0, they had a mobility of $-2.49 (\pm 0.31) \mu\text{m V}^{-1} \text{s}^{-1} \text{cm}^{-1}$ for the reference strain 20501 and $-2.69 (\pm 0.37) \mu\text{m V}^{-1} \text{s}^{-1} \text{cm}^{-1}$ for the strain TM300. Thus, at this pH no significant difference was noticed between the 3 strains of *S. carnosus* confirming the choice of one strain representative of the group for the following study.

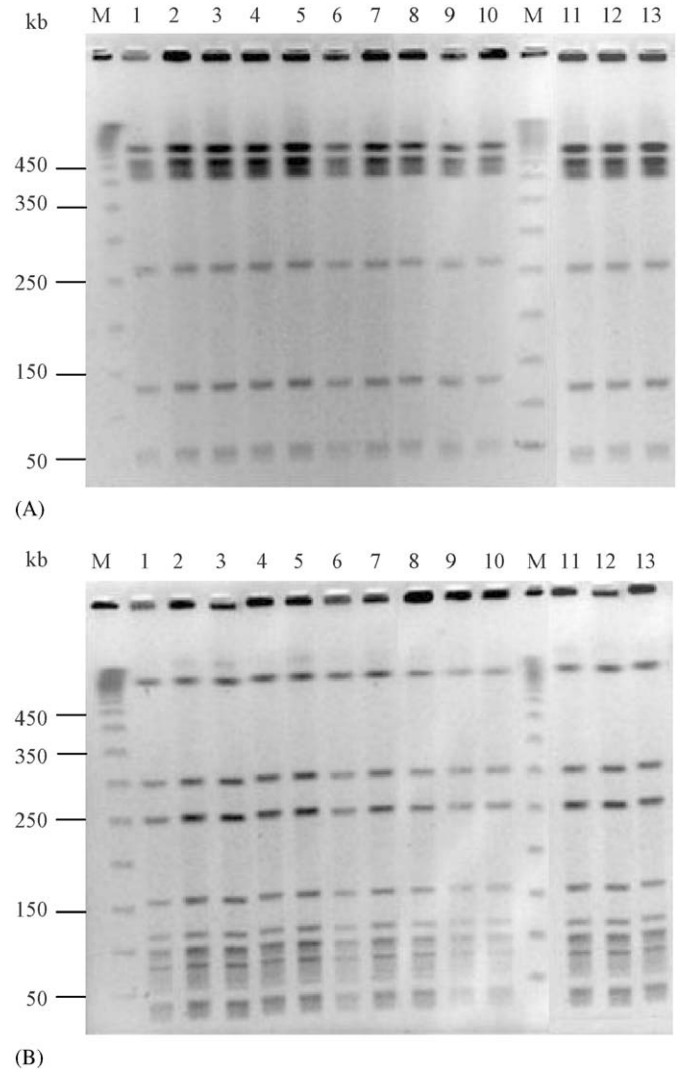


Fig. 1. PFGE profiles of *ApaI* (A) and *SmaI* (B) restricted genomic DNA of *Staphylococcus carnosus* strains. Lanes M: Lambda ladder (Promega); lane 1: CIT 833; lane 2: DSM 20501; lane 3: CIT S01014; lane 4: CIT S01015; lane 5: CIT S01018; lane 6: UB M427; lane 7: UT TM300; lane 8: UB M431; lane 9: UB M429; lane 10: CIT 836; lane 11: UB M433; lane 12: CIT S01016; lane 13: CIT S01017.

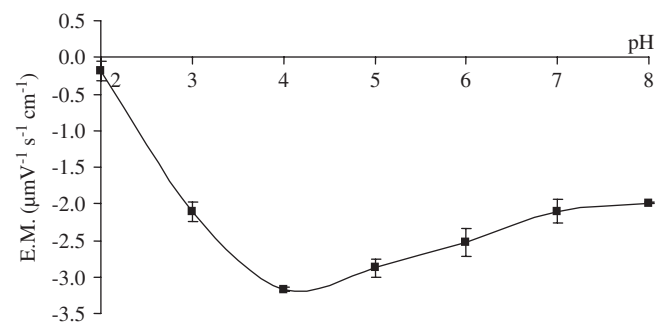


Fig. 2. Electrophoretic mobility (EM) of the *S. carnosus* strain CIT 833 (—■—) with standard deviation.

3.3. Adhesion and growth on stainless steel and PTFE chips by the *S. carnosus* strain CIT 833

The *S. carnosus* strain CIT 833 did not adhere on PTFE chip except in the Hussain medium with salt (Table 2). Adhesion was moderate on stainless steel, reaching 17% in MCDB and 15% in Hussain media but it was very low in TSB one. Addition of NaCl, to TSB or Hussain media, improved adhesion on stainless steel. Similarly, the adhesion realized in SW reached 30% on this support. But addition of divalent cations to TSB did not improve the adhesion.

The growth of *S. carnosus* was different on the two supports and also varied according to the media (Table 3). On PTFE chip, growth of 1.2 and 1.5 log was observed in TSB/SW and TSB/NaCl, respectively after 48 h of

incubation. Then the population remained stable. No significant growth was noticed in MCDB and in Hussain media with or without NaCl. In TSB and TSB/Ca–Mg medium, the population increased of 0.6 and 1.0 log, respectively, in a progressive way during all the incubation.

On stainless steel chip, the opposite behaviour of the strain was noticed in the media TSB/NaCl and TSB/SW, the population dropped of 1.5 log until 48 h and then was stable until 120 h (Table 3). In MCDB and Hussain media, a slight decline (–0.5 log) was noticed at 48 h and the population stayed constant up to 120 h. In Hussain medium with NaCl, no growth was noticed as observed on PTFE chip. In TSB and TSB/Ca–Mg media, the growth of the strain, with a progressive increase of about 1.0 log during all the incubation, was quite similar to the one observed on PTFE.

Table 2
Adhesion (2 h) of the *S. carnosus* strain CIT 833 to stainless steel and PTFE chips

Media	Support	Stainless steel		PTFE	
	Bacteria loaded	Adhered	% Adhesion ^a	Adhered	% Adhesion
<i>Complex media^b</i>					
TSB	10.0	0.1 (0.03) ^c	1	0.1 (0.07)	1
TSB/SW	9.0	2.8 (0.7)	31	0.01 (0.00)	0.1
TSB/NaCl	9.0	2.9 (0.8)	32	0.02 (0.01)	0.2
TSB/Ca–Mg	9.0	0.2 (0.1)	2	0.1 (0.06)	1
<i>Chemical defined media^b</i>					
MCDB	10.0	1.7 (0.5)	17	0.5 (0.2)	5
Hussain	6.8	1.0 (0.4)	15	0.4 (0.03)	6
Hussain/NaCl	7.9	1.6 (0.8)	20	1.7 (0.3)	22

^aAdhesion was calculated by the following equation: adhesion = (bacteria adhered/bacteria loaded) × 100, bacteria adhered is the population expressed as cfu × 10⁷/cm² of chip after 2 h of adhesion, bacteria loaded is the initial population loaded on the chip expressed as cfu × 10⁷/cm² of chip.

^bTSB, tryptic soy broth with yeast extract, TSB/SW adhesion done in saline water (SW) and growth in TSB, MCDB, Molecular Cellular Development of Biology 202 supplemented with 1% yeast nitrogen base without amino acids.

^cStandard deviation, *n* = 6 values.

Table 3
Growth of the *S. carnosus* strain CIT 833 to stainless steel and PTFE chips in different media

Support	Stainless steel				PTFE	
	2	48	120	2	48	120
<i>Complex media^a</i>						
TSB	5.92 ^b (0.16) ^c	6.48 (0.10)	7.08 (0.13)	6.12 (0.29)	6.59 (0.24)	6.75 (0.02)
TSB/SW	7.41 (0.11)	6.36 (0.20)	6.22 (0.42)	5.18 (0.41)	6.03 (0.31)	6.38 (0.24)
TSB/NaCl	7.46 (0.11)	6.39 (0.44)	6.63 (0.23)	5.08 (0.39)	6.54 (0.31)	6.36 (0.24)
TSB/Ca–Mg	6.29 (0.22)	6.45 (0.15)	7.23 (0.26)	5.96 (0.25)	6.30 (0.19)	7.01 (0.07)
<i>Chemical defined media^a</i>						
MCDB	7.23 (0.11)	6.66 (0.18)	6.67 (0.16)	6.69 (0.14)	6.85 (0.04)	6.50 (0.28)
Hussain	7.02 (0.16)	6.56 (0.18)	6.65 (0.11)	6.60 (0.03)	6.70 (0.14)	6.70 (0.34)
Hussain/NaCl	7.20 (0.22)	7.34 (0.11)	7.17 (0.02)	7.23 (0.08)	6.79 (0.16)	7.17 (0.05)

^aTSB, tryptic soy broth with yeast extract, TSB/SW adhesion done in saline water (SW) and growth in TSB, MCDB, Molecular Cellular Development of Biology 202 supplemented with 1% yeast nitrogen base without amino acids.

^bMean of six chips (log cfu cm^{–2}).

^cStandard deviation, *n* = 6 values.

3.4. Synthesis of exopolysaccharides

The quantity of polysaccharides linked to the *S. carnosus* strain CIT 833 cells was weak and did not vary during all the time of incubation, it was 11.5 ± 1.7 , 10.9 ± 4.4 , $15.8 \pm 6.8 \mu\text{g}$ per $\log 10^8$ cfu, after 2, 48 and 120 h of incubation, respectively. So, this strain did not synthesize polysaccharides during its growth on stainless steel.

3.5. SEM observations

These observations showed that the *S. carnosus* strain CIT 833 weakly colonized the surface of the two supports after 48 h of incubation (Fig. 3). Cells were organized in tetrads, in pairs or single on stainless steel (Fig. 3a) and PTFE (Fig. 3b) chips. Cells were isolated and did not aggregate between them. No mucous matrix was visualized confirming the absence of exocellular polymers. Cells of *S. carnosus* strain CIT 833 were not able to aggregate between them to build a three-dimensional structure with multi-layer of cells.

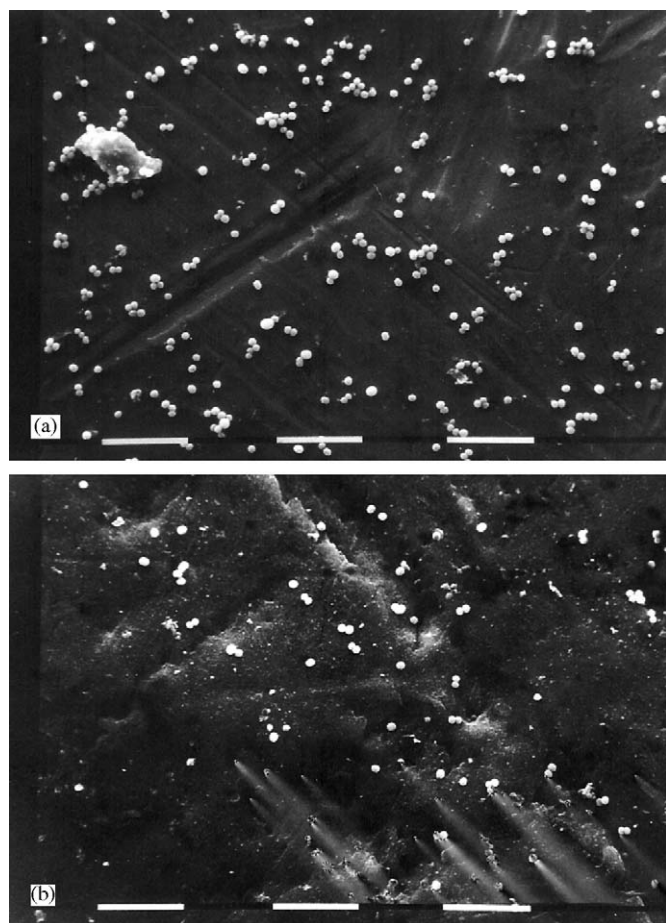


Fig. 3. Observation by scanning electron microscopy of colonization by *S. carnosus* strain CIT 833 on stainless steel chip (a) and on PTFE chip and (b) size bar: 10 μm .

4. Discussion

Genetic diversity analysed by pulsed-field gel electrophoresis showed that the strains of *S. carnosus* isolated from meat products constituted a remarkably homogeneous genetic group by comparison to others species of *Staphylococcus* such as *S. xylosus*, *S. epidermidis* and *S. aureus* (Morot-Bizot et al., 2003; De Mattos et al., 2003; Rabello et al., 2005). For *S. epidermidis*, *S. aureus* and *S. xylosus* diversity in biofilm formation within species was observed (Mack et al., 1992; Planchon et al., 2006). In our study, all the strains of *S. carnosus* had the same physicochemical surface characteristics measured by MATS method which compares the microbial cell affinities for monopolar and apolar solvents. Similarities between strains of the same species with regard to their physicochemical properties lead to think that they have probably similar surface architectures.

Attachment, the first step of biofilm formation, is mainly governed by physicochemical interactions between the support and the bacterial surface including hydrophobicity, Van der Waals forces and Lewis acid-base properties (Bellon-Fontaine et al., 1996; Briandet et al., 1999). The physicochemical surface properties of *S. carnosus* were studied to estimate its potential of adhesion. *S. carnosus* was hydrophilic with a strong basic character and thus it adhered preferentially on hydrophilic support such as stainless steel. Such results were already observed for some strains of *S. xylosus* (Planchon et al., 2006) and *S. aureus* (Lerebour et al., 2004). While other strains of *S. xylosus*, *Staphylococcus* spp., *S. sciuri* and *S. epidermidis* presented a basic character surface with moderate hydrophobic properties, thus they adhered preferentially on hydrophobic and moderately on hydrophilic supports (Briandet, 1999; Leriche and Carpentier, 2000; Planchon et al., 2006). This basic character is due to the negatively charged surface of micro-organisms. Indeed, most of bacteria are negatively charged in the range of pH usually encountered in food ($\text{pH} < 7$). Similarly, negative charges were found for *S. carnosus* with an isoelectric point at pH 2. Some strains of *S. xylosus* and *S. aureus* were found to reach their isoelectric point at pH 2.0 while other strains of *S. xylosus*, *S. aureus*, *S. epidermidis* and *Staphylococcus* spp. isolated from food industry did not reach it between pH 2 and 7 (Sonohara et al., 1995; Briandet, 1999; Planchon et al., 2006). Bacterial cells generally reached isoelectric points around pH 2 or 3.5. But some staphylococci encapsulated or not, did not have an isoelectric point (Giovannacci et al., 2000). The negative cell surface charge of bacteria is mainly due to the composition of teichoic acids (Gross et al., 2001). They are highly charged cell wall polymers, composed of alternating phosphate and ribitol or glycerol groups, which are substituted with D-alanine and N-acetylglucosamine (Peschel et al., 2000).

Even if bacteria and surfaces are negatively charged, Van der Waals forces can overcome repulsion and lead to adhesion (Cramton et al., 1999). The Van der Waals forces

are generally attractive, and ionic forces can be either attractive or repulsive. The repulsive electrostatic interactions between bacterial cells and negative charged surfaces (stainless steel) could be reduced by treatment of the support with positively charged ions. In our study, the addition of NaCl improved significantly the adhesion of *S. carnosus* on stainless steel. Similar results have been shown for *Staphylococcus* spp. (Briandet, 1999).

Primary adhesion is followed by growth and accumulation on the surface of the support. We have shown that *S. carnosus* adhered preferentially on hydrophilic support but it grew of approximately 1.0 log both on hydrophilic and hydrophobic supports in TSB medium. Addition of NaCl during growth had contrary effects: release on stainless steel (hydrophilic) and enhancement on PTFE (hydrophobic) supports. Such positive effect of NaCl had been already shown on the biofilm growth of different coagulase negative staphylococci on hydrophobic support (Moret et al., 2003). For *S. xylosus*, NaCl improved and stabilized the biofilm growth on stainless steel and PTFE supports (Planchon et al., 2006). Even if growth was observed for *S. carnosus*, no aggregation between cells was observed in scanning electron microscopy (SEM) and no polysaccharides were assayed by the Dubois method. These results are in agreement with those of Heilmann et al. (1996) which showed that the *S. carnosus* strain UT TM300 achieved the primary attachment to a hydrophilic surface but was unable to accumulate on the surface and so to form aggregates. Authors thought that this adhesion could be mediated by hydrophilic interaction of wall or lipo-teichoic acid.

Absence of synthesis of polysaccharides could explain the lack of formation of three-dimensional network. The polysaccharides play a key role in biofilm formation of staphylococci because they consolidate the adhesion of bacterial cells to surfaces and cell–cell adhesion. The polysaccharide intercellular adhesin (PIA) described for *S. epidermidis* and *S. aureus* strains, is responsible for intercellular aggregation and could mediate adherence to hydrophilic surfaces (Heilmann et al., 1996; Cramton et al., 1999). PIA is encoded by *ica* locus and a mutation in this locus resulted for *S. aureus* in a loss of the ability to form biofilm (Cramton et al., 1999). *S. carnosus* UT TM300 strain is devoided of the *icaA* gene, one gene of the locus *ica*, and is described like “non-biofilm forming” strain by several authors (Cramton et al., 1999; Gross et al., 2001; Götz, 2002). Since all the *S. carnosus* strains are genetically homogeneous and exhibit similar surface properties, we can assume that these strains do not have the ability to produce PIA as the UT TM300 strain.

In conclusion, it appeared that *S. carnosus* adhered on different abiotic surfaces which can be encountered in food factories but was not able to accumulate on these surfaces. This observation could explain why *S. carnosus* is rarely isolated in the environment and never mentioned as coagulase negative staphylococci isolated in the clinical area.

Acknowledgements

We express our gratitude to the University of Tübingen (F. Götz) for providing the *S. carnosus* strain UT TM300 and the University of Bath for providing four strains. We thank N. Garrel and B. Duclos for their technical assistance. This work was financially supported by a national contract: Aliment Qualité Sécurité (AQS R01/09) and was a part of ECOS project (A03B02). Stella Planchon has a research fellowship from the French Ministry of “Education Nationale et Recherche”.

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