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CHANGE IN SURFACE PROPERTIES OF *LACTOBACILLUS REUTERI* DSM 17938 AND *SACCHAROMYCES BOULARDII* UNDER METHYLPREDNISOLON TREATMENT

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Abstract: We undertake this study in the aim to give new insight about the surface properties of microorganism under Medrol® (methylprednisolon) treatment. The several properties of the cell surface of *Lactobacillus reuteri* was determined by a variety of physico-chemical methods. *Saccharomyces boulardii* yeasts as another control of probiotic to Medrol interaction with the cell membrane was used. The study was carried out by microelectrophoresis, dynamic light scattering and laser Doppler velocimetry. We discuss the importance of properly selecting the suspending medium of zeta potential of bacterial cells are to be determined.

Medrol altered the surface electrical charges and size of *Lactobacillus reuteri* DSM 17938 (L. reuteri Protectis®) membrane at doses of 0.5 μg Medrol/mL and at 0.3 – 0.8 mg Medrol/mL. We suggest that the negative charges on the surface of *L. reuteri* were increased by the alteration in specific conductivity. Medrol could penetrate the bacterial membrane and changed the permittivity of the *L. reuteri* membrane surface and changed the particle volume.

There was an enhancement of zeta potential (ζ) of *S. boulardii* yeasts and volume under the action of 0.2 and 0.4 μg Medrol/mL and significant reduction of ζ and volume upon 1 μg Medrol/mL or 5 μg Medrol/mL in distilled water media.

It is pointed out that the above methods form a unique set of techniques for studying microbial cell surfaces, in the sense that the results of the various methods allow an interpretation of the physicochemical properties of the cells in terms of their chemical composition.

INTRODUCTION

Physicochemical phenomena on bacterial surfaces are widely investigated. Most of the modern studies on determining the bacteria electrokinetic properties concern the significance of their electrophoretic mobility and zeta potentials (Lin et al., 2006; Lee, 2009; Strauss, 2009) as necessary parameters for determination of the value of density of surface charge and stability of the cellular suspension. The value of surface charge density set an example for the average quantity of electricity on the unit cell surface. The value of this magnitude is calculated assuming that the electrical charges are equally distributed on the surface.

There are only few studies interpreting the bacterial surface charge density because of the complexity of factors included in their determination. It is not possible to make a conclusion from its value concerning the equalization of the electrical charge distribution on the cellular or sub-cellular surface. Surface charge density is determined by the calculation of the appropriate dependence between its value and that of the ζ potential. Authors reported about abnormal values of zeta potential of *Streptococcus thermophilus* and *Leuconostoc mesenteroides* (two fouling microorganisms from dairy industry (Busscher et al., 1990). Long-range electrostatic forces substantially influence bacterial interactions during the preliminary steps of biofilm formation (Dague et al., 2006).

We suggest that the negative charges on the surface of *L. reuteri* were increased by the alteration in specific conductivity. Medrol could penetrate the bacterial membrane and changed the permittivity of the *L. reuteri* membrane surface and changed the particle volume.

Lactic acid bacteria are acid-tolerant gram-positive bacteria, non-sporulating rods or cocci and are able to produce lactic acid during heterofermentative metabolism (Klaenhammer et al., 2002). They are defined as living cells, prokaryote, heterothrophic and chemo-organothrophic, i.e. they require complex organic molecules as an energy source (Axelsson, 2009; Von Wright and Axelsson, 2012). Lactic acid bacteria exhibit a thick layer varying between 30 and 100 nm of peptidoglycan (Vollmer and Seligman, 2010; Delcour et al., 1999).

The charge of bacterial cell walls mostly originates from carboxylic, phosphate and amino groups (Kleijn and van Leeuwen, 2000; Harden and Harris, 1952). The degree of protonation of these anionic and cationic groups is determined by the pH. Almost all bacterial cells are negatively charged, because the number of deprotonated carboxylate and phosphate groups is generally higher than that of the protonated amino groups. The compensating charge mainly consists of (positive) counterions that penetrate the porous wall, and, to a minor extent, of (negative) co-ions that tend to be expelled from it.

Bacteria migrate toward the opposite charge at a certain speed according to their charge. The more bacteria are charged, the more its electrophoretic mobility (EPM) is high, and they will move faster toward the electrode (Grare et al., 2007). In the case of hard sphere, the EPM of the particles is directly proportional to the zeta potential from the Smoluchowski equation (Duval and Gaboriaud, 2010). In any case, this equation is valid for biological systems due to their soft, heterogeneous and ion-permeable features. Bacteria are a typical

example of soft particles (Oshima, 1995). Bacteria possess the physicochemical surface characteristics such as hydrophobicity, Lewis acid/base and charge which are involved in the physicochemical interactions between cells and interfaces.

Food matrices are complex and heterogenic media, with a microstructure depending on interactions between the components in media (van der Waals, electrostatic or structural forces, etc.). The effect of bacteria on the emulsion stability depends on the surface properties of the strains. Flocculation and aggregation phenomena were observed in emulsion at pH for which the bacterial surface charge was opposed to the one of the proteins. The effects of bacteria on the stability of emulsion depend also on the concentration of cations present in media such as Ca^{2+} (Ly et al., 2008). Thus bacteria, depending on their surface properties, could interact with other components of matrices and consequently affect the stability of emulsions.

Based on Gouy-Chapman theory, we examined the influence of Medrol (methylprednisolon) on the surface electrical charge, particle size and particle volume of *Saccharomyces boulardii* yeasts and of *Lactobacillus reuteri* bacteria.

Lactobacillus species are the major components of starter cultures used in food fermentation or as probiotics (Axelsson et al., 1998). The phenomenon of adhesion to epithelial cells has been considered a priority for selection of probiotics, and it is mediated by the physicochemical properties of the bacterial surface like the microbial cell surface hydrophobicity, known as one of the determining factors in microbial adhesion, but also by specific molecular interactions (Upadrasta et al., 2011). The other factor is the zeta potential of bacterial surface and significance of the surface electrical charges on it. Retaining lactobacillus viability through the intestine represents major technological and biological hurdles, given the extreme conditions the lactobacillus encounter during production and storage and in the harsh environment of the stomach. During production and processing, lactobacillus undergo several stresses such as substrate depletion (end of fermentation), osmolarity and temperature shock (freeze- or spray-drying) (Teixeira et al., 1995; Lian et al., 2002; Santivarangkna et al., 2007). When exposed to such conditions, bacteria activate stress responses that involve up- and downregulation of certain genes. Generally, bacteria have a natural tendency to adhere to surfaces as a survival mechanism that help bacterial strains to continue to be present in the bacteria flora of the gut several days and to be active during intestinal transit, participate in digestion process, inhibit and/or prevent the colonization of pathogens and create a healthy environment (Larpent et al., 1994; Servin and Coconnier 2003).

However, cell surface properties including electrophoretic mobility, electrokinetic (zeta) potential and surface electrical charge as impacted by the composition of the membrane can be altered through exposure to additional environmental stresses including drug administration (Medrol addiction). These changes alter membrane electrostatics and the cell's ability to interact with medicine and survive in the surrounding environment (isotonic media or low ionic strength buffered solution as well as water media). Membrane treatment by methylprednisolon could results in sustained local concentrations of Medrol® at the membrane interface. *S. boulardii* yeasts or *L. reuteri* DSM 17938 bacteria

could be used as carriers for Medrol as an immunomodulator.

In order to give new insight about the adaptive strategies developed by *Lactobacillus reuteri* to overcome the unfavourable growth and media conditions and to find the relationship between surface characteristics – induced changes in membrane and size and volume to Medrol treatment, the effects of methylprednisolon on cell membrane electrokinetic properties and polydispersity were investigated.

In this work, we determined: 1) dose effects on cell membrane of *S. boulardii* and *L. reuteri* of physicochemical properties at lower and higher concentrations of Medrol treatment; 2) effect of vesicle size or length of bacteria on electrokinetic parameters of *S. boulardii* and *L. reuteri* cells, respectively; 3) simultaneous effect of cell volume on yeast or on bacteria electrophoretic mobility, zeta potential and surface electrical charge.

MATERIALS AND METHODS

Microorganisms, preparation conditions

Lactobacillus reuterii DSM 17938 (*L. reuteri* Protectis®) was prepared after 4 times washing with PBS (Phosphate Buffered Saline free, 7.4) of the BioGaia Protectis drops, purchased by BioGaya AB, Stockholm medicine, to remove the additives. *Lactobacillus reuteri* (5 drops) equivalent to 100 million active bacteria were suspended in 1 mL of Phosphate Buffered Saline free, (pH 7.4). The samples were washed for 4 min at 4500 g using Eppendorf centrifuge (MiniSpin). The pellets were then dissolved in 250 μL PBS without or in the presence of Medrol treatment (μg/mL). After incubation at 37°C for 30 minutes in ThermoMix (Analytik Jena) a bacterial suspension was stored at 25°C until use (2 hours). To determine particle size, as well as EPM, ζ or σ, 100 μL of the incubated samples were dissolved in 1 mL of Saline Sorbitol Buffer (pH 7.5) where the final concentration of 4,17 x 10^5 cells/mL was determined.

Saccharomyces boulardii was purchased from medicine from Biocodex, France (Enterol®). *Saccharomyces boulardii* yeasts (equivalent to 250 mg lyophilized cells of *S. boulardii*) were dissolved in 10 mL of PBS, pH 7.4 for an hour at 25 °C (concentration of 1,85 x 10^9 cells/mL).

Saccharomyces boulardii cells (100 μL) were diluted in 1 mL of double distilled water (with conductivity æ = 0,68 μS cm^{-1}) where the final concentration of 1,85 x 10^8 cells of *S. boulardii* per milliliter was used in the presence of Medrol (μg/mL). The *S. boulardii* suspension in the presence of Medrol (μg/mL) were incubated for 30 min at 25°C and stored at 25°C until DLS and LDV measurements for ~ 2 hours (concentration of 1,85 x 10^8 cells/mL).

Microelectrophoresis measurements

The electrophoretic mobility (EPM) was measured using a Cytopherometer (OPTON, Feintechnik GmbH, Wien, Austria) using a rectangular cell and platinum electrodes. Electrophoretic migration of 15 - 25 particles was timed for both forward and backward (reversed field) runs over a known distance (32 μm) at a constant electric current of 0.1 mA, voltage of 440 V (*S. boulardii*), as well

as of 2 mA, 320 V (*L. reuteri*). The S. *boulardii* cells were suspended in distilled water and *L. reuteri* were suspended in Saline Sorbitol Buffered (SSB) medium: Sorbit 4.5%, NaCl 0.0145 M, $NaHCO_3$ 6 x 10^{-4} M, pH 7.50. The bacteria were observed under a light microscope, connected to a Sony video camera, providing 800-times magnification. The observation light (with intensity of 13 μmol quanta m-2 s-1) was filtered through a blue interference filter. The images were recorded on a Sony video recorder RDR-GX700/S. The results were expressed as means of the EPM per 10^{-8} m^2 $V^{-1}s^{-1}$ ± standard error for each probe. The standard errors of the electrophoretic mobility u were 2 - 7 %. The electric conductivity of the suspension medium was measured using a Cyber Scan PC510 (Eutech Instruments, USA,Singapore) pH/Conductivity meter. Its value (SSB) was 1.8 mS cm^{-1}. The experiments were carried out at 25°C.

The zeta (electrokinetic) potential ζ was calculated from the electrophoretic mobility u using Helmholtz-Smoluchowski equation (Hiemenz, 1977):

$$\zeta = \frac{4\pi.\eta \times u}{\varepsilon_r \times \varepsilon_0}$$

where ζ is the electrokinetic potential in mV, ε_r is the dielectric constant of the aqueous phase (at 25 °C), ε_o is the permittivity of free space (ε_o = 8.8542 ε10^{-12} F m^{-1}), η is the viscosity of the aqueous phase (η = 0.0010 Pa s at 25°C).
The electrostatic potential in the aqueous phase at the surface of the membrane located at x = 0, and σ (charge density) was calculated:

$$\frac{A\sigma}{C^{1/2}} = \sinh\left(\frac{ze\psi_0}{2kT}\right)$$

where k is the Boltzmann constant, T is temperature, e is electronic charge, z is the valence of the symmetrical electrolyte solution, and C is the bulk aqueous electrolyte concentration.

$$A = \frac{1}{(8N\varepsilon_r\varepsilon_0 kT)^{1/2}}$$

where N is Avogadro's number, ε_r the dielectric constant, ε_o the permittivity of free space; constant A = 136.6 and kT/c = 25.69 at 25°C.
If surface electrical charge (σ expressed in electronic charge/square angstrom) was calculated according to McLaughlin (1977) and C in moles/liter, then:

$$\frac{136.6\sigma}{C^{1/2}} = \sinh\left(\frac{z\psi}{51.38}\right)$$

where the bulk aqueous electrolyte concentration C is in moles/dm^{-3} (M), ψo= mV and ψo≈ ζ. (McLaughlin, 1977).
The Debye Length, δ^{-1} (nm) (specifically defined as the distance over which the electric field and potential decay to (1/e) of their value at x = 0) is δ^{-1} = 964 nm for the case of water solution or δ^{-1} = 3.0 nm for the case of SSB media.

Dynamic light scattering and laser Doppler velocimetry

The size of the *L. reuteri* (length of bacteria) and *S. boulardii* cells (diameter of sphere) were measured with MICROTRAC Zetatrac ZS instrument (Model NPA152, Largo, FL, USA) with a 2 Solid-State Diode Lasers, providing a 780 nm light and an optical output power of 3 mW, nominal grade 111B. The ZetatracTM uses the dynamic light scattering method. The velocity distribution of a sample of particles suspended in a medium is a known function of the particle size. Light from the laser diode is coupled to the sample through an optical power splitter assembly. Light scattered from each particle is Doppler-shifted by particle motion (Brownian motion). The Doppler-shifted scattered light is mixed with coherent, unishifted light; and the optical system sends these mixed signals to a silicon photodetector. The detector output signal is then amplified, filtered, digitized, and mathematically analyzed by the Microtrac ® FLEX Windows Software, using proprietary algorithms, to provide the particle size distribution. For measuring particle size distribution, the *L. reuteri* and *S. boulardii c*ells were properly dispersed in the solvent (distilled water or Saline Sorbitol Buffer) using Vortex (Labnet, Labnet International, Inc.). The ionic strength (I) of the deionized water is reported as 1 x 10^{-7} M (Gregory, 2005) and I = 0,0151 M for SSB media.

The Zetatrac™ analyzer measures the additional velocity impaired to the charged particles when placed in an electric field. Particle electrophoretic mobility used accepted relationships between mobility and zeta potential. The relationship between zeta potential and mobility is given by the Smoluckowski equation: $\zeta = u.\eta/\varepsilon$, where ζ = zeta potential, u = electrophoretic mobility, η = viscosity, ε = dielectric constant for water at 25 ^{0}C and zeta potential (mV) ~ 12.8 x electrophoretic mobility (μ/sec/Volt/cm). The absolute values of the electrophoretic mobility, zeta potential, as well as charge were represented.

Statistical Analysis

Results represented the mean ± standard deviation or standard error of mean. The statistical analysis of data was performed using the t-test, while the statistical significance of data was set at $p \leq 0.05$.

RESULTS AND DISCUSSION

In the present work the Lactic acid bacteria would be used as a model system for study the surface properties of the membranes and to examination the mechanisms by which the Medrol® (methylprednisolon) affect the membrane. In order to understand the bacterial interactions, electrokinetic and optic approaches would be applied with a special focus on the environmental conditions (pH, ionic strength) and concentration affecting surface electrical properties of bacteria. Several techniques have been implemented to evaluate bacterial surface and methylprednisolon interaction, such as particle microelectrophoresis, dynamic light scattering (DLS) and laser Doppler velocimetry (LDV) methods.

The investigations on the influence of immunomodulators were made (Лазаров и сътр., 2010). Medrol activates the anti-inflammatory program of the immune system and organism is protected by the inflammatory processes, which

could affect the mucous membrane and tissues of the respiratory organs in depth, to provide necrosis or liquid keeping (Feinberg et al., 1957).

There was a slight increase in the Medrol induced EPM change of *S. boulardii* yeasts in distilled water at concentrations of 0.2 μg/mL and 0.4 μg/mL by 10% due to the relative effectiveness of Medrol binding to the membrane surface (Fig. 1A). The values changed from – 3.07 x 10^{-8} $m^2V^{-1}s^{-1}$ (without Medrol) to – 3.37 x 10^{-8} $m^2V^{-1}s^{-1}$ (in the presence of 0.2 μg/mL), and to – 3.42 x 10^{-8} $m^2V^{-1}s^{-1}$ (in the presence of 0.4 μg/mL). The relative mobility reduction of EPM of *S. boulardii* yeasts was estimated at 12.4%. There was a decrease in EPM of *S. boulardii* cells in the presence of 1 μg Medrol/mL (u = – 2.69 x 10^{-8} $m2V^{-1}s^{-1}$), where the minimal surface electrical charge was calculated (σ = – 0.0470 $C.m^{-2}$) (Fig. 1C).

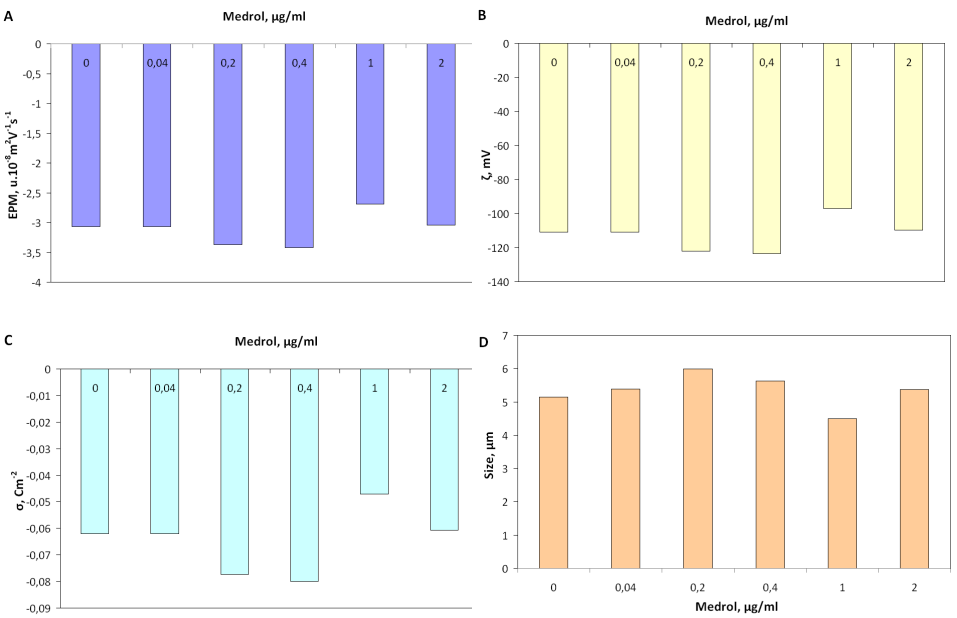

Fig. 1. Influence of Medrol at doses of (0.04 – 2 μg/mL) on electrophoretic mobility (A), zeta potential (B), surface charge density (C) and particle size (D) of *Saccharomyces boulardii* yeasts in Distilled Water.

The small effect of increase in negative zeta potential by about of 10% at 0.2 μg/ml and 11.5% at concentrations of 0.4 μg/mL was established at low ionic strength media. *S. boulardii* yeasts possess high negative charges during incubation in distilled water and altered sizes without and after treatment with Medrol (0.04 – 2 μg/mL). The large difference in the electrokinetic behavior of *S. boulardii* yeasts in water solution is important to be noticed. An increase of zeta potential (ζ) by approximately 12 – 13 mV at acting concentration of 0.2 and 0.4 μg Medrol/mL, where ζ reaches – 122 mV to -124 mV in comparison to control

value of – 110.88 mV (Fig. 1B). These data could be due to a sharp increase in size of *S. boulardii y*easts of 16.5% and 9%, respectively, under the upper doses of Medrol (Fig. 1D). Dose of 1 μg Medrol/mL, however, significantly decreased the zeta potential with 12.45%, like reaching to – 97.07 mV. It was connected by the strong decrease in particles volume of 33% and decrease in particles size with 12.45%.

We observed a significant decrease in the net negative surface electrical charge of *S. boulardii* yeasts in the presence of 1 μg Medrol/mL (σ varied from control level of $\sigma = -0.062$ C.m^{-2} without Medrol to $\sigma = -0.047$ C.m^{-2} in the presence of 1 μg Medrol/mL).

There was a strong increase in surface charge of *S. boulardii* yeasts after lower concentrations of Medrol in distilled water (σ varied from – 0. 077 C.m^{-2} in the presence of 0.2 μg Medrol/mL to – 0.080 C.m^{-2} in the presence of 0.4 μg Medrol/mL) (Fig. 1C).

There was no significant alteration on the EPM of *S. boulardii* cells in the presence of 2 μg Medrol/mL. The lack of electrostatic effect at upper dose was due to a smaller level of Medrol penetration through the yeast's surface for Medrol molecules (Fig. 1C).

There was a sharp increase in volume of S. boulardii yeasts of 23%, 58%, 31% and 14% at doses of Medrol: 0.04 μg/mL, 0.2 μg/mL, 0.4 μg/mL and 2 μg/mL, respectively, where one fraction was registered (Fig. 1D).

On contrary, the significant decrease in sizes of *S. boulardii* yeasts of 25%, 11% and 17% at doses of 1 μg Medrol/mL or doses of 50 μg Medrol/mL, 5 μg Medrol/mL and 10 μg Medrol/mL, respectively, was registered (Fig. 2 D). There was a strong decrease in volume of *S. boulardii* cells upon higher doses of treatment of Medrol (μg /mL). Our results showed a reduction in volume of S. boulardii yeasts upon 1 μg/mL and 50 μg Medrol/mL of 58,7%, in the presence of 5 μg Medrol/mL of ~ 30% and after treatment with 10 μg Medrol/mL – of ~ 43%. Only treatment with 25 μg Medrol/mL led to an increase in volume of *S. boulardii* cells with 14.9%. There was a tendency of increase in EPM, ζ and σ with around 12% compared to control values (u varied from 1.78×10^{-8} m^{2}V^{-1}s^{-1} without Medrol in yeasts suspension to 2.00×10^{-8} m^{2}V^{-1}s^{-1} in the presence of 25 μg Medrol/mL; ζ varied from 22.84 mV without Medrol in *S. boulardii* suspension to 25.55 mV in the presence of 25 μg Medrol/mL; σ varied from 0.709 fC in the absence of Medrol in yeasts suspension to 0.799 fC in the presence of 25 μg Medrol/mL) (Fig. 2 A,B).

There was a decrease in EPM of *S. boulardii* in distilled water at concentrations of 1 μg Medrol/mL and 5 μg Medrol/mL of 37% and 32%, respectively. There was a reduction of zeta potential of the upper yeasts with values of change of 37% and 33% upon the same doses of treatment. But Medrol induced a large reduction in surface electrical charge of *S. boulardii* suspension (preliminary incubated in PBS, pH 7.4 and then dissolved in distilled water) upon 1 μg/mL (σ varied from 0.709 fC without Medrol to 0.320 fC in the presence of 1 μg/mL) where a decrease of ~ 55% was observed (Fig. 2 C). The significant decrease in σ of *S. boulardii* in the presence of 5 μg Medrol/mL was measured ($\sigma = 0.393$ fC) which characterized a reduction of 44% compared to control values without

Medrol in suspending medium. Higher concentrations of 10 µg Medrol/mL led to a smaller effect of change in surface charge density where 22% decrease in surface electrical charges was registered. Higher concentration of 50 µg Medrol/mL of treatment of *S. boulardii* cells induced no significant changes in EPM and zeta potential, but a reduction of σ with 32% compared to control values of surface charge density of S. boulardii without Medrol in suspending media was registered

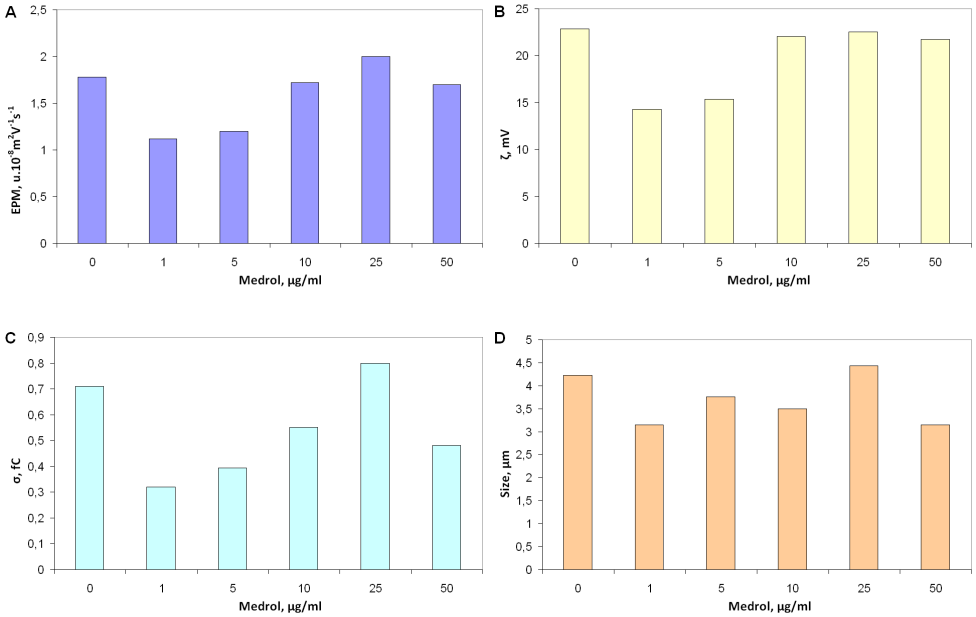

Fig. 2 Influence of Medrol at concentrations of (1 – 50 µg/mL) on electrophoretic mobility (A), zeta potential (B), surface charge density (C) and particle size (D) of *Saccharomyces boulardii* yeasts in Distilled Water.

A great dynamic in particle size distribution of *L. reuteri* (suspended in PBS, pH 7.4 and then diluted in Saline Sorbitol Buffer, pH 7.50) in the presence of Medrol treatment (0.1 µg/mL – 2.0 µg/mL) was observed (Fig. 3). Dose of 0.1 µg/mL induced 97% decrease in volume of bacteria (size of 0.614 µm). Concentrations of 0.75 µg Medrol/mL on *L. reuteri* bacteria were characterized by size of 2.418 µm, 1.0 µg Medrol/mL of treatment with size of 1.457 µm and 1.5 µg Medrol/mL dose of treatment led to a size of 1.446 µm. Hence, the volume of *L. reuteri* was strongly altered by ~ 64% reduction. There was a decrease in volume of *L. reuteri* upon 0.25 µg Medrol/mL (size of 1.961 µm) in suspending media of 12%. On contrary, higher concentration of 0.50 µg Medrol/mL and 0.75 µg Medrol/mL induced a significant increase in volume of bacteria of 64,4% and 19.6% enhancement, respectively. Highest dose of treatment of 2.0 µg Medrol/mL (size of 1.811 µm) led to a 30% decrease in volume of *L. reuteri* bacteria.

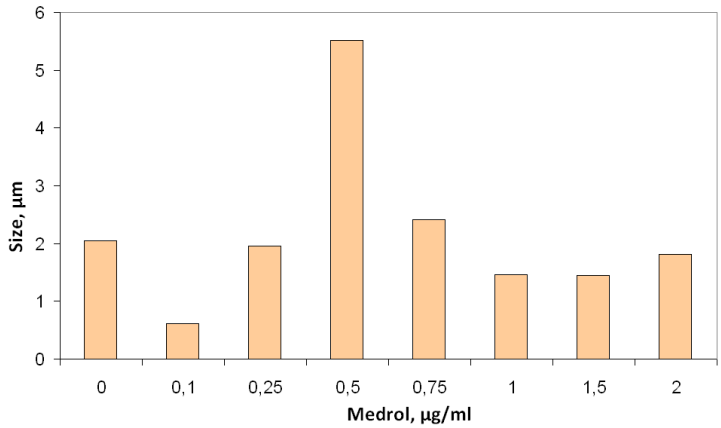

Fig. 3 Influence of Medrol at doses of (0.1 – 2 µg/mL) on particle size of *Lactobacillus reuteri* DMS 17938 bacteria in Saline Sorbitol Buffer, pH 7.50.

Fig. 4 (A,B,C) illustrated LDV parameters of *L. reuteri* bacteria suspended in low ionic strength media at pH 7.50 and concentrations of Medrol. We found a drop in EPM ($u = 0.89 \times 10^{-8}$ $m^2V^{-1}s^{-1}$) (Fig. 4A), zeta potential ($\zeta = 11.34$ mV) (Fig. 4B) and surface charge density ($\sigma = 0.454$ fC) (Fig. 4C) at 0.4 mg Medrol/mL. Lower doses of Medrol (0.1 – 0.3 mg/mL) as well as higher doses of Medrol (0.6 and 0.8 mg/mL) induced a large increase in all the electrokinetic parameters in contrast to the values of EPM, ζ and σ without Medrol in suspending media of *L. reuteri* bacteria compared to control value without Medrol in suspending medium. There was a maximal increase of EPM ($u = 6.58 \times 10^{-8}$ $m^2V^{-1}s^{-1}$ at 0.1 mg Medrol/mL, and $u = 5.12 \times 10^{-8}$ $m^2V^{-1}s^{-1}$ at 0.2 mg Medrol/mL, which corresponded to $\zeta = 84.28$ mV and to $\zeta = 65.51$ mV, respectively. The surface electrical charged was changed from $\sigma = 0.004$ fC for L. reuteri in the absence of Medrol to $\sigma = 0.478$ fC at 0.1 mg Medrol/mL and to $\sigma = 0.146$ fC at 0.2 mg Medrol/mL but the value increased to $\sigma = 1.701$ fC at 0.3 mg Medrol/mL because of the different fractions observed at that dose.

Increasing the Medrol doses of treatment, a rapid change in particle size of *L. reuteri* bacteria occurred at 0.3 mg Medrol/mL (size of 4.52 µm, (volume 48.35 µm3)), at 0.4 mg Medrol/mL (size of 5.29 µm, (volume 77.51 µm3)), at 0.6 mg Medrol/mL (size of 2.744 µm, (volume 10.82 µm3)) and at 0.8 mg Medrol/mL (size of 1.619 µm, (volume 2.22 µm3)). Control value of particle size measurement of L. reuteri bacteria in the absence of Medrol in suspending medium was characterized by sizes of 0.62 µm and volume (4.87 µm3) (Fig. 4 D).

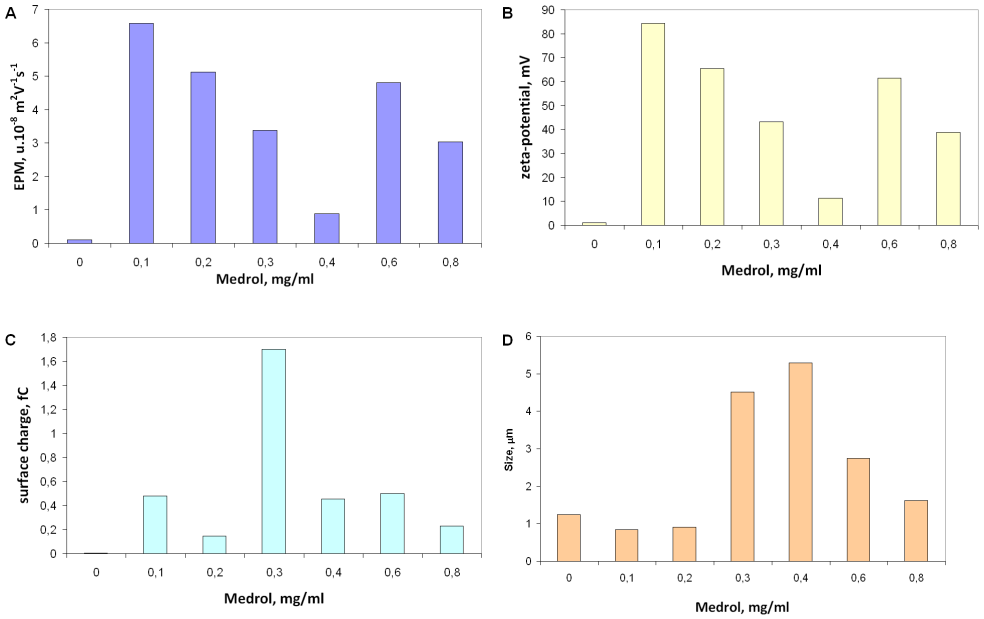

Fig. 4 Influence of Medrol at concentrations of (0.1 – 0.8 mg/mL) on electrophoretic mobility (A), zeta potential (B), surface charge density (C) and particle size (D) of *Lactobacillus reuteri* DMS 17938 bacteria in Saline Sorbitol Buffer, pH 7.50.

It was found that control bacteria of *L. reuteri* had one main fraction with size of 1.245 µm (volume 1.01 µm3) (Fig 4D). There was a decrease in size of *L. reuteri* bacteria under lower concentrations of Medrol treatment. The size of the particle is 0.838 µm (volume 0.31 µm3) at 0.3 mg Medrol/mL and 0.907 µm (volume 0.39 µm3) at 0.2 mg Medrol/mL. Middle doses of Medrol caused a large increase in size of *L. reuteri* bacteria. It was characterized by 47.9 fold enhancement in volume (size was 4.52 µm (volume 48.35 µm3) at 0.3 mg/mL, as well as by 76.7 fold increase in volume (size was 5.29 µm (volume 77.51 µm3) at 0.4 mg Medrol/mL of treatment compared to control volume of *L. reuteri* bacteria without Medrol in suspending media. Higher doses of Medrol led to a smaller effect in volume changes of 10.7 fold increase (size of 2.744 µm (volume 10.82 µm3) at 0.6 mg/mL and of 2.2 fold enhancement in volume (size of 1.619 µm (volume 2.22 µm3) at 0.8 mg/mL compared to control volume of *L. reuteri* bacteria without Medrol in suspending media. The results demonstrated the shrinkage (at 0.1 and 0.2 mg Medrol/mL) and swelling (at 0.3 – 0.8 mg Medrol/mL) of *L. reuteri* bacteria where the highest concentration of Medrol treatment decreased the level of swelling in comparison to control particles without Medrol (Fig. 4D).

There were a large difference in EPM (Fig. 4A), zeta potential (Fig. 4B) and surface electrical charge of *L. reuteri* bacterial membrane in the presence of methylprednisolon (Fig. 4C). Lower concentrations of 0.2 mg Medrol/mL had

strong electrostatic effect of increase in electrical charge density on the outer surface of the bacterial membrane. There was a specific drop in zeta potential of *L. reuteri* after treatment with concentration of 0.4 mg Medrol/mL (Fig. 4B) where a highest increase in size of 4.25 fold compared to size of untreated bacteria was registered (Fig. 4D).

CONCLUSION

Medrol® altered the surface properties of *S. boulardii* yeasts as well as of *L. reuteri* bacteria. The strong enhancement of electrophoretic mobility, zeta potential and surface electrical charge was accompanied by an increase in volume, i.e. swelling of the S. boulardii yeasts suspended in distilled water under Medrol® treatment (at doses of 0.2 – 0.4 μg/mL). It could be expected a lack of aggregation processes because of the higher net negatively charged surface of *S. boulardii* yeasts. The specific shrinkage of S. boulardii cells in the presence of 1 μg Medrol/mL or 5 μg Medrol/mL showed the role of electrokinetic charge in Medrol binding to the membrane where the membrane stability was reduced and the aggregation processes could be expected.

Medrol® molecules can destabilized the bilayer structure of *L. reuteri* bacterial membranes at concentrations of mg/mL and pH 7.5. The intervention of the Medrol® molecules with their three hydroxyl groups significantly affected the electrokinetic potential and the distribution of surface charges and therefore may cause neutralizing the electrical charges due to the presence of calcium ions as additives. Increasing the electrical charge density of the L. reuteri bacterial membranes was followed of the process of domination of Van der Waals strengths of repulsion over the Van der Waals strengths of attraction between membranes. Higher charged L. reuteri bacteria upon 0.1 – 0.8 mg Medrol/mL treatment did not allow the conglomeration of bacteria in low ionic strength media.

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Abbreviations

EPM	electrophoretic mobility
ζ	zeta (electrokinetic) potential
σ	surface charge density
DLS	dynamic light scattering
MI	Mean Intensity diameter
LDV	laser Doppler velocimetry

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