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Probing surface properties of lactic acid bacteria - Comparative modification by anhydride and aldehyde grafting

Xiaoyi Jiang [a], Elhamalsadat Shekarforoush [a], Musemma Kedir Muhammed [a], Nico Bovet [b], Marcel Ceccato [c], Kathryn A. Whitehead [d], Nils Arneborg [a], Jens Risbo [a,*]

[a] University of Copenhagen, Department of Food Science, Rolighedsvej 30, DK-1958, Copenhagen, Denmark
[b] Technical University of Denmark, Centre for oil and gas, Elektrovej, 2800 Kgs. Lyngby, Denmark
[c] Aarhus University, Interdisciplinary Nanoscience Center, Gustav Wieds Vej 14, DK-8000, Åarhus C, Denmark
[d] Manchester Metropolitan University, Department of Life Sciences, Chester St, Manchester M15GD, United Kingdom

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ABSTRACT

Surface of *Lactobacillus crispatus* DSM 20584 (LBC) and *Lactobacillus rhamnosus* GG (LGG) from stationary and exponential phase were chemically modified using hexanoic anhydride (HA) and octanal via grafting hydrophobic moieties onto the bacterial surface hydroxyl and amine groups. The physicochemical properties of the bacteria were measured using a range of surface-sensitive methods including x-ray photoelectron spectroscopy (XPS), zeta potential measurement, contact angle measurement (CAM) and microbial adhesion to solvents (MATS). Before modification, the surface of two strains was distinctly different, where LBC was covered by hydrophobic surface-layer proteins (SLPs) while LGG was hydrophilic with the rich presence of polysaccharides. Surface hydrophilic polymers rendered steric hindrance of LGG against autoaggregation, whereas LBC lacking polysaccharides showed strong autoaggregation. After HA and octanal modifications, the intrinsic surface differences between two strains were reduced according to the Principal Component Analysis (PCA). The enhancement of hydrophobicity by HA and octanal was most likely derived from the lowered Lewis acid-base characters via elimination of hydroxyl and amine groups. Chemical modification using the two treatments can be a useful tool to tune the surface of lactic acid bacteria, which might be further applied to other microorganisms, enabling applications such as altered bacterial adhesive behaviors and biofilm formation.

1. Introduction

Lactic acid bacteria are extensively used as food components due to their applications in both the processing and preservation of dairy products, as well as their health-promoting effects on the human gastrointestinal system. Surface properties of lactic acid bacteria can influence the manufacturing of fermented dairy products, and can manifest itself as either desirable autoaggreation of e.g. *Lactobacillus. diacetylactis* assisting continuous fermentation [1], or in unwanted biofouling and contamination by e.g. *Streptococcus thermophiles* due to biofilm formation [2–4]. The surface properties and hence the adhesion of bacteria to the human gastrointestinal epithelium also determine their probiotic effects due to interactions with other microorganisms [5,6].

The composition and conformation of cell wall constituents play important roles in bacterial surface properties. As Gram-positive bacteria, the cell wall of lactic acid bacteria consists of a thick layer of peptidoglycan, decorated by lipoteichoic acid, polysaccharides and surface-bound proteins [7]. Lipoteichoic acid is a linear polymer consisting of long hydrophobic tails and strongly acidic phosphate groups, contributing to its polyelectrolyte character [8,9]. The abundant polysaccharides on the surface of the cell wall are either neutral or acidic, leading to the intrinsically hydrophilic nature of most lactic acid bacterial strains [10]. Surface proteins (mainly S-layer proteins) are non-covalently bound and strongly basic with high isoelectric points [11]. For example, S-layers occur in many strains of *Lactobacillus. crispatus* and govern their adhesion behaviors [12,13]. In comparison, the surface of species like *Lactobacillus. rhamnosus* is predominated by long and polymeric polysaccharides, even though the limited presence of proteinaceous components has also been reported [14,15].

Surface modification of lactic acid bacteria allows the tuning of surface properties for various purposes such as enhanced tolerance

* Corresponding author.
E-mail address: jri@food.ku.dk (J. Risbo).

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during industrial production [16] and improved functionality in probiotic interactions with intestinal tract and pathogenic bacteria [9]. Modification of bacterial surface can be accessed by biologically altering growth conditions such as growing phase [10], temperature [17] and composition of medium [18], or by physical coating of surface-active polymers [19,20]. Chemical grafting is another modifying approach that connects hydrophobic moieties via covalently binding to bacterial functional groups that are hydrophilic. Compared to biological and physical routes, chemical modification enables more accurate targeting of surface hydrophilic groups, covalently adding hydrophobic parts without largely altering the bacterial cell wall compositions.

Chemical modification by long-chain carboxylic acid anhydrides and aldehydes has been reported on polysaccharide particles. Anhydride modification of polysaccharides has been extensively studied on starches [21–23], celluloses [24,25] and chitins [26]. Using anhydride modification, hydrophobicity of polysaccharides can be increased through connection of hydrophobic chains onto hydroxyl groups, enabling the formation of lipophilic films [22]. Similar principles have been applied to bacteria where lactic acid bacteria modified using octenyl succinic anhydride (OSA) were able to produce stable Pickering foams and emulsions [27]. In comparison, aldehyde modification grafts result in hydrophobic chains via reductive amination of primary or secondary amines forming imines. One example of aldehyde modification was on chitosan particles, where substitution degrees from 0.3 to 0.7 of surface amine groups were obtained by using aldehydes bearing long chains from C3 to C12 [28–30]. Since aldehyde modification requires the presence of primary or secondary amines, the relevant researches are still limited to chitosan.

In this study, we aim to tune the surface of lactic acid bacterial strains *Lactobacillus rhamnosus* GG (LGG) and *Lactobacillus crispatus* DSM20584 (LBC) by applying either hexanoic anhydride (HA) or octanal modification, and to probe changes in surface properties induced by modifications using a series of surface-sensitive techniques. Due to the constitutional difference between the cell walls of the two strains, it is hypothesized that the hydrophobization by HA and octanal modification should accompany different changes in other physicochemical properties such as charge density and Lewis acid-base properties. The idea is that a deeper understanding of the modified physicochemical properties of bacteria can be gained by probing the bacterial surface using a combination of chemical and physicochemical approaches including XPS, zeta potential measurement, CAM and MATS assay.

2. Materials and methods

2.1. Materials and chemicals

Hexanoic anhydride (HA), octanal, glycerol, dimethyl sulfoxide (DMSO), hexadecane, chloroform, ethyl acetate, decane, sodium chloride (NaCl), potassium chloride (KCl), disodium hydrogen phosphate (Na_2HPO4), potassium dihydrogen phosphate (KH_2PO_4), formamide, 1-bromonaphthalene, lithium chloride (LiCl), Coomassie Brilliant Blue G-250 were purchased from Sigma-Aldrich, Steinheim, Germany. NuPAGE™ Lithium dodecyl sulfate (LDS) sample buffer (4X), NuPAGE™ 12% Bis-Tris Mini Protein gel (1.0 mm, 15 wells), Pierce™ BCA Protein Assay Kit was purchased from Thermo Fisher Scientific, USA. *Lactobacillus rhamnosus* GG (LGG) was bought from Collections of Micro-organisms BCCM, Ghent, Belgium and *Lactobacillus crispatus* DSM 20,584 (LBC) was kindly obtained from strain collection of Department of Food Science, University of Copenhagen (Finn Kvist Vogensen, Personal communication). MRS broth (de Man, Rogosa and Sharpe) was bought from Oxoid, Basingstoke, England. All the chemicals were used as received, except for MRS broth which was sterilized in an autoclave (115 °C, 10 min) before use. MilliQ water (18.2 MΩcm at 25 °C) was used in all the experiments.

2.2. Growth of bacteria and dry biomass determination

Lactobacillus rhamnosus GG and *Lactobacillus crispatus* DSM20584 from the previous stock were inoculated in MRS broth at 37 °C for 24 h, and then culture stocks were prepared by mixing equal volumes of culture and glycerol (40 v/v%). The prepared culture stocks were kept at −80 °C.

Bacteria from frozen stock were anaerobically propagated (100 μL) in 10 mL MRS broth at 37 °C for 24 h. Then, 250 μL of the bacterial preculture was anaerobically incubated in 50 mL MRS broth at 37 °C. Finally, cells were collected from late exponential phase (16 h) and stationary phase (24 h) by centrifugation at 5000 × g for 5 min at 4 °C, and washed twice with sterile MilliQ water.

The dry weight of bacteria was determined following a standard method [31]. Briefly, after growth, cells were collected by centrifugation at 5000 × g for 5 min at 4 °C, followed by washing twice with MilliQ water passed through a 0.2 μm-pore-size cellulose membrane. The harvested cell pellets were re-suspended in 3 mL sterile MilliQ water. One milliliter of cell suspension was transferred to a pre-weighed sterile aluminum boat and the bacteria were dried in a hot air oven at 105 °C, and the total weight was measured regularly until a stable dry weight was obtained. The procedure was carried out in triplicate. In the end, the dry biomass of bacteria from the exponential phase and stationary phase corresponding to 250 μL preculture in 50 mL broth was 75.5 ± 2.7 and 77.9 ± 1.7 mg for LGG, and 31.1 ± 1.3 and 34.8 ± 0.5 mg for LBC, respectively.

2.3. Extraction and analysis of bacterial surface-layer proteins (SLPs)

To investigate the intrinsic SLP profiles of LBC and LGG, the extraction of bacterial SLPs was conducted using LiCl solution based on a previous work with some modifications [32]. Briefly, bacteria grown in 50 mL MRS broth were collected after 16 and 24 h incubation by centrifugation. After washing twice with MilliQ water, bacterial pellets (15 mg in dry biomass) were re-suspended in 3 mL LiCl solution (5 M) and the SLPs were allowed to be extracted for 1 h at 37 °C with vigorous shaking. Then, cell pellets were removed by centrifugation at 5000 × g for 15 min at 4 °C and the supernatant was collected by filtering through a 0.2 μm-pore-size cellulose membrane and transferred to a Amicon 10-kDa centrifugal filter (Merck Millipore Ltd, USA). The supernatant was dialyzed and concentrated by centrifugation at 4000 × g for 20 min at 4 °C, and the concentrated supernatant was continuously dialyzed twice by adding same amount of MilliQ water. Finally, the dialyzed LiCl extract was transferred to a new Eppendorf tube for further analyses.

The analysis of SLPs using SDS-PAGE was performed as described previously [33]. Briefly, LiCl extract was mixed undiluted with NuPAGE™ LDS Sample Buffer (4X) at a volume ratio of 3:1 and the sample was then heated under 80 °C for 10 min to denature the proteins. Ten microliter of sample was loaded into each well, and the gel was let to run at 200 V for 45 min and finally stained overnight with Coomassie Brilliant Blue. In addition, total protein concentration of the LiCl extracts was determined using BCA assay [34] according to the Pierce™ BCA Protein Assay Kit protocol provided by the manufacturer.

2.4. Anhydride and aldehyde modification of bacteria

Anhydride modification of bacteria was adapted from a previous protocol [27]. Briefly, after bacteria grown in 50 mL broth for 16 h and 24 h were collected and washed twice with MilliQ water, the cell pellets were re-suspended in phosphate buffered saline (PBS), pH 7.4 with a final bacterial concentration of 0.4 wt% and the pH of bacterial suspension was adjusted to approximately 7.8. Then, hexanoic anhydride (20 w/w% based on the cell dry weight) in DMSO solution was slowly added into the cell suspension while stirring (final DMSO conc. in bacterial suspension = 2%). During the modification, the pH was maintained between 7.4 - 7.9 by adding 0.1 M NaOH solution. When the pH

was constant for at least 15 min, cells were harvested by centrifugation at 5000 × g for 5 min at 4 °C, and washed twice with sterile MilliQ water.

Aldehyde modification of bacteria was conducted by firstly re-suspending the cell pellets in citric acid-sodium citrate buffer (pH 5) with a final bacterial conc. of 0.4 wt%. A predetermined amount of octanal (30 w/w%) was dissolved in DMSO and this solution was added to the bacterial suspension while stirring (final DMSO concentration in bacterial suspension = 2%). During the modification process, the pH was maintained between 4.9 – 5.1 by adding 1 M NaOH or HCl solution. The reaction mixture was stirred under room temperature for 24 h and the reaction was ceased by adjusting pH to 7. Finally, bacteria were collected by centrifugation at 5000 × g for 5 min at 4 °C, and washed twice with sterile MilliQ water.

2.5. X-ray photoelectron spectroscopy (XPS)

For XPS analysis, bacteria grown in 50 mL broth for 16 h and 24 h, with and without modifications were washed twice and re-suspended in MilliQ water to a cell concentration of 1×10^9 CFU/mL. Then, 50 μL of this cell suspensions was carefully transferred onto the surface of a silicon wafer and air-dried in a clean fume hood. After the formation of a bacterial layer on the surface, the silicon wafers were stored at room temperature in a desiccator containing phosphorous pentoxide for further analysis. The XPS experiments were carried out in a Kratos Axis Ultra DLD fitted with a monochromatic AlKα X-rays source (Kratos Scientific, Manchester, UK). The charge neutralizer of the instrument was used against charging of the samples. Data were analyzed using the commercial software CasaXPS and its associated sensitivity factor library (Casa Software Ltd, UK). Energy step for high resolution scan was 0.1 eV. The binding energy was calibrated using aliphatic C 1 s line fixed at 285 eV.

2.6. Zeta potential measurements

The surface charge of the bacteria was investigated in terms of zeta potential using a zeta sizer (Malvern Zetasizer, Nano ZSP, UK) at 25 °C. The used background electrolyte solution was 10 mM KH_2PO_4 (pH 3–11). For the measurement, unmodified and modified bacterial pellets corresponding to 250 μL preculture grown in 50 mL MRS broth for 16 h and 24 h were re-suspended in 50 mL KH_2PO_4 and the suspensions were diluted 10 times with the same buffer. Then, 1 mL of this suspension was injected into the capillary cell using a disposable syringe. The capillary cell was rinsed subsequently with MilliQ water and sample before starting the measurement.

2.7. Microbial adhesion to solvents (MATS)

Evaluation of bacterial surface properties was carried out as described in previous reports [2,35] with modifications. Briefly, bacterial pellets grown for 16 h and 24 h with or without chemical modification were re-suspended in 10 mM KH_2PO4 solution and an initial optical density (OD) of around 0.8 at 600 nm was obtained (SpectraMax i3x, Molecular Devices LLC, USA), which indicated an approximate cell density of 10^8 CFU/mL. An aliquot of cell suspension (250 μL) was mixed with 42 μL solvent in an Eppendorf tube. After 10 min incubation under room temperature, the mixtures were simultaneously vortexed at highest speed for 90 s using a vibrating shaker (IKA Shakers VXR basic Vibrax®, Germany), which consisted of three times of 30 s vortexing with an interval of 1 min rest between each 30 s. After vortexing, mixtures were let to stand for 15 min at room temperature to allow complete phase separation. Then, 200 μL of the aqueous phase was carefully transferred to the 96-well plate to measure OD values without disturbing the interface. The percentage of microbial adhesion to solvents (A) was calculated by the following equation;

$$\boldsymbol{A}\ (\%) = \left(1 - \frac{\boldsymbol{A}_1}{\boldsymbol{A}_0}\right) \times 100 \tag{1}$$

where A_0 is the initial OD600 of the bacterial suspension and A_1 is the OD600 value of the lower aqueous phase after phase separation. The used solvents included chloroform, a monopolar acidic solvent paired with hexadecane, a nonpolar solvent; and ethyl acetate, a monopolar basic solvent paired with decane, a nonpolar solvent. The hydrophobicity of bacteria was evaluated by their adhesion to hexadecane (A_H), and the Lewis base (A^-) and acid (A^+) properties were calculated by the adhesion difference within the selected solvent pairs;

$$\boldsymbol{A}^- = \boldsymbol{A}_C - \boldsymbol{A}_H \tag{2}$$

$$\boldsymbol{A}^+ = \boldsymbol{A}_{EA} - \boldsymbol{A}_D \tag{3}$$

where A_C, A_H, A_{EA} and A_D represent bacterial adhesion to chloroform, hexadecane, ethyl acetate and decane, respectively.

2.8. Contact angle measurements (CAM)

Contact angles of unmodified, 20 w/w% HA-modified and 30 w/w% octanal-modified bacteria grown for 16 h and 24 h were measured according to a previous protocol with minor modifications [36]. Briefly, bacterial pellets (80 mg in dry biomass) were re-suspended in 50 mL KH_2PO_4 (10 mM) solution and a thick layer of bacteria was deposited on a 0.45 μm (pore size) polyvinylidene difluoride membrane filter using negative pressure. Then, the wet filters with mounted bacterial lawns were glued onto glass slides and air-dried for approximately 90 min, which allowed the formation of plateau contact angles [37]. The contact angle measurements were carried out at room temperature by using sessile drop method with water, formamide and 1-bromonaphthalene as probe liquids (OCA 25, Dataphysics Instruments, Stuttgart, Germany). For each measurement, at least three filters were prepared and five droplets were dispensed on the dry areas of the same filter.

The Lifshitz–van der Waals (γ_B^{LW}), Lewis base (γ_B^-) and Lewis acid (γ_B^+) components of the surface free energy of bacteria (B) were calculated from the contact angles (θ) of three reference liquids using Van Oss-Good model [38]. In this model, the contact angle (θ) of pure liquid (L) can be expressed as;

$$\cos\boldsymbol{\theta} = -1 + \frac{2\sqrt{\boldsymbol{\gamma}_B^{LW}\boldsymbol{\gamma}_L^{LW}}}{\boldsymbol{\gamma}_L} + \frac{2\sqrt{\boldsymbol{\gamma}_B^+\boldsymbol{\gamma}_L^-}}{\boldsymbol{\gamma}_L} + \frac{2\sqrt{\boldsymbol{\gamma}_B^-\boldsymbol{\gamma}_L^+}}{\boldsymbol{\gamma}_L} \tag{4}$$

where the contact angle (θ) was experimentally measured, and the Lifshitz–van der Waals (γ_L^{LW}), Lewis acid (γ_L^+) and base (γ_L^-) components, and surface tension (γ_L) of all three reference liquids (L) were obtained from the database of SCA software (Dataphysics Instruments).

The overall Lewis acid-base surface tension component (γ_B^{AB}) of bacteria is defined as;

$$\boldsymbol{\gamma}_B^{AB} = 2\sqrt{\boldsymbol{\gamma}_B^+\boldsymbol{\gamma}_B^-} \tag{5}$$

Moreover, the hydrophobicity of bacteria was evaluated by determining the cohesive energy of bacteria in water (ΔG_{BWB}), based on the approach proposed by Van Oss [39,40]. According to the Dupré equation, ΔG_{BWB} is only related to the interfacial tension between bacteria and water (γ_{BW}) and is defined by;

$$\Delta\boldsymbol{G}_{BWB} = -2\boldsymbol{\gamma}_{BW} \tag{6}$$

The interfacial tension (γ_{BW}) in Van Oss-Good model can be further expressed as;

$$\boldsymbol{\gamma}_{BW} = \left(\sqrt{\boldsymbol{\gamma}_B^{LW}} - \sqrt{\boldsymbol{\gamma}_W^{LW}}\right)^2 + 2\left(\sqrt{\boldsymbol{\gamma}_B^+\boldsymbol{\gamma}_B^-} + \sqrt{\boldsymbol{\gamma}_W^+\boldsymbol{\gamma}_W^-} - \sqrt{\boldsymbol{\gamma}_B^+\boldsymbol{\gamma}_W^-} - \sqrt{\boldsymbol{\gamma}_B^-\boldsymbol{\gamma}_W^+}\right) \tag{7}$$

where the Lifshitz–van der Waals (γ^{LW}), Lewis acid (γ^{+}) and base (γ^{-}) components of water (W) and bacteria (B) were known from the database and previous calculations, respectively. Therefore, ΔG_{BWB} can be obtained by inserting Eq. (7) to (6), and this value, if negative, indicates that bacteria tend to attract each other in water so their surface is considered hydrophobic, and vice versa.

2.9. Bacterial autoaggregation assay

The bacterial autoaggregation assay was performed according to a previous method [41] with a minor modification. Briefly, bacterial pellets grown for 16 h and 24 h in 50 mL MRS broth were washed twice with MilliQ water and resuspended in PBS (pH 7.2) to obtain an initial OD of around 1 at 600 nm, which corresponded to a cell concentration of 2×10^8 CFU/mL. Then, the bacterial suspensions were let to stand for certain time intervals (2, 4, 8, 18, 24, 48 h) and the upper part of suspension was carefully pipetted without disturbing the lower bacterial suspension and the OD600 was measured spectrophotometrically. The kinetics of bacterial sedimentation termed as autoaggregation coefficient (ACt) was calculated based on the equation;

$$\boldsymbol{ACt}\ (\%) = \left(1 - \frac{\boldsymbol{A_t}}{\boldsymbol{A_i}}\right) \times 100 \tag{8}$$

where A_i represents the initial OD of the bacterial suspension at 600 nm and A_t is the OD_{600} of the upper part of bacterial suspension at time t.

2.10. Statistical analysis

Statistical analysis was carried out using Origin Software 9.4 (Origin Lab Coorporation, USA). Principal Component Analysis (PCA) of all the samples was carried out in terms of selected surface properties. All the data reported are the averages of at least two samples and the results are presented as average ± standard error.

3. Results

3.1. Analysis of surface-layer proteins (SLP)

Two bacterial strains, LGG and LBC, were harvested each from exponential phase and stationary phase, giving rise to four combinations of unmodified (UM) bacteria (LGG-E, LGG-S, LBC-E and LBC-S). Before modifications, the SLPs of four unmodified bacteria were extracted and analyzed to examine the distinct surface protein profiles of the two strains.

The band patterns of LGG and LBC by SDS-PAGE (Fig. 1A) showed completely different SLPs profiles, although the effect of growth phases was not evident. Compared to LBC, the band patterns of LGG showed a significantly reduced number and intensity, indicating their lack of non-covalently bound SLPs. In contrast, SLPs of LBC were efficiently extracted using 5 M LiCl, where a similar pattern but slightly decreased intensity was found for LBC from exponential phase than from stationary phase. The same quantitative information on the total protein concentration in the LiCl extracts was also confirmed by BCA assay (Fig. 1B). Even though bands were observed in various molecular weights (from 12 to 70 kDa), SLPs of LBC were still dominated by protein of molecular weight ranging from 40 to 70 kDa, with the strongest intensity shown at around 45 kDa and 55 kDa. It was also reported by previous studies that the SLPs of *Lactobacillus* species varied in molecular mass between about 40 and 55 kDa [32,42,43].

3.2. Surface chemical composition by XPS analysis

The four unmodified bacteria were treated using two modification schemes, hexanoic anhydride (HA) and octanal (O) modification, which yielded totally 12 types of unmodified and modified bacteria. The concentration of HA and octanal was optimized according to the induced changes in hydrophobicity and zeta potentials of bacteria, and finally 20 w/w% and 30 w/w% were selected for HA and octanal modification, respectively (Figs. S1 and S2).

The elemental concentration of unmodified and modified bacterial

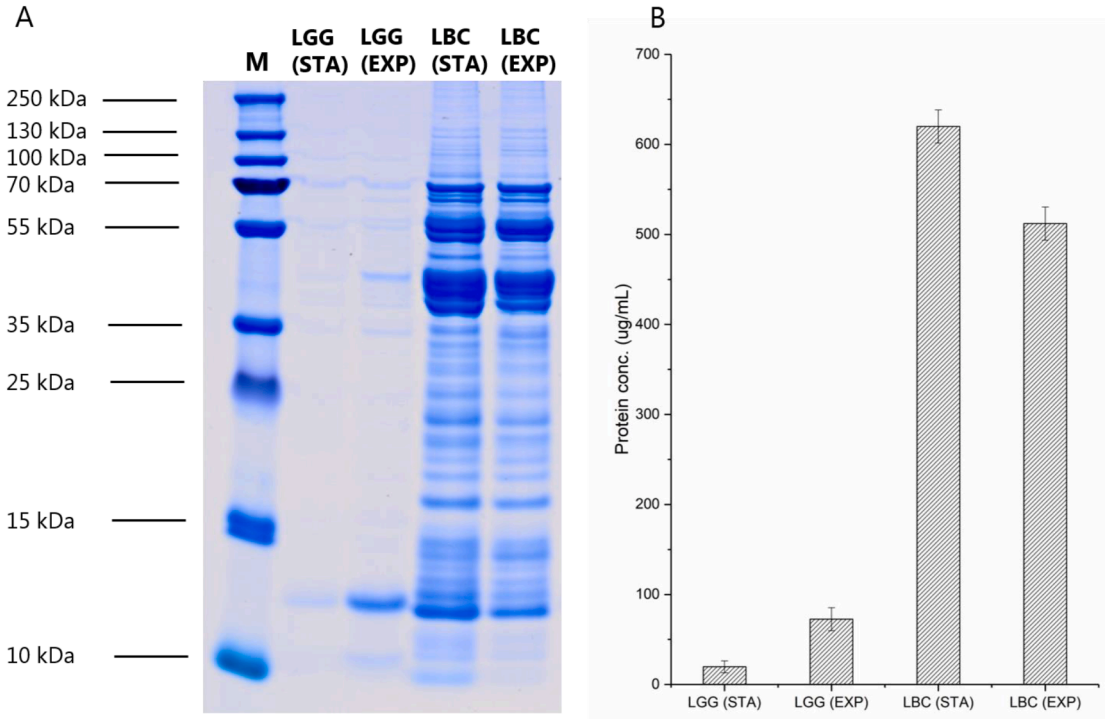

Fig. 1. SDS-PAGE analysis (A) and BCA-quantified protein concentrations (B) of LiCl extracts from the surface of unmodified LGG and LBC from stationary and exponential phase. Error bars represent standard errors ($n = 2$).

surface expressed as percentage ratios of three elements O, N and P to C was demonstrated (Table 1). For unmodified bacteria, the N/C ratios of LBC, regardless of growth phases, were systematically three times higher than LGG, indicating a rich amount of proteinaceous components on LBC surface, as also confirmed by SDS-PAGE and BCA assay. In contrast, higher O/C ratios displayed by LGG than LBC suggested a larger presence of oxygen-rich compounds such as phosphate groups, carboxylate groups and polysaccharides on the surface of LGG. Combining with the rather low occurrence of phosphorus on LGG compared to LBC, the high amount of oxygen-rich compounds were more likely attributed to carboxylates or polysaccharides instead of phosphate groups. Even though modification by either HA or octanal caused slight reductions in the N/C, O/C and P/C ratios of both strains probably due to the grafting of carbon chains, these changes were still seen not significant.

3.3. pH dependence of surface charge

Fig. 2 shows that unmodified LGG and LBC exhibited different pH dependence of surface charges, whereas no obvious difference was observed in terms of difference between growth phases within each strain. Unmodified LGG showed an almost neutrally-charged surface over the whole pH range, and zeta potential was not further influenced by modifications. In contrast, the surface charge of unmodified LBC showed a stronger pH dependence, where a highly positive and negative zeta potential was observed at pH 3 and 10, respectively, even though the overall charge profile was still dominated by acidic pH from 3 to 7, indicating the deprotonation of amines in SLPs or peptide stems of peptidoglycan. For LBC in stationary phase, octanal modification did not bring evident effects, whereas HA modification slightly lowered the steepness of the curve, mainly by introducing more positive charges at high pH above 7. For LBC in exponential phase, a similar effect on lowering the steepness by HA modification was observed and even exaggerated, where the zeta potential decreased from approx. 20 to −0.2 mV at pH 3 and increased from approx. −21.8 to −14.2 mV at pH 10. Octanal modification, on the other hand, seemed to pose an opposite effect to HA modification, as seen from the higher maximum value (from 20 to 23.7 mV) and lower minimum value (from −21.8 to −34 mV). Moreover, all the bacteria possessed a clearly-defined isoelectric point (IEP), which was directly read from the crossover with x axis. Generally, the IEPs of LBC bacteria were slightly higher than LGG, due to more positively-charged surface of LBC at lower pH range.

3.4. Cell hydrophobicity by MATS and CAM

In MATS, cell hydrophobicity can be theoretically reflected by bacterial adhesion to nonpolar solvents such as hexadecane and decane [44]. Hexadecane was considered here because it is the standard model solvent for MATS to describe cell hydrophobicity [35,45].

According to Fig. 3A, an overall higher affinity to hexadecane was demonstrated by unmodified LBC (> 60%) than LGG (~10%), regardless of growth phases, indicating an intrinsically higher surface hydrophobicity of LBC than LGG. Also, no obvious growth dependence of hydrophobicity was found for unmodified LGG, while slightly higher adhesion to hexadecane was observed for LBC in exponential phase (75.3%) than stationary phase (63.9%). After both of the modifications, a rise in adhesion to hexadecane was shown by all the modified bacteria, where HA modification was typically more capable of improving hydrophobicity than octanal modification, and LBC with intrinsically higher hydrophobicity, also showed a stronger response (approx. 90%) to the two modifications than LGG (approx. 20%) in terms of adhesion to hexadecane.

As a complementary method for MATS, the contact angles of bacterial surface against three solvents were measured to calculate the three surface energy components, Lifshitz–van der Waals (γ_B^{LW}), Lewis base (γ_B^-) and acid (γ_B^+) property, which were used to further deduce the surface hydrophobicity expressed in the cohesive free energy of bacteria in water (ΔG_{BWB}). In Fig. 3B, the unmodified LBC, regardless of growth phases, showed more negative values of ΔG_{BWB} (−23.38 and −47.27 mJ/m^2 for stationary and exponential phase, respectively) than LGG (−6.62 and −6.79 mJ/m^2), indicating again the higher intrinsic hydrophobicity of LBC, as also confirmed by MATS. After modification of LBC and LGG in two growth phases, all bacteria demonstrated more negative ΔG_{BWB} values, confirming the effective hydrophobization by HA and octanal. In contrast to MATS, the improved hydrophobicity by CAM was more evident for LGG than LBC. For LGG, the enhanced hydrophobicity was also strongly dependent on growth phase, where HA brought a better enhancement for LGG in exponential phase whilst octanal caused more evident changes for LGG in stationary phase.

3.5. Lewis acid-base properties by MATS and CAM

The Lewis acid (A^+) and base (A^-) properties by MATS after two types of modifications were demonstrated (Fig. 4A). Almost all the bacteria expressed higher adhesions to chloroform than to hexadecane and lower adhesions to ethyl acetate than to decane, suggesting their predominantly basic surface. The only exception was unmodified LGG, which regardless of growth phase, displayed a stronger Lewis acidic than basic character, and this acidic property was totally eliminated after HA and octanal modification. However, modifications in the basic property of LGG were greatly dependent on the growth phases, where only LGG in stationary phase showed a dramatic reduction (almost to zero) after either of the two modifications. For LBC, no acidic character was detected for any samples. A stronger basic property was displayed by unmodified LBC in exponential phase, which was also more impaired after both modifications.

Regarding the total Lewis acid-base properties (γ_B^{AB}) deduced from CAM (Fig. 4B), unmodified LGG exhibited much stronger acid-base interactions in both stationary phase (21.16 mJ/m^2) and exponential phase (22.45 mJ/m^2) than unmodified LBC (8.15 and 5.57 mJ/m^2). After two modifications, the acid-base interactions were diminished for all bacteria. The constituents Lewis acid (γ_B^+) and base (γ_B^-) parameter components were separately presented (Fig. 4C). Unlike MATS, which did not describe the acidic property for most of the samples, the acidic property was detected by CAM, even though the values were still low (0.02 - 6.63 mJ/m^2) compared with that of Lewis base components (0.03 - 19.61 mJ/m^2), in line with the findings of MATS. Another agreement of the two methods was that the strong acid character of unmodified LGG at both growth phases was suppressed upon modifications. Likewise, the basic character of all bacteria was impaired by two modifications and the degree of reduction was related to both the growth phases and modification approaches. For example, a stronger effect of HA modification was found for LGG in stationary phase (12.24 mJ/m^2) than exponential phase (0.49 mJ/m^2), whilst the opposite was observed for octanal modification.

Table 1
Concentrations of carbon, oxygen, nitrogen and phosphorus measured by XPS analysis.

Samples		O/C	N/C	P/C
LGG-S	UM	0.533 ± 0.036	0.055 ± 0.009	0.002 ± 0.002
	HA	0.489 ± 0.007	0.063 ± 0.001	0.000 ± 0.000
	O	0.467 ± 0.024	0.061 ± 0.024	0.000 ± 0.000
LGG-E	UM	0.544 ± 0.040	0.067 ± 0.011	0.005 ± 0.007
	HA	0.478 ± 0.002	0.053 ± 0.006	0.003 ± 0.004
	O	0.448 ± 0.041	0.056 ± 0.017	0.000 ± 0.000
LBC-S	UM	0.351 ± 0.013	0.195 ± 0.017	0.014 ± 0.002
	HA	0.330 ± 0.012	0.171 ± 0.008	0.011 ± 0.001
	O	0.309 ± 0.006	0.174 ± 0.011	0.013 ± 0.000
LBC-E	UM	0.330 ± 0.019	0.202 ± 0.007	0.015 ± 0.001
	HA	0.345 ± 0.020	0.188 ± 0.016	0.013 ± 0.001
	O	0.318 ± 0.001	0.179 ± 0.002	0.012 ± 0.004

Values are represented as mean values resulted from duplicated experiments.

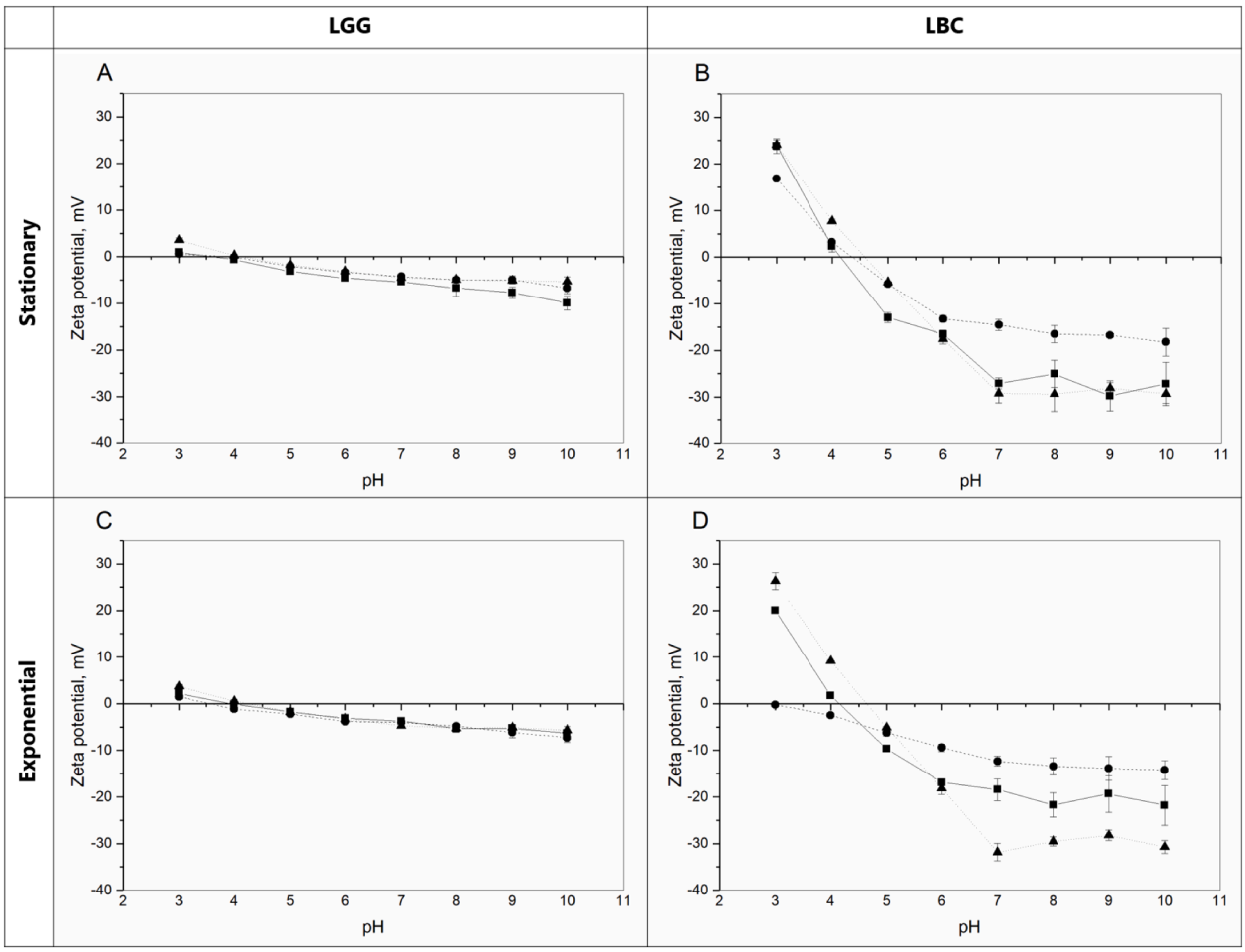

Fig. 2. Zeta potential profiles as a function of pH (3–11) for unmodified (squares), HA-modified (circles) and octanal-modified (triangles) LGG in stationary (A), exponential phase (C) and LBC in stationary (B) and exponential phase (D). Error bars represent standard errors ($n = 2$).

3.6. Bacterial autoaggregation

The autoaggregation of bacteria was measured based on the rate of cell sedimentation over 48 h. Almost all the bacteria sedimented after 48 h and the autoaggregating ability of two strains seemed independent on the growth phase (Fig. 5). Generally, LBC regardless of growth phases, exhibited better ability to autoaggregate than LGG over the observed period. After HA and octanal modification, autoaggregation ability was notably reduced for LBC, where the weakest autoaggregation ability was observed for HA-modified bacteria with an initial Act (2 h) of only 0.26. In comparison, no such remarkable changes were found for LGG after HA and octanal modification except that the autoaggregation within 5 h was, in contrast to LBC, slightly improved by two ways of modifications. However, the overall autoaggregation rate of LGG was still reduced over the later period of observation.

3.7. Relationships among surface properties of bacteria by PCA analysis

Bacteria before and after modifications were grouped in terms of surface properties assessed in different investigations using PCA analysis. It should be noted that among all the investigated properties, only ΔG_{BWB} values are negatively correlated with hydrophobicity, and therefore the absolute values of ΔG_{BWB} ($|\Delta G_{BWB}|$) were used in the PCA analysis, to be more consistent with other properties.

The two strains with and without modifications were differentiated by selected surface properties using PCA analysis, with the first two components explaining 74.04% of the total variance (Fig. 6). Regarding the vectors, the $|\Delta G_{BWB}|$ values deduced in CAM were negatively correlated with Lewis acid (γ_B^+) and base (γ_B^-) characters, whilst no such negative correlation was found between the hydrophobicity (A_H) and Lewis acid (A^+), base (A^-) characters evaluated by MATS, even though the two methods yielded positively-correlated acid properties. Moreover, O/C ratio, as one of the only two factors pointing to the lower quadrants, was negatively correlated with N/C ratio, autoaggregation, IEP and hydrophobicity by MATS. In terms of bacteria, the unmodified LGG samples were clearly separated by Lewis acid properties, whereas unmodified LBC bacteria were grouped in another quadrant by N/C ratios, autoaggregation and IEPs. After modification, the first two dominant factors differentiating modified LGG became O/C ratio and hydrophobicity in terms of $|\Delta G_{BWB}|$ value. Likewise, hydrophobicity also well grouped the modified LBC samples, where HA-modified and octanal-modified LBC were better separated using CAM and MATS, respectively.

4. Discussion

The two investigated strains LBC and LGG displayed intrinsically different surface chemical compositions and physicochemical properties. Unmodified LBC possessed large amount of SLPs with molecular weights in the regions of 45 and 55 kDa. SLPs of especially 45 kDa were previously reported to be responsible for the bacterial surface hydrophobicity, adhering properties and autoaggregation ability [41,46,47]. The proteinaceous surface of unmodified LBC rendered their naturally-high hydrophobicity, as evaluated by MATS and CAM. In

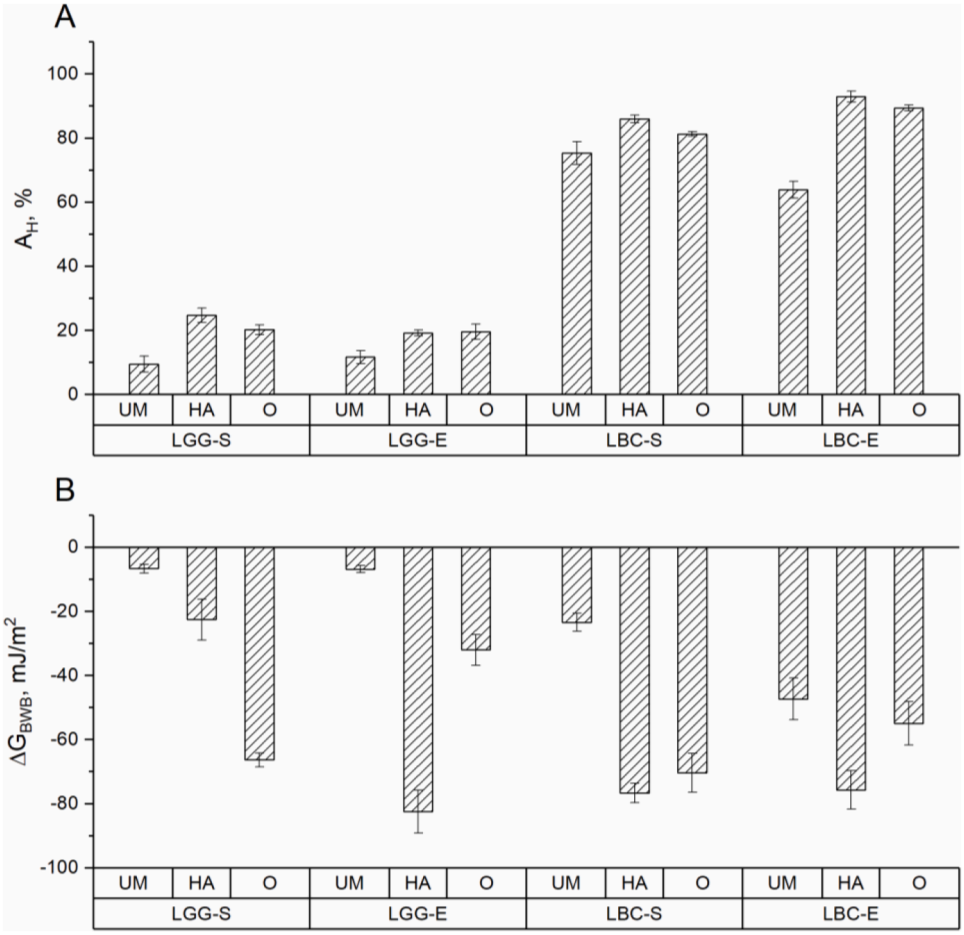

Fig. 3. Hydrophobicity of unmodified and modified bacteria in stationary and exponential phase by MATS and CAM method. A: Hydrophobicity in terms of bacterial adhesion to hexadecane, A_H by MATS. B: Hydrophobicity in terms of cohesive energy of bacteria in water, ΔG_{BWB} by CAM. Error bars represent standard errors (n = 2).

contrast, for unmodified LGG, the abundance of oxygen-rich groups such as carboxylic acids, carboxylates and hydroxyls together with their neutrally-charged surface over the whole pH range, suggested the dominance of hydrophilic anionic or neutral polysaccharides on their surfaces.

The two modification schemes, according to PCA analysis, greatly diminished the abovementioned intrinsic surface differences of LBC and LGG, mainly by enhancing hydrophobicity. Concomitantly with this, the Lewis acid-base properties of bacteria were also reduced to different extents depending on the involved strains, growth phases and modification schemes, as confirmed by both CAM and MATS. These findings were in line with several studies demonstrating that bacterial hydrophobicity was increasingly recognized originating from Lewis acid-base properties and highly-hydrophobic cells were typically weak electron donors (Lewis bases) [36,39,48].

In terms of Lewis acid-base properties, basic characters were detected for all the bacteria by both MATS and CAM, suggesting abundant presence of basic (electron donating) groups such as phosphates from e.g. lipoproteins and (lipo-)teichoic acids, carboxylates and amine groups associated with surface-bound proteins [49,50]. Hydroxyl groups in cell wall polysaccharides were considered as both strongly basic and weakly acidic groups [51]. Therefore, HA and octanal modification removing –OH and $–NH_2$ groups should in principle lower the acidic and basic characters of bacteria.

Indeed, after HA modification, CAM showed a clear reduction or even elimination of basic character for all the HA-modified bacteria, and a stronger reduction of LGG from exponential phase than from stationary phase possibly revealed a richer content of –OH and/or $–NH_2$ on their surfaces. A higher N/C ratio and surface protein concentration of unmodified LGG from exponential phase confirmed the larger presence of $–NH_2$ groups. Similar findings were previously reported that the surface protein concentration of LGG decreased along with growth [10]. In MATS, the basic character after HA modification showed a similar decrease but was nearly unchanged for LGG in exponential phase. It should be noted that the pK_a of $–NH_2$ is about 10 - 11 [52], and the acidic pH (~4.2) used in MATS would therefore protonate $–NH_2$, forming $–NH_3^+$ groups that were on the other hand more acidic than basic [49]. Consequently, if there were high amount of $–NH_2$ on the unmodified surface, MATS might underestimate the basic character of unmodified cells compared to the corresponding modified cells. In view of this, a somewhat unchanged basic character of HA-modified LGG at exponential phase shown by MATS, could be attributed to the higher presence of $–NH_2$ groups on the unmodified surface, compared with LGG at stationary phase. This inference was supported by the results from CAM, XPS and SDS-PAGE analysis.

Octanal modification is based on the reduction of primary amines into imines or imine derivatives. In this reaction, the reductive products were not always imines, because the last step involving deprotonation of iminium ions is very much dependent on the chemical properties of connected variable groups [53]. Therefore, the elimination of $–NH_2$ groups by octanal could either diminish basic character by forming imines or increase the acidic character by generating intermediate iminium cations. Similar to HA modification, the weakest response to octanal modification assessed by MATS was again found for LGG in

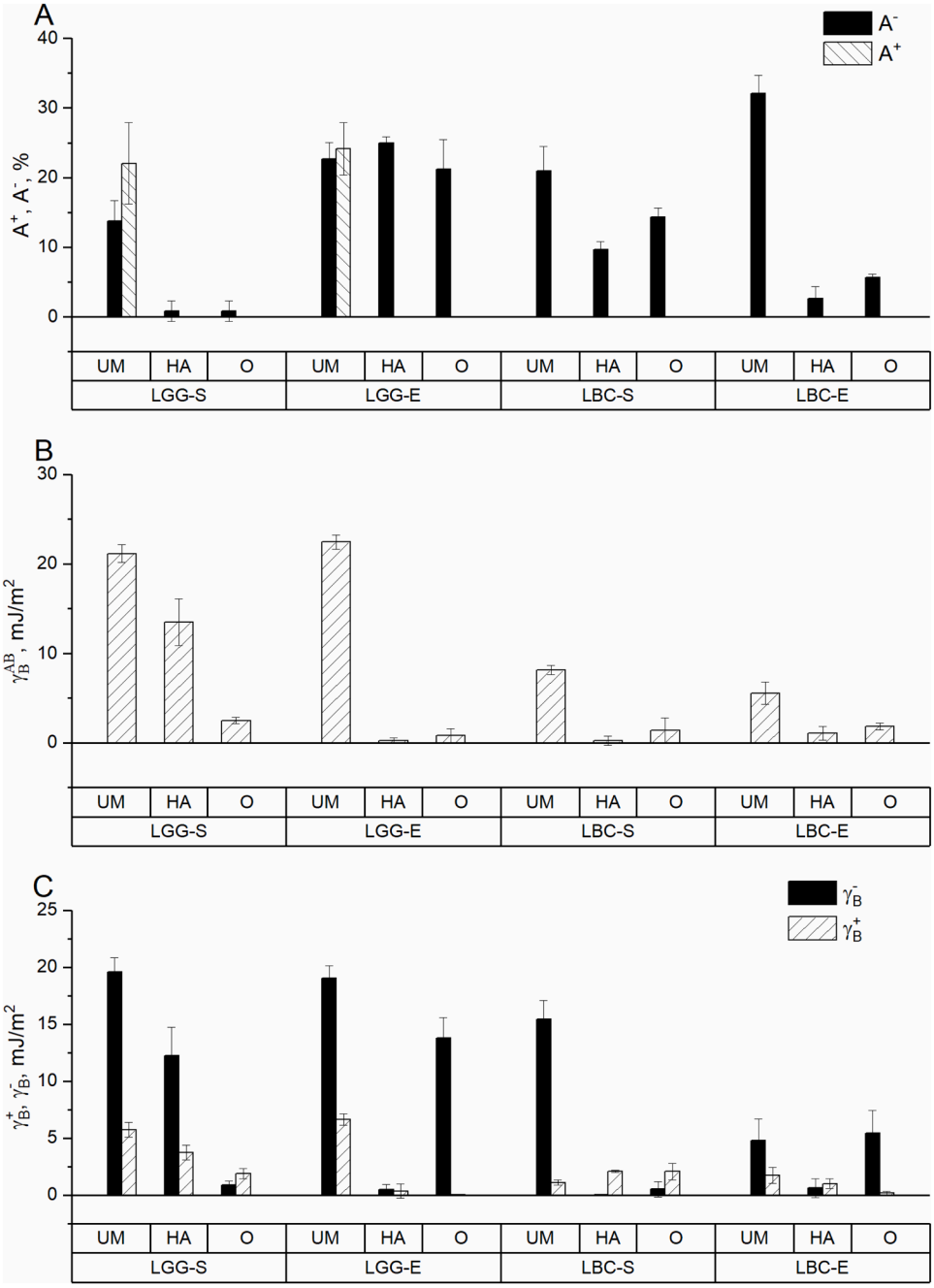

Fig. 4. Surface Lewis acid and base properties of unmodified and modified bacteria in stationary and exponential phase assessed by MATS and CAM method. A: Lewis acid (A^+) and base (A^-) properties by MATS, where all the negative values of A^- and A^+ were assigned the value of "zero", indicating no Lewis acid or base property displayed by bacteria. B: The total Lewis acid-base components (γ_B^{AB}) by CAM. C: Lewis acid (γ_B^+) and base (γ_B^-) parameter components by CAM. Error bars represent standard errors ($n = 2$).

exponential phase due to the potential underestimation of amount of $-NH_2$ groups on the unmodified cells. In CAM, this was no longer a problem and the reduced basic character after octanal modification was demonstrated, but typically less reduced than HA modification, which substituted both –OH and $-NH_2$ groups.

The induced changes in Lewis acid-base properties enables the understanding of the autoaggregation behaviors of LGG and LBC affected by the two modifications. Seeing bacteria as colloidal particles, the aggregation can be induced by long-range forces including electrostatic and steric interactions, as well as short-range Lewis acid-base interactions [41]. We propose the following hypothesis: In the setup of the autoaggregation assay involving high ionic strength (0.15 M PBS), the electrostatic interactions were greatly suppressed, and thus the steric interactions took the determinant roles among the long-range forces. For unmodified LGG, the hydrophilic polysaccharides on the surface formed steric hindrance against cell autoaggregation, while SLPs possessed by LBC were crystalline arrays with high compactness [43,54]. This may have consequently led to the insufficient steric repulsion causing more severe autoaggregation of unmodified LBC. The instability of unmodified LBC may have enabled the further approach of cells and ultimately short-range Lewis acid-base interactions started to become prevalent [55]. Therefore, the reduced autoaggregation of LBC after two modifications could be explained by the reduced Lewis acid-base attractions, as confirmed by MATS and CAM, where HA with a stronger effect in lowering acid-base interactions also resulted in lower autoaggregation than octanal. In contrast to the LBC, the two modifications showed no significant influence on the autoaggregation of LGG. Hence, even though the grafting of hydrophobic moieties might slightly decrease the entropy of hydrophilic polysaccharides, the effect seemed still too small to overcome the energy barriers rendered by strong steric repulsions, and therefore short-range acid-base interactions may have had less effect.

Interestingly, MATS and CAM as complimentary methods, did not

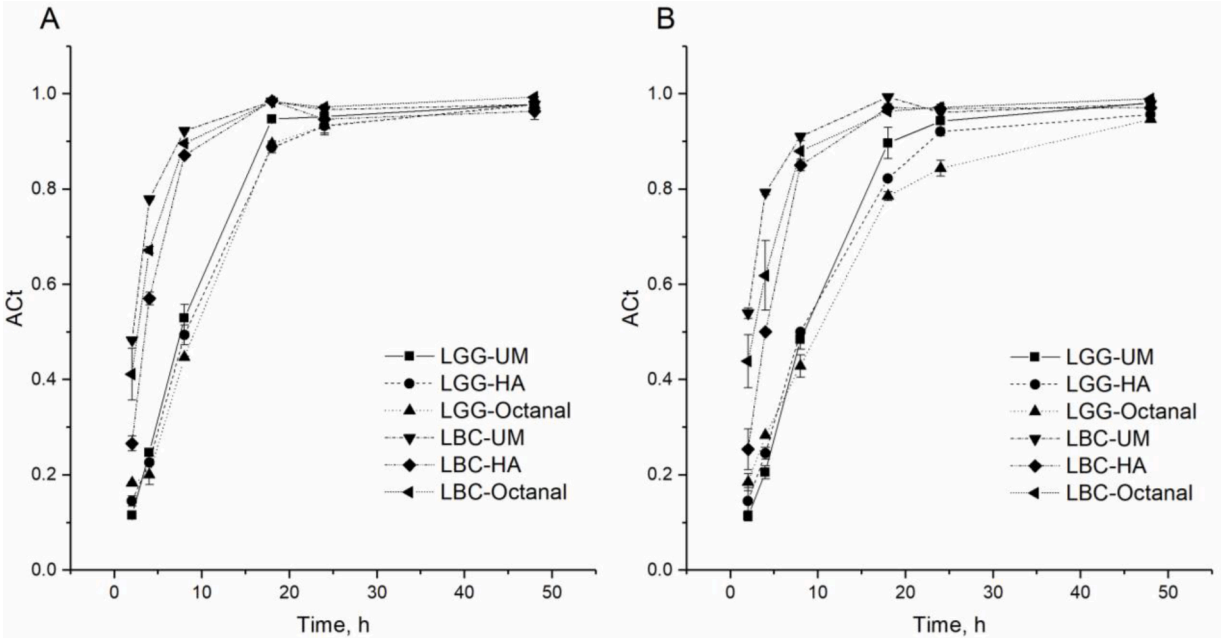

Fig. 5. Autoaggregation ability of unmodified, HA-, octanal-modified LGG and LBC cells from stationary (A) and exponential (B) phase. Error bars represent standard errors ($n = 2$).

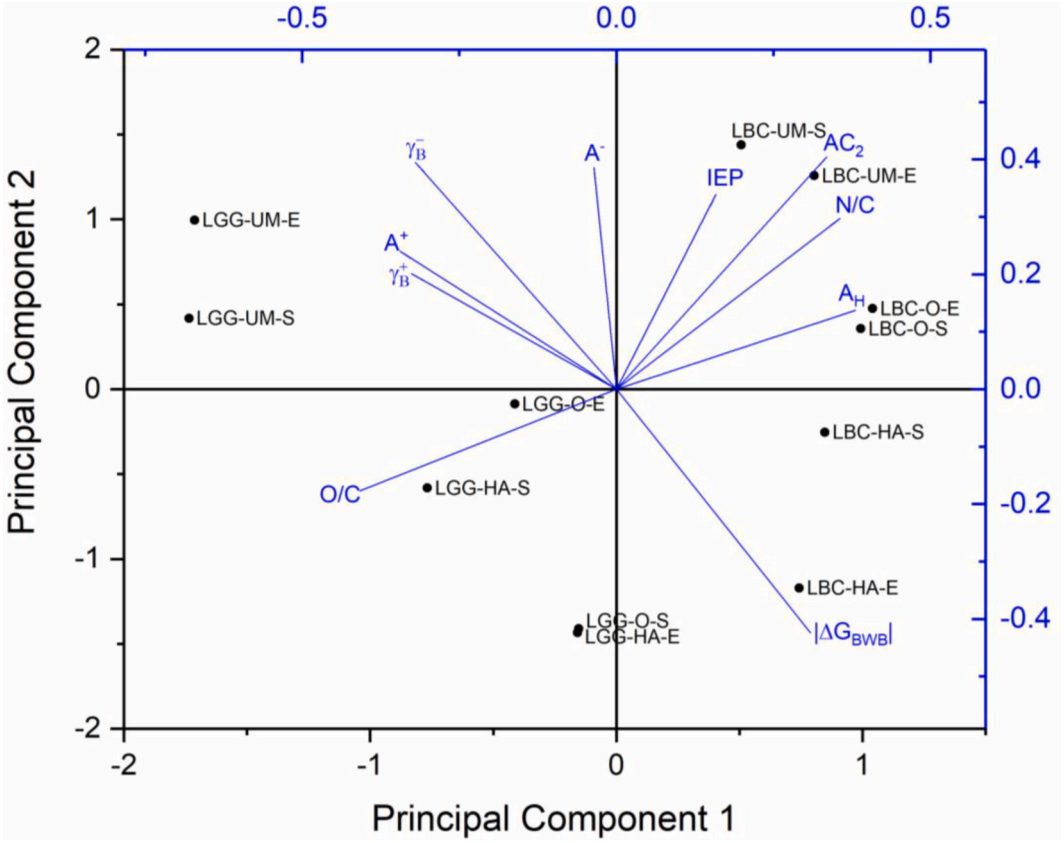

Fig. 6. Principal component analysis (PCA) biplot showing relationships among surface properties of unmodified and modified bacteria including isoelectric point (IEP), autoaggreation after 2 h (AC_2), N/C and O/C ratios by XPS analysis, cell hydrophobicity in terms of adhesion to hexadecane (A_H) and absolute cohesive energy of bacteria in water ($|\Delta G_{BWB}|$), as well as Lewis acid-base properties evaluated by MATS (A^+, A^-), and CAM (γ_B^+, γ_B^-). The first two principal components explained 54.46% and 19.58% of the total variance, respectively.

always lead to the same conclusions. According to PCA analysis, a negative correlation was obtained between the acid/basic properties (γ_B^+, γ_B^-) and hydrophobicity ($|\Delta G_{BWB}|$) by CAM, whereas such correlation was not found in MATS. Also, for LGG samples, CAM revealed notably-improved hydrophobicity after modifications, whilst only slight improvement was detected by MATS. Such discrepancies might be explained by different measuring principles and performances of two methods. MATS is based on the Pickering stabilization of hydrocarbon droplets by bacteria, so a minimum water contact angle of 35° - 40° is required to ensure bacterial adsorption at interface [56]. Therefore, MATS might show low sensitivity in evaluating hydrophilic cells, which complied with our finding that unmodified LGG with a water contact angle of around 55° showed low adhesion to hexadecane. In addition, because of a Pickering mechanism, it is difficult for MATS to well distinguish the effects of e.g. electrostatic interactions and van der Waal attractions. Despite these weaknesses, MATS allowed the investigation of naturally-hydrated bacterial cells, meaning that the steric effects provided by surface polymers could also be captured in this method. Hence, for LGG samples, an overall low adhesion to hexadecane (< 25%) could also be attributed to the strong steric repulsions by surface hydrophilic polysaccharides. In contrast, CAM measuring on dry bacterial lawns might oppositely lead to the conformational changes of such polymers by limiting their mobility. Therefore, the steric effect of LGG might become not fully-detectable in CAM as the hydrophilic polymers would collapse upon drying.

The PCA plot has revealed some potential correlations between different surface properties. For example, the IEPs of bacteria were negatively correlated with O/C ratios and positively correlated with aggregation and N/C ratios. Indeed, a high O/C ratio suggested the presence of a large number of oxygen-rich groups, namely phosphates, carboxylates or carboxylic acid and sulfate groups, which are normally contained in strong polyelectrolytes contributing to the surface negative charges [57,58]. In contrast, SLPs with considerably high pI of 9 – 11 can oppositely raise the IEP of bacteria [7]. This was confirmed by the highly distinguishable unmodified LBC from unmodified LGG according to factors of IEPs, aggregation and N/C ratios, which were mostly related to the presence of SLPs in LBC. However, these factors again became less dominant after HA and octanal modification, which in a way gave rise to the similarity of SLP-carrying LBC and non-SLP-carrying LGG based on their increased hydrophobicity and decreased Lewis acid/base properties.

Understanding the mechanisms behind HA and octanal modification lays the fundamental aspects for the potential applications of such modifications in future. Regarding applications, one essential question is that if the modifications kill the bacteria. According to our previous studies where lactic acid bacteria were surface-modified using acid anhydrides, the viability of bacteria was greatly affected by the dosage of chemical agents, and applying proper concentrations succeeded in improving cell hydrophobicity without compromising their viability [59,60]. Hence, living bacteria with desirable surface properties allows for different functionalities in food, pharmaceutical and biotechnological applications. For example, tuned autoaggregation ability helps the adhesion and colonization of lactic acid bacteria in human gastrointestinal tract, providing the prerequisites of their health-promoting functionality as probiotics. Moreover, micron-sized *Lactobacillus* bearing partial hydrophobicity were reported as effective Pickering stabilizers, creating various colloidal structures including foam [20,61], emulsion [59,62], double emulsion [63] and colloidosomes [60].

5. Conclusion

The present study modified the surface of lactic acid bacterial strains, LBC and LGG, using two chemical modifying schemes, which induced changes in surface properties as confirmed by using a series of physicochemical approaches. Unmodified LGG possessed a hydrophilic and neutrally-charged surface rich in polysaccharides, rendering sufficient steric repulsions against cell autoaggregation, while unmodified LBC was covered by compact SLPs and polyelectrolytes containing phosphates which displaying high charge density, hydrophobicity and autoaggregation ability. However, these surface differences of two strains were greatly suppressed after HA and octanal modifications, mainly by enhancing hydrophobicity and reducing Lewis acid-base properties due to the grafting of hydrophobic moieties onto bacterial functional groups. Growth phases affected the degree of such outcomes based on the amount of –OH and $–NH_2$ groups on the unmodified cells. Regarding this, MATS and CAM were in agreement when evaluating hydrophobic LBC samples, but lacking systematic correlations for LGG due to the different mechanisms and experimental performances of two methods. By understanding the modification of two strains using HA and octanal, this study provides insights into the probing of physicochemical properties of lactic acid bacteria, along with further possibilities to precisely tune the surface of microorganisms, in order to serve purposes in applications such as improving probiotic adhesion to intestinal tract, biofilm formation and Pickering stabilization of colloidal structures.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

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References

[1] C.P. Champagne, C. Lacroix, I. Sodini-Gallot, I. Sodini-Gallop, Immobilized Cell Technologies for the Dairy Industry, Crit. Rev. Biotechnol. 14 (1994) 109–134, https://doi.org/10.3109/07388559409086964.
[2] C. Pelletier, C. Bouley, C. Cayuela, S. Bouttier, Cell Surface Characteristics of Lactobacillus casei subsp.casei, Lactobacillus paracasei subsp.paracasei, and Lactobacillus rhamnosus Strains, Appl. Environ. Microbiol. 63 (1997) 1725–1731.
[3] Christophe.J.P. Boonaert, Paul.G. Rouxhet, Surface of Lactic Acid Bacteria: relationships between Chemical Composition and Physicochemical Properties, Appl. Environ. Microbiol. 66 (2000) 2548–2554, https://doi.org/10.1128/AEM.66.6.2548-2554.2000.
[4] S.H. Flint, P.J. Bremer, J.D. Brooks, Biofilms in dairy manufacturing plant - description, current concerns and methods of control, BIOFILMS IN DAIRY MANUFACTURING PLANT-, Biofouling 11 (1997) 81–97, https://doi.org/10.1080/08927019709378321.
[5] G. Deepika, D. Charalampopoulos, Surface and Adhesion Properties of Lactobacilli, 1st ed., Elsevier Inc., 2010 https://doi.org/10.1016/S0065-2164(10)70004-6.
[6] G. Deepika, E. Karunakaran, C.R. Hurley, C.A. Biggs, D. Charalampopoulos, Influence of fermentation conditions on the surface properties and adhesion of Lactobacillus rhamnosus GG, Microb. Cell Fact. 11 (1) (2012), https://doi.org/10.1186/1475-2859-11-116.
[7] P. Schär-Zammaretti, J. Ubbink, The Cell Wall of Lactic Acid Bacteria : surface Constituents and Macromolecular Conformations, Biophys. J. 85 (2003) 4076–4092, https://doi.org/10.1016/S0006-3495(03)74820-6.
[8] M.-.P. Chapot-Chartier, S. Kulakauskas, Cell wall structure and function in lactic acid bacteria, Microb. Cell Fact. 13 (1) (2014) S9, https://doi.org/10.1186/1475-2859-13-S1-S9. Suppl.
[9] P. Schär-zammaretti, M. Dillmann, D. Amico, M. Affolter, J. Ubbink, P. Scha, N. D. Amico, Influence of fermentation medium composition on physicochemical surface properties of lactobacillus acidophilus, Appl. Environ. Microbiology 71 (2005) 8165–8173, https://doi.org/10.1128/AEM.71.12.8165.
[10] G. Deepika, R.J. Green, R.A. Frazier, D. Charalampopoulos, Effect of growth time on the surface and adhesion properties of Lactobacillus rhamnosus GG, J. Appl. Microbiol. 107 (2009) 1230–1240, https://doi.org/10.1111/j.1365-2672.2009.04306.x.

[11] J. Delcour, T. Ferain, M. Deghorain, E. Palumbo, P. Hols, The biosynthesis and functionality of the cell-wall of lactic acid bacteria, Antonie van Leeuwenhoek, Int. J. General Mol. Microbiol. 76 (1999) 159–184, https://doi.org/10.1023/A:1002089722581.

[12] T. Toba, R. Virkola, B. Westerlund, Y. Bjorkman, J. Sillanpaa, T. Vartio, N. Kalkkinen, T.K. Korhonen, A collagen-binding S-layer protein in Lactobacillus crispatus, Appl. Environ. Microbiol. 61 (1995) 2467–2471, https://doi.org/10.1128/aem.61.7.2467-2471.1995.

[13] H.C. Van Der Mei, B. Van De Belt-Gritter, P.H. Pouwels, B. Martinez, H.J. Busscher, Cell surface hydrophobicity is conveyed by S-layer proteins - A study in recombinant lactobacilli, Colloids Surf. B Biointerfaces 28 (2003) 127–134, https://doi.org/10.1016/S0927-7765(02)00144-3.

[14] G. Deepika, E. Karunakaran, C.R. Hurley, C.A. Biggs, D. Charalampopoulos, Influence of fermentation conditions on the surface properties and adhesion of Lactobacillus rhamnosus GG, 2012. 10.1186/1475-2859-11-116.

[15] G. Francius, D. Alsteens, V. Dupres, S. Lebeer, S. De Keersmaecker, J. Vanderleyden, H.J. Gruber, Y.F. Dufrêne, Stretching polysaccharides on live cells using single molecule force spectroscopy, Nat. Protoc. 4 (2009) 939–946, https://doi.org/10.1038/nprot.2009.65.

[16] A. Ampatzoglou, B. Schurr, G. Deepika, S. Baipong, D. Charalampopoulos, Influence of fermentation on the acid tolerance and freeze drying survival of Lactobacillus rhamnosus GG, Biochem. Eng. J. 52 (2010) 65–70, https://doi.org/10.1016/j.bej.2010.07.005.

[17] A.E. Zeraik, M. Nitschke, Influence of Growth Media and Temperature on Bacterial Adhesion to Polystyrene Surfaces, Arch. Biol. Technol. v. 55 (2012) 569–576, https://doi.org/10.1590/S1516-89132012000400012.

[18] F. Vaningelgem, M. Zamfir, T. Adriany, L. De Vuyst, Fermentation conditions affecting the bacterial growth and exopolysaccharide production by Streptococcus thermophilus ST 111 in milk-based medium, J. Appl. Microbiol. 97 (2004) 1257–1273, https://doi.org/10.1111/j.1365-2672.2004.02418.x.

[19] P. Wongkongkatep, K. Manopwisedjaroen, P. Tiposoth, S. Archakunakorn, T. Pongtharangkul, M. Suphantharika, K. Honda, I. Hamachi, J. Wongkongkatep, Bacteria interface pickering emulsions stabilized by self-assembled bacteria−chitosan network, Langmuir 28 (2012) 5729–5736, https://doi.org/10.1021/la300660x.

[20] C.Y. Falco, X. Geng, M. Cárdenas, J. Risbo, Edible foam based on Pickering effect of probiotic bacteria and milk proteins, Food Hydrocoll 70 (2017) 211–218, https://doi.org/10.1016/j.foodhyd.2017.04.003.

[21] X. Song, G. He, H. Ruan, Q. Chen, Preparation and properties of octenyl succinic anhydride modified early indica rice starch, Starch/Staerke 58 (2006) 109–117, https://doi.org/10.1002/star.200500444.

[22] R.L. Shogren, A. Viswanathan, F. Felker, R.A. Gross, Distribution of octenyl succinate groups in octenyl succinic anhydride modified waxy maize starch, Starch/Staerke 52 (2000) 196–204, https://doi.org/10.1002/1521-379X(200007)52, 6/7<196::AID−STAR196>3.0.CO;2-4.

[23] M. Matos, A. Marefati, G. Gutiérrez, M. Wahlgren, M. Rayner, Comparative emulsifying properties of octenyl succinic anhydride (OSA)-modified starch: granular form vs dissolved state, PLoS One 11 (2016) 1–16, https://doi.org/10.1371/journal.pone.0160140.

[24] C.L. Mccormick, P.A. Callais, Derivatization of cellulose in lithium chloride and N-N-dimethylacetamide solutions, Polymer (Guildf) 28 (1987) 2317–2323, https://doi.org/10.1016/0032-3861(87)90393-4.

[25] J. Wu, J. Zhang, H. Zhang, J. He, Q. Ren, M. Guo, Homogeneous acetylation of cellulose in a new ionic liquid, Biomacromolecules 5 (2004) 266–268, https://doi.org/10.1021/bm034398d.

[26] K. Gopalan Nair, A. Dufresne, A. Gandini, M.N. Belgacem, Crab Shell Chitin Whiskers Reinforced Natural Rubber Nanocomposites. 3. Effect of Chemical Modification of Chitin Whiskers, (2003). 10.1021/bm030058g.

[27] X. Jiang, C.Y. Falco, K.N. Dalby, H. Siegumfeldt, N. Arneborg, J. Risbo, Surface engineered bacteria as Pickering stabilizers for foams and emulsions, Food Hydrocoll 89 (2019) 224–233, https://doi.org/10.1016/j.foodhyd.2018.10.044.

[28] E.I. Rabea, M.E. Badawy, T.M. Rogge, C.V. Stevens, W. Steurbaut, M. Höfte, G. Smagghe, Enhancement of fungicidal and insecticidal activity by reductive alkylation of chitosan, Pest Manag Sci 62 (2006) 890–897, https://doi.org/10.1002/ps.1263.

[29] M.E. Badawy, Chemical modification of chitosan: synthesis and biological activity of new heterocyclic chitosan derivatives, Polym. Int. 57 (2008) 254–261, https://doi.org/10.1002/pi.2333.

[30] J. Desbrières, C. Martinez, M. Rinaudo, Hydrophobic derivatives of chitosan: characterization and rheological behaviour, Int. J. Biol. Macromol. 19 (1996) 21–28, https://doi.org/10.1016/0141-8130(96)01095-1.

[31] E. Li, R. Mira de Orduña, A rapid method for the determination of microbial biomass by dry weight using a moisture analyser with an infrared heating source and an analytical balance, Lett. Appl. Microbiol. 50 (2010) 283–288, https://doi.org/10.1111/j.1472-765X.2009.02789.x.

[32] S. Lortal, J. Van Heijenoort, K. Gruber, U.B. Sleytr, Slayer of Lactobacillus helveticus ATCC 12046: isolation, chemical characterization and re-formation after extraction with lithium chloride, J. General Microbiol. 138 (1992) 61–62, https://doi.org/10.1099/00221287-138-3-611.

[33] U.K. Laemmli, Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4, Nature 227 (1970) 680–685, https://doi.org/10.1038/227680a0.

[34] P.K. Smith, R.I. Krohn, G.T. Hermanson, A.K. Mallia, F.H. Gartner, M. D. Provenzano, E.K. Fujimoto, N.M. Goeke, B.J. Olson, D.C. Klenk, Measurement of protein using bicinchoninic acid, Anal. Biochem. 150 (1985) 76–85, https://doi.org/10.1016/0003-2697(85)90442-7.

[35] M.N. Bellon-Fontaine, J. Rault, C.J. Van Oss, Microbial adhesion to solvents: a novel method to determine the electron-donor/electron-acceptor or Lewis acid-base properties of microbial cells, Colloids Surf. B Biointerfaces 7 (1996) 47–53, https://doi.org/10.1016/0927-7765(96)01272-6.

[36] F. Hamadi, H. Latrache, Comparison of contact angle measurement and microbial adhesion to solvents for assaying electron donor-electron acceptor (acid–base) properties of bacterial surface, Colloids Surf. B Biointerfaces 65 (2008) 134–139, https://doi.org/10.1016/j.colsurfb.2008.03.010.

[37] H.J. Busscher, A.H. Weerkamp, H.C. van Der Mei, A.W. van Pelt, H.P. de Jong, J. Arends, Measurement of the surface free energy of bacterial cell surfaces and its relevance for adhesion, Appl. Environ. Microbiol. 48 (1984) 980–983, https://doi.org/10.1128/aem.48.5.980-983.1984.

[38] C.J. Van Oss, M.K. Chaudhury, R.J. Good, Interfacial Lifshitz-van der Waals and Polar Interactions in Macroscopic Systems, Chem. Rev. 88 (1988) 927–941, https://doi.org/10.1021/cr00088a006.

[39] C.J. van Oss, Hydrophobicity of biosurfaces - Origin, quantitative determination and interaction energies, Colloids Surf. B Biointerfaces 5 (1995) 91–110, https://doi.org/10.1016/0927-7765(95)01217-7.

[40] C.J. van Oss, R.J. Good, M.K. Chaudhury, Additive and nonadditive surface tension components and the interpretation of contact angles, Langmuir 4 (1988) 884–891, https://doi.org/10.1021/la00082a018.

[41] M. Polak-Berecka, A. Wasko, R. Paduch, T. Skrzypek, A. Sroka-bartnicka, The effect of cell surface components on adhesion ability of Lactobacillus rhamnosus, Antonie Van Leeuwenhoek (2014) 751–762, https://doi.org/10.1007/s10482-014-0245-x.

[42] Y.J. Goh, M. Andrea Azcárate-Peril, S. O'flaherty, E. Durmaz, F. Valence, J. Jardin, S. Lortal, T.R. Klaenhammer, Development and application of a upp-based counterselective gene replacement system for the study of the s-layer protein SlpX of Lactobacillus acidophilus NCFM †, Appl. Environ. Microbiol. 75 (2009) 3093–3105, https://doi.org/10.1128/AEM.02502-08.

[43] E. Smit, F. Oling, R. Demel, B. Martinez, P.H. Pouwels, The S-layer protein of Lactobacillus acidophilus ATCC 4356: identification and characterisation of domains responsible for S-protein assembly and cell wall binding, J. Mol. Biol. 305 (2001) 245–257, https://doi.org/10.1006/jmbi.2000.4258.

[44] M. Rosenberg, Bacterial adherence to hydrocarbons: a useful technique for studying cell surface hydrophobicity, FEMS Microbiol. Lett. 22 (1984) 289–295, https://doi.org/10.1111/j.1574-6968.1984.tb00743.x.

[45] M.C. van Loosdrecht, J. Lyklema, W. Norde, G. Schraa, A.J. Zehnder, The role of bacterial cell wall hydrophobicity in adhesion, Appl. Environ. Microbiol. 53 (1987) 1893–1897, https://doi.org/10.1128/aem.53.8.1893-1897.1987.

[46] B. Kos, J. Šušković, S. Vuković, M. Sĭmpraga, J. Frece, S. Matošić, Adhesion and aggregation ability of probiotic strain Lactobacillus acidophilus M92, J. Appl. Microbiol. 94 (2003) 981–987, https://doi.org/10.1046/j.1365-2672.2003.01915.x.

[47] J. Frece, B. Kos, I.K. Svetec, Z. Zgaga, V. Mrša, J. Šušković, Importance of S-layer proteins in probiotic activity of Lactobacillus acidophilus M92, J. Appl. Microbiol. 98 (2005) 285–292, https://doi.org/10.1111/j.1365-2672.2004.02473.x.

[48] H.C. Van Der Mei, R. Bos, H.J. Busscher, A reference guide to microbial cell surface hydrophobicity based on contact angles, Colloids Surf. B Biointerfaces 11 (1998) 213–221, https://doi.org/10.1016/S0927-7765(98)00037-X.

[49] R. Djeribi, Z. Boucherit, W. Bouchloukh, W. Zouaoui, H. Latrache, A study of pH effects on the bacterial surface physicochemical properties of Acinetobacter baumannii, Colloids Surf. B Biointerfaces 102 (2013) 540–545, https://doi.org/10.1016/j.colsurfb.2012.08.047.

[50] F. Hamadi, H. Latrache, A. El Ghmari, M. Ellouali, M. Mabrrouki, N. Kouider, Effect of pH and ionic strength on hydrophobicity and electron donor and acceptor characteristics of Escherichia coli and Staphylococcus aureus, Ann Microbiol 54 (2004) 213–225, 10.1.1.545.9461.

[51] K.M. Pelzer, L. Cheng, L.A. Curtiss, Effects of Functional Groups in Redox-Active Organic Molecules: A High-Throughput Screening Approach, (2017). 10.1021/acs.jpcc.6b11473.

[52] P. Vollhardt, N. Schore, Acidity and Basicity of Amines, Organic Chemistry (2019) 21, 4.1-21.4.7.

[53] J. Crugeiras, A. Rios, E. Riveiros, T.L. Amyes, J.P. Richard, Glycine enolates: the effect of formation of iminium ions to simple ketones on α-amino carbon acidity and a comparison with pyridoxal iminium ions, NIH Public Access 130 (2009) 2041–2050, https://doi.org/10.1021/ja078006c.Glycine.

[54] B. Johnson, K. Selle, S. O'flaherty, Y.J. Goh, T. Klaenhammer, Identification of extracellular surface-layer associated proteins in Lactobacillus acidophilus NCFM, Microbiology (N Y) 159 (2013) 2269–2282, https://doi.org/10.1099/mic.0.070755-0.

[55] J. Burgain, J. Scher, G. Francius, F. Borges, M. Corgneau, A.M. Revol-junelles, C. Cailliez-grimal, C. Gaiani, Lactic acid bacteria in dairy food : surface characterization and interactions with food matrix components, Adv. Colloid Interface Sci. 213 (2014) 21–35, https://doi.org/10.1016/j.cis.2014.09.005.

[56] M.C.M.V.A.N. Loosdrecht, J. Lyklema, W. Norde, G. Schraa, A.J.B. Zehnder, Electrophoretic Mobility and Hydrophobicity as a Measure To Predict the Initial Steps of Bacterial Adhesion, Applied and Enviromental Microbiology 53 (1987) 1898–1901, https://doi.org/10.1128/aem.53.8.1898-1901.1987.

[57] R. Briandet, T. Meylheuc, C. Maher, M.N. Bellon-Fontaine, Listeria monocytogenes Scott A: cell surface charge, hydrophobicity, and electron donor and acceptor characteristics under different environmental growth conditions, Appl. Environ. Microbiol. 65 (1999) 5328–5333, https://doi.org/10.1128/aem.65.12.5328-5333.1999.

[58] J. Patel, M. Sharma, S. Ravishakar, Effect of curli expression and hydrophobicity of Escherichia coli O157: H7 on attachment to fresh produce surfaces, (2011) 737–745. 10.1111/j.1365-2672.2010.04933.x.

[59] X. Jiang, C. Yucel Falco, K.N. Dalby, H. Siegumfeldt, N. Arneborg, J. Risbo, Surface engineered bacteria as Pickering stabilizers for foams and emulsions, Food Hydrocoll 89 (2019) 224–233, https://doi.org/10.1016/j.foodhyd.2018.10.044.
[60] X. Jiang, H.J. Martens, E. Shekarforoush, M.K. Muhammed, K.A. Whitehead, N. Arneborg, J. Risbo, Multi-species colloidosomes by surface-modified lactic acid bacteria with enhanced aggregation properties, J. Colloid Interface Sci. 622 (2022) 503–514, https://doi.org/10.1016/J.JCIS.2022.04.136.
[61] X. Jiang, E. Shekarforoush, M.K. Muhammed, K.A. Whitehead, N. Arneborg, J. Risbo, Lactic acid bacteria as structural building blocks in non-fat whipping cream analogues, Food Hydrocoll (2023) 135, https://doi.org/10.1016/j.foodhyd.2022.108137.
[62] H. Firoozmand, D. Rousseau, Microbial cells as colloidal particles : pickering oil-in-water emulsions stabilized by bacteria and yeast, Food Res. Int. 81 (2016) 66–73, https://doi.org/10.1016/j.foodres.2015.10.018.
[63] X. Jiang, E. Shekarforoush, M.K. Muhammed, K. Whitehead, A.C. Simonsen, N. Arneborg, J. Risbo, Efficient chemical hydrophobization of lactic acid bacteria – One-step formation of double emulsion, Food Res. Int. 147 (2021), https://doi.org/10.1016/j.foodres.2021.110460.