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IMPACT OF THE SURFACE PROPERTIES OF LACTIC BACTERIA ON THE STABILITY OF EMULSIONS

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Abstract

Bacteria have physicochemical surface properties which depend on the chemical composition of the cell surface. These characters proceed from several type of physicochemical interactions and are involved in attachment processes of microorganisms to surfaces. Thus they are of interest in several areas, as biomedicine, formation of biofilms and adhesion to apolar surfaces.
Moreover, food matrix are complex heterogeneous media, which structure settles on interaction forces between molecules (van der Waals, electrostatic or structural forces…). When bacteria are present in a matrix, it is probable that their surface interacts with the other constituents. So far, few studies have mentioned this subject.
In order to understand the involvement of cells surface properties in a food matrix, the effect of surface properties of lactic bacteria on the stability of model emulsions were studied. The results showed that the choice of a bacterium according to its surface properties may have a strong impact on the stability and on the behavior of an emulsion.

Keywords: Food emulsion, Lactic bacteria, Surface properties.

1. Introduction

The hydrophobicity of microorganisms has been studied since 1924, with the work of Mudd and Mudd (cited by Rosenberg (1991)), who observed the repartition of microorganisms at the oil/water interface. From the seventies, the phenomenon of the attachment of microorganisms to surfaces or to animal or plant tissus has been more and more studied, since it became of great interest in different areas such as pathology, depollution processes… In food industry, this subject has been studied mainly in order to understand the formation of biofilms. It is now well established that microorganisms possess physicochemical properties (hydrophobic, hydrophilic, Lewis acid/base…) that depend on the chemical composition of the cell wall and membrane (Boonaert and Rouxhet, 2000; Pelletier et al., 1997).

Food matrix are complex and heterogene media, whose structure settles on interaction forces between molecules (van der Waals, electrostatic or structural forces…) (Gupta and Muralidhara, 2001). When bacteria are present in a medium, it is probable that their surface interacts with the other components through physicochemical interactions. Kiely and Olson (2000) studied the cell surface properties of five *Lactobacillus casei* strains in a attempt to find the factors influencing the adsorption of lactic bacteria on fat droplets, in order to improve the retention of flavors in fat-light Cheddar. Lubers *et al.* (1994)

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showed that the impact of yeast wall on aroma compounds in wine depends on the nature of the volatile compounds and on the composition of the wall. This apart, it remains a scarcely studied subject.

Lactic bacteria are widely used in food industry for milk fermentations.

In order to understand the impact of physicochemical properties of microorganisms on a food matrix, we first evaluated the influence of lactic bacteria properties in a model emulsion. These results were then used to study the impact of their surface properties on the stability of a food emulsion and milk gels.

2. Materials and Methods

2.1. Lactic bacteria strains

The strains used in this study come from the Microbiology Laboratory "UMR UB/INRA 1232", some of these strains come from ARILAIT or CNRZ/INRA (Jouy-en-Josas) collections (Table 1).

2.2. Physicochemical caracterisation of cells surface

MATS (Microbial Adhesion To Solvents) tests were applied (Boonaert and Rouxhet, 2000); the method consists in the comparison of the affinity of the cells towards a couple of solvents: one monopolar which can be electron donor or acceptor, and one apolar, the van der Waals component of the surface free energy of the solvents within a couple being similar.

For the experiments, a bacterial suspension (with an absorbance = Abs1) was mixed with a solvent in the proportion of 1:6, in order to form an emulsion. This mixture was then left until the separation of two phases. The absorbance of the aqueous phase was measured (Abs2) and the adhesion was expressed as: % adhesion = (1 – Abs2/Abs1)*100.

The couples of solvents used, were: chloroform (monopolar and electron-acceptor) and hexadecane (apolar) or ethyl acetate (monopolar and electron-donnor) and decane (polar). All these solvents were of high purity (Sigma-Aldrich, St Quentin-Fallavier, France).

2.3. Preparation of the emulsions

In order to obtain emulsions containing droplets with different surface charges, the following surfactants were used:

- Tween 20 (polyoxyethylene-sorbitane monolaurate): non-ionic
- CTAB (dodecyltrimethyle-ammonium bromure): cationic
- SDS (sodium dodecyle sulfate): anionic

The emulsions were obtained by te homogenisation of n-hexadecane (20% v/v) and the aqueous phase (80% v/v) containing a surfactant (0.5 %), with the aid of ultrasounds (3 times 3 min.). The emulsion was then diluted 10 times in phosphate buffer (0.01 M; pH 6.5) or citrate buffer (0.01M; pH 4.5).

The bacteria were grown until the stationnary phase and then washed once (7000 *g* during 4 min.). They were then added to the emulsion at a final cell concentration of about 10^9 cell.mL^{-1}.

2.4. Caracterisation of the emulsions

The size of the droplets in each emulsion was determined by laser granulometry (Malvern Mastersiser 2000), with the aid of the manual method. The refractive index of hexadecane was 1.41.

2.5. Observation by fluorescence microscopy

The emulsion was observed with the aid of a microscope and an image acquisition system. The droplets of hexadecane and the bacteria were stained respectively with Nile Red and Dapi (Molecular Probes, USA).

3. Results

3.1. Surface properties of the lactic bacteria strains

Table 1: Lactic bacteria strains used in this study.

Strain	Code	
Lactoccocus lactis ssp. *lactis*	SL01*	SL04*
	SL05*	SL06*
	SL02*	SL03*
Lactoccocus lactis ssp. *cremoris*	SC 07*	SC 10*
	SC 08*	SC 10*
	SC 09*	SC 12*
Lactoccocus lactis ssp. *lactis subvariant diacetylactis*	SD 13*	SD 17*
	SD 14*	SD 18*
	SD 15*	CNRZ125**
	SD 16*	CNRZ 126**
Streptococcus thermophilus	TA 60***	
Lactobacillus delbrueckii ssp. *bulgaricus*	LB 340***	

Origin of the strains: *ARILAIT, **CNRZ, ***our laboratory

The variations in the percentages of adhesion to chloroform (between 0 and 80%) of 22 lactic bacteria strains (Fig. 1), evidenced a diversity in the basic caracter of the strains. Most of the tested bacteria are hydrophilic. The percentages of adhesion to apolar solvents did not exceed 40%.

Following these measurements for the whole lactic bacteria, two strains with opposite characters were selected in order to study the effect of bacteria on the stability of an emulsion:

- one adhering to solvents: *L. diacetylactis 18* (SD18)
- one not adhering to any solvent: *L.diacetylactis 16* (SD16)

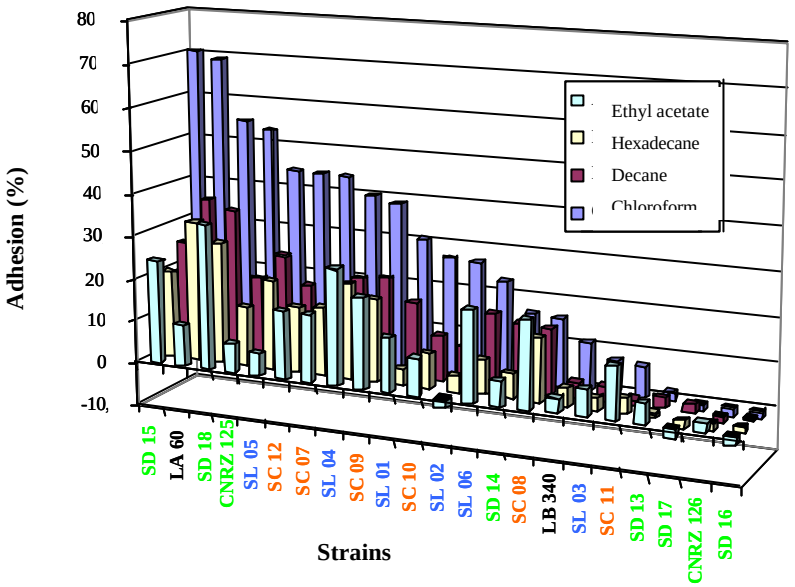

Fig. 1. MATS test results for 22 strains of lactic bacteria, in phosphate buffer (0.01 M; pH 6.5). The strains are ordered by decreasing percentage of adhesion to chloroform.

3.2. Impact of lactic bacteria on the stability of an emulsion

The presence of bacteria had no effect on the emulsions prepared with the anionic surfactant (SDS) or with Tween 20, as shown by laser granulometry measurements and by microscopy (not presented). However, microscopy observations showed a clear effect within the emulsion prepared with the cationic surfactant (CTAB).

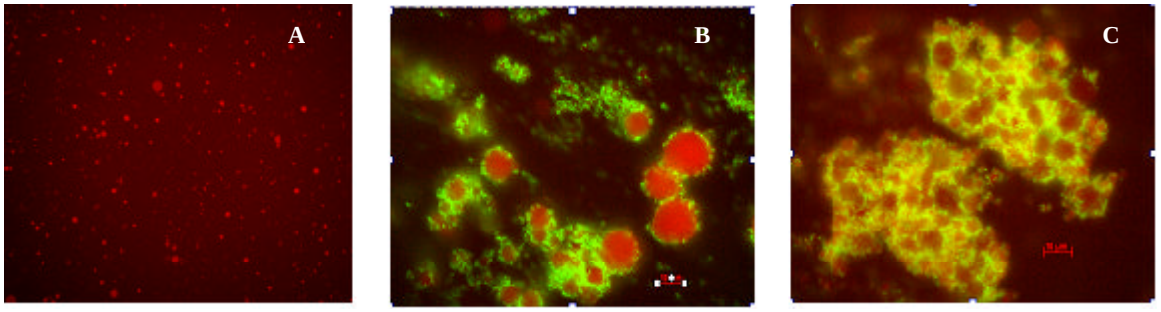

Fig. 2. Images de microscope fluorescente de l'émulsion préparée avec CTAB dans e tampon citrate 4,5: A, reference without bacteria; B, with *L. diacetylactis* SD18; C, with *L. diacetylactis* SD16.

The emulsion without bacteria (Fig. 2A) exhibited homogene small droplets. When the bacteria were present in the emulsion, the droplets became bigger and coalesced (Fig. 2B and 2C).

The fluorescence images showed that the bacteria were located on the surface of the droplets, acting as bridges between the droplets. Moreover, this phenomenon was more clearly observed in the presence of *L. diacetylactis* SD16 than with *L. diacetylactis* SD18.

After 24 h, the emulsion without bacteria was still stable, whereas in the presence of the bacteria, there was a separation of the two phases, this effect being clearer for *L. diacetylactis* SD16 than for *L.*

diacetylactis SD18 (Fig. 3). Also, the emulsions prepared with phosphate buffer (Fig. 3A) were slightly more stable in the presence of bacteria than that prepared with citrate buffer (Fig. 3B).

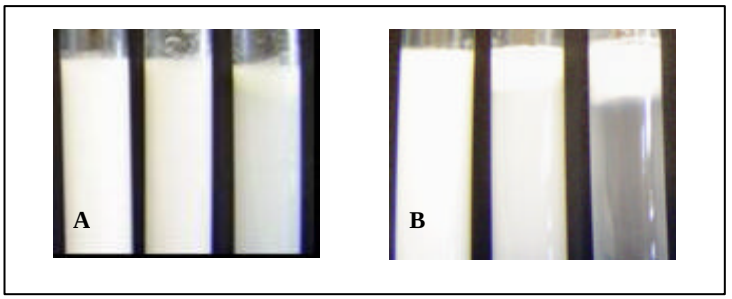

Fig 3. Photographies of emulsions after 24 h, without bacteria (left tube), with *L. diacetylactis* SD18 (midle tube) and *L. diacetylactis* SD16 (right tube): A, emulsion with phosphate buffer pH 6.5 (0.01 M); B, emulsion with citrate buffer pH 4.5 (0.01 M).

4. Discussion

The adhesion to solvents in MATS tests is the result of van der Waals, electrostatic and Lewis acid/base interactions between the microorganisms and the particles in the medium. According to Bellon-Fontaine *et al*. (1996), van der Waals forces play a minor role compared to Lewis acid/base or electrostatic forces. According to the wide range of adhesion percentages obtained for the tested strains (Fig. 1), the relative importance of each of the forces intervening in the adhesion process of microorganisms, varies with the strain.

The bacteria have a weak affinity for apolar solvents, which suggests that they have a relatively hydrophile global character. Some of the strains exhibited a basic character (high affinity for chloroform which is an acidic solvent), whereas some others did not have this character. The basic character may result from the presence of carboxylic groups on the surface of microorganisms (Pelletier *et al*., 1997; Bellon-Fontaine *et al*., 1996).

Pelletier *et al* (1997) showed that the surface of eight *Lactobacillus* strains were in all cases strongly basic and hydrophilic. Similar results were obtained with *Lactococus lactis* subsp. *lactis* bv. *diacetilactis* and *Lactobacillus helveticus* by Boonaert and Rouxhet (2000). According to these authors, the chemical groups of proteins, polysaccharides, peptidoglycans and (lipo)teichoique acid of the cell surface are at the origin of their physicochemical properties. The hydrophilic character is notably correlated with a high concentration of polysaccharides and a weak concentration of carbonated compounds (Van der Mei *et al*., 2001; Boonaert and Rouxhet, 2000; Pelletier *et al*., 1997).

The formation and the stability of an oil-in-water emulsion, depend on the composition of the adsorption layer around the droplets (Van Aken, 2003). According to the results obtained concerning the stability of the emulsions, they were not modified in the presence or absence of the bacteria, when prepared with SDS or Tween. An effect on the stability of emulsions of the addition of bacteria was only observed with the emulsion prepared with CTAB, which contained positively charged droplets. Also that effect was clearer at pH 4.5 than at pH 6.5, indicating that the total surface charges (increasing with a decrease in pH), influenced the aggregation process. The bacteria were able to form

bridges between droplets, causing their aggregation (Fig. 2). Thus, the appearing of big droplets may be due to the gathering of small droplets. These results could be compared to that of Ye and Singh (2000) about the stabilisation of whey protein emulsions by calcium ions (Ca^{2+}): a floculation and an increase in the size of the droplets were observed, moreover Ca^{2+} also gave rise to the formation of bridges. Still according to Ye and Singh (2000) the addition of Ca^{2+} in the emulsion decreases the electrostatic repulsion between the droplets and increases the potential associations and aggregations. Van Aken (2003) also suggested that calcium bridges can decrease the charge of the adsorption layer of the droplets, thus enabling connections.

4. Conclusion

We suggest that the effects of lactic bacteria on the stability of an emulsion prepared with CTAB are notably due to the surface charges of bacteria. If negatively charged bacteria are added in an emulsion containing positively charged droplets, a decrease in the electrostatique repulsions between the emulsion droplets may be provoked, forming aggregations.

The whole results show that the choice of a bacterium according to its surface properties can have an important impact on the stability and the behaviour of an emulsion, which can be of great interest in many food processes.

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