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MORPHOLOGICAL AND RHEOLOGICAL IDENTIFICATION OF ROD LACTIC ACID BACTERIA

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ABSTRACT
Cell morphological shape and size of four strains of rod LAB grown in two different media (MRS and RSM) were studied using scanning electron microscope (SEM) and image analysis technique. Topographical images reveal rod structures with height profiles and variation in size which occurred singly or in pairs and in chains. Rod cells had a wide range of breadth (0.40 - 1.07 µm) with wide cells perimeters of 12.26 up to 5.51 µm. Both areas of *Lb. helveticus* 764N and *Lb. casei* 761N were reduced when grown in RSM than in broth medium. On the contrary, cells areas of *Lb. acidophilus* 791N and *Lb. Paracasei* 72MP were increased when grown in RSM than in broth medium. The least elongated cells are reported for *Lb. acidophilus* 791N in broth medium. As far as strains are concerned, the elongation varies between 0.572 and 0.790 µm. rod cells had a different orientation angles and surfaces seemed to have heights or extrudes with external boundary which was either a sharp outline or a rough surface. Cells hardness and wall thickness effected negatively or positively depending on the strain and growing medium. Rheological properties (viscosity, shear stress and torque) recorded different values. Shear stress followed the same trend as viscosity with the maximum reached at different times (11 up to13 hrs). Clotting time was 11 up to13 hrs depending on the strain. According to acid production development, the fastest strain reached the iso-electric point (pH 4.6) was *Lb. helveticus* (11 hrs).

KEYWORDS: Lactic Acid Bacteria, Morphology, Topographical, Rheological properties, Shear stress.

INTRODUCTION
Determination of morphological and physiological parameters at the level of single cells is a major challenge. Cell morphology is important in the current descriptions of lactic acid bacteria (LAB) and species genera. On these bases, the shape of a bacterial cell influences many aspects of its life, including nutrient access, motility, chemotaxis, and resistance to predation.

Morphology is easy to study and analyze, particularly in microorganisms. In addition, morphological comparisons are valuable because structural features which depend on the expression of many genes are usually genetically stable.[1]

Bacterial cell size is an ecologically important factor because it determines many aspects of its metabolism, food size range and susceptibility to predators. Thus, size can be used to define individual and population characteristics of groups of organisms. Size is also a factor required for converting concentrations of organisms to biomass, a value necessary to assess the role of the organisms in the carbon cycle.[2] For the smallest organisms, size is a parameter easier to determine than genetic identity and characterize bacterial communities.[3]

The most commonly employed method for measuring bacterial size as well as other bacterial characteristics is by means of an ocular micrometer. During the last decades electron microscope is becoming increasingly more useful in imaging techniques for microbial study.[4]

Moreover; image analysis, a new and developing technique, is now applicable in almost all sciences dealing with images in great detail. The major scope of the image analysis is to define parameters such as size, number, shape, position and optical density of the objects which are recognized in an image. In collaboration with image processing that improves the image in viewing (e.g., zooming, sharpening, segmentation) it is becoming a powerful tool for scientists because of its great accuracy, repetition and fidelity.[5]

The purposes of this study were developing a set of data for LAB using a computationally quick procedure for extracting textural features of images and discussing the usefulness of these features as a tool for their

characterization, in addition to determining the differences in rheological properties of lactic acid bacteria under the study.

MATERIALS AND METHODS

Bacterial strains

Four strains of LAB (pure frozen cultures of *Lactobacillus casei* 761N, *Lactobacillus paracasei* 72MP, *Lactobacillus acidophilus* 791N and *lactobacillus helveticus* 764N) were obtained from the laboratory of Dairy Biotechnology of Faculty of Agriculture, Alexandria University**.**

Growth media

DE MAN, ROGOSA, SHARPE (MRS) broth medium.

It composes of the following ingredients (Formula gm/L): Peptone (10.0) – yeast extract (4.0) - glucose (20.0) - sorbitan mono-oleate (1.0 ml) – dipotassium hydrogen phosphate (2.0) – sodium acetate (5.0) – triammonium citrate (2.0) – magnesium sulphate (0.2), pH 6.9 ± 2.[6]

Reconstituted skim milk (RSM)

Reconstituted low heat skim milk powder (Mefad, Egypt), with 11% T.S (w/v), was sterilized at 115°C/7 min, then cooled overnight (pH 6.7).[7]

PREPARATION OF BACTERIAL CELLS SUSPENSIONS

MRS broth cells suspensions

Cultures of Lactic acid bacteria strains (frozen stocks) were separately sub - cultured in MRS broth at 37ºC for *Lb. acidophilus* 791N, *Lb.casei* 761N, *Lb.paracasei* 72 MP and *Lb.helveticus* 764N to the third subculture of the strains.

RSM cells suspensions

The activated cultures (in MRS broth), were grown in reconstituted skim milk (RSM) under the same conditions of MRS broth until a pH of 4.7. Casein was precipitated by 0.2% (w/v) EDTA (1:10, v/v). Cells suspension was obtained from the collected supernatant.

SAMPLE PREPARATION FOR SCANNING ELECTRON MICROSCOPE

Bacterial cells suspensions of MRS broth and RSM were centrifuged at 3000 rpm for 1 min. To fix the protein, pellets (bacterial cells) were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer at pH 7.2 for 2 hr., followed by washing several times in 0.1 M phosphate buffer for 15 min intervals. Samples were dehydrated in series of aqueous ethanol solutions (25%, 50%, 75%, 95% and 100%) for 5 min each, dried to critical point using CO_2 in a Critical Point Dryer (Polaron, Waterford, England), and mounted on aluminum SEM stubs, sputter-coated with gold (Spi module sputter coater, spi supplies division of structure probe. Inc.).[8] Samples were examined at 10 - 25 KV with SEM. Because the method relies on the accuracy of measurements, care was taken to always use the microscope at the same viewing angle.

IMAGE ANALYSIS

For image analysis, Scanning Prop Image Processor (SPIP) program 6.0.6 (BETA, Denmark) was used which enables the user to manipulate lateral calibration and unit cell detection to account for magnification differences in each image. After calibration of the program according to the scale bar on the micrographs, cells were magnified for better definition of the cell edges; the cells were then measured by moving the pointer from one cell edge to the other side edge. Detection and quantification of bacterial cell were done using the polygon measure shape "Fig. 1".

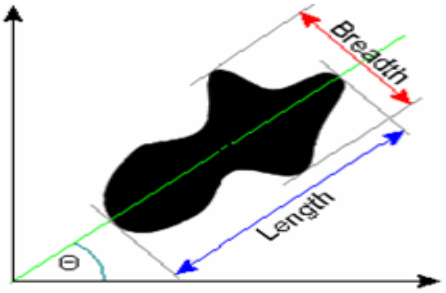

Figure 1. Polygon shape used for measuring the morphological parameters

Several morphological and geometrical parameters such as (area, length, breadth, perimeter, roundness, elongation, compactness, solidity, hardness, roughness and wall thickness) were calculated by the system as follows:

- **Area**

The Area is calculated from the shapes periphery, i.e. the closed polygon that surrounds the feature. The area is calculated using:

$$Area(polygon) = \frac{\sum_{i\in}(\chi_i + \chi_{i-1}).(y_i - y_{i-1})}{2}$$

- **Length**

Length is defined as the longest cord along the angle θ given by the moment's axis to the x-axis "Fig. 1".

- **Breadth**

Breadth (or width) is defined as the longest cord perpendicular to the angle θ given by the moment's axis to the x-axis "Fig. 1". Breadth is the extension of the bounding rectangle in the vertical direction.

- **Perimeter**

For polygon shapes, the perimeter is calculated from the shape's contour as:

$$\text{Perimeter} = \sum_i \sqrt{(xi - xi - 1)^2 - (yi - yi - 1)^2}$$

- **Aspect Ratio**

Aspect Ratio is defined as Length over Breadth. From this definition the aspect ratio will always be greater than

or equal to 1.0. The aspect ratio of both a circle and square is 1.0, whereas other shapes will have a value less than 1.0.

$$\text{Aspect Ratio} = \frac{Length}{Breadth}$$

- **Compactness**

Compactness is a measure expressing how compact a feature is

$$Compactness = \frac{Diameter}{Length} = \frac{\sqrt{\frac{4.Area}{\pi}}}{Length}$$

- **Solidity**

Solidity is a measure describing the resemblance of the shape's area with its convex area.

$$Solidity = \frac{Area}{Convex\ Area}$$

- **Elongation**

Elongation is a measure indicating how elongated a shape is. A square or circle will return the value zero. As these shapes change toward a long rectangle or ellipse the returned value converges towards 1.0.

$$\text{Elongation} = \frac{Length - Breadth}{Length}$$

RHEOLOGICAL PARAMETERS MEASUREMENTS

Rheological measurements of milk inoculated with bacteria (viscosity, shear stress and torque) were carried out in triplicates over temperature of 25ºC using a concentric cylinder Brookfield Programmable viscometer (Model DV–II; Brookfield Engineering Laboratories, USA) with UL adaptor and ULA spindle over a shear rate of 12.2s^{-1}. WinGather version 1.1, (Brookfield Engineering Laboratories, Inc., Copyright© 1995) software was used to collect, store and plot the data on a personal computer connected to the viscometer.

Acidifying activity of LAB strains

3% of the activated strains were inoculated into sterile reconstituted skim milk (RSM) and used for periodically monitoring pH each 2 hrs[9], acidity was determined according to.[10] All treatments were done in triplicates.

Statistical Analysis

The two-way statistical analysis of variance (ANOVA), mean separation and correlation required subprogram of MSTAT microcomputer statistical program was applied to evaluate morphological and rheological parameters of various rod strains of lactic acid bacteria. Simple and multiple linear regression analysis were applied and the student "t" test was used to test mean at $p<0.05$.[11]

RESULTS AND DISCUSION

Four lactobacilli strains *(Lb.acidophilus* 791N, *Lb.helveticus* 764N, *Lb.casei* 761N *and Lb.paracasei* 72MP) were morphologically investigated using SEM. The cells were photographed and representative images were shown in" Fig.2". Topographical images revealed rod structures with height profiles and variation in size which occurred singly, pairs and chains. Images produced from scans were analyzed using SPIP software. However, most of the functions in different software were similar and thus the results were comparable. Bright and dark areas in the images corresponded to peaks and troughs in the objects. Commonly, different scales are used in the vertical and horizontal axes. There were many offline operations for getting object properties. For example, sectional profile analysis was used to measure depth, height, and width of specimens. Roughness analysis was performed over the entire image or a selected part of the image to describe the surface status of the bacteria. When viewing an entire cell, large numbers of individual components were visualized depending on the cell size.[12]

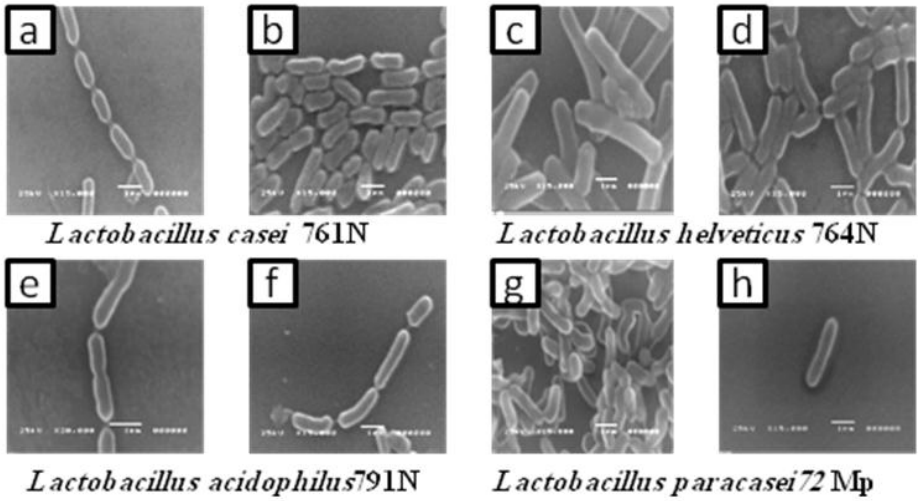

Figure 2. Micrograph of the Lactobacillus strains grown in MRS broth (a, c, e & g) and in RSM (b, d, f & h)

MORPHOLOGICAL CHARACTERSTICS OF LAB STRAINS

Bacterial cells breadth

Maximum cells breadth was obtained with *Lb. helveticus* in broth medium, with the highest mean breadth of 1. 07 µm. Sharp drop in breadth of *Lb. helveticus* was noticed when grown in RSM which reported the least breadth (0.403 µm). On the contrary, *Lb. paracasei* cells breadth increased when grown in broth (0.83 µm) than in RSM medium. Significant differences were found within *Lb. helveticus* and other lactobacillus strains in cells breadth (LSD= 0.1006) at 0.05 α level. Type of medium the bacteria grown in did not affect cells breadth of *Lb. acidophilus* and it didn't differ from *Lb. paracasei* cells breadth when grown in broth medium. Both type of growing medium and bacterial strain had a significant effects ($P<0.05$ and $P<0.001$, respectively) on bacterial cells breadth. Moreover, the interaction between the two factors influenced significantly ($P<0.001$) cells breadth of growing strains in broth medium. Variations in cells width are much less pronounced.

Bacterial cells length

As far as the four lactobacillus strains are concerned, great differences in their cells length are found. This can be partly explained with the fact that, with few exceptions, young cells are much longer than old or mature ones. *Lb.helveticus* grown in broth medium recorded the maximum cells length, while it was reduced to less than half its value by growing in RSM. A decrease in *Lb. helveticus* and *Lb. casei* cells length caused by growing medium type appears to be due to a variety of factors. The major causes appear to be changes in the environment with the accumulation of waste products. An increase in the osmotic pressure of the medium will also cause a decrease in cell size and may possibly be the most important factor. It is worth mentioning that it was impossible to measure the length of many cells due to their curved shape. Additional1y, the cel1 edges were, in many cases, difficult to distinguish and therefore measurement was impossible. Length varied considerably between 2.8 µm and 5.35 µm.[13] reported that *Lactobacillus delbrueckii* cells were 0.5 to 0.8 µm in width and 2 to 9 µm in length, which is quite similar to our results (0.5-1.2 µm width x 1-10 µm length).

The interaction between growing medium type and bacterial strain significantly ($P<0.001$) affected the length of the cells. Additionally, significantly differences were obtained within treatments (LSD value = 0.7974) at $\alpha= 0.050$.

Bacterial cells elongation

The least elongated cells were reported for *Lb. acidophilus* 791N in broth medium. As far as strains are concerned, the elongation varies between 0.572 and 0.790 µm.

Great differences were found in the elongation of the cells. This can be partly explained with the fact that, with few exceptions, young cells are much longer than old or mature ones. Least Significant Difference Test (LSD value = 0.06704 at $\alpha= 0.050$) showed no differences in cells of *Lb. helveticus* 764N elongation in both media the bacteria grown in. Additionally, there were no statistical differences between *Lb. helveticus* 764N grown in RSM and *Lb. casei* grown in broth medium.

Bacterial strain affected significantly the cells elongation ($P<0.001$), while growing medium and the interaction between the two factors did not influence ($P>0.05$) the cells elongation.

Bacterial cells perimeter

By comparing the data of the four rod strains, it becomes evident that there is a significant difference in the perimeters of their cells. The *Lb. helveticus* 764N strain seems to be the biggest (12.267 µm), with the *Lb. casei* and *Lb. paracasei* strains being smaller (7.694 and 5.516 µm, respectively), whereas the *Lb. acidophilus* 791N (0.770 µm) being the smallest when grown in broth medium**.** The cells perimeter varies between 9.469 µm and 15.710 µm. Great differences are found in the perimeters as an effect of cell strain.

Differences were also verified by applying the ANOVA (analysis of variance) statistical method to the mean values of the perimeters of the different strains of Lactobacillus. Growing medium and the interaction between it and bacterial strain affected significantly the cells perimeters (LSD value = 1.673 at $\alpha= 0.050$).

Bacterial cells surface area

Both areas of *Lb. helveticus* 764N and *Lb. casei* 761N were reduced when grown in RSM than in broth medium. On the contrary, cells areas of *Lb. acidophilus* 791N and *Lb. paracasei* 72MP were increased when grown in RSM than in broth medium. Moreover, *Lb. helveticus* 764N had the highest cells area ($4.550 \pm 1.107 \mu m^2$) after grown in broth medium and *Lb. casei* 761N had the lowest ($2.599 \pm 1.809 \mu m^2$).

Morphologically, the four lactobacillus strains are clearly identified. Areas had the same trend as length and elongation. It can be safely says that there is a significant difference between the areas of the four rod strains (LSD value = 0.7925 at $\alpha= 0.050$). Nutritional stress can change bacterial morphology. It can increase or decrease a cell's uptake–surface area. Moreover, it benefits bacterial cells attaching to a surface because it increases specific surface area in direct contact with the solid medium. In addition, it may allow bacterial cells to access nutrients by enhancing the possibility that part contact a nutrient-rich zone and pass compounds to the rest of the cell's biomass.

Bacterial cells solidity

Solidity is a measure describing the resemblance of the shape's areas with their convex area. RSM as a growing medium significantly decreased the cells solidity of all rod strains tested over that of growing in broth medium**.** Growing in RSM increased the convexity of the cells which resulted in a reduction in their solidity.

Table 1: Mean, standard deviation (SD), coefficient of variation (Co. Var.) of morphological and geometrical parameters of LAB grown in MRS

Bacterial strains	Mean ± Sd								
	Breadth (µm)	Length (µm)	Elongation (µm)	Perimeter (µm)	Area (µm2)	Aseptic ratio (0-1)	Solidity (0-1)	Compactness (0-1)	Wall Thickness (µm)
Lb.helveticus	1.07±0.16	5.21±0.81	0.79±0.04	11.70±2.28	4.19±1.10	4.88±0.72	0.86±0.93	0.43±0.3	0.667±0.13
Lb.acidophilus	0.85±0.11	2.76±1.72	0.57±0.01	5.16±0.11	1.30±0.21	2.34±0.53	0.93±0.77	0.64±0.05	0.292±0.07
Lb.casei	0.79±0.09	2.89±2.08	0.72±0.04	7.31±0.79	1.76±0.29	3.65±0.60	0.89±0.88	0.51±0.61	0.284±0.10
Lb.paracasei	0.83±0.10	0.41±0.35	0.58±0.10	5.62±0.53	1.27±0.28	2.52±0.28	0.01±0.05	0.04±0.07	0.321±0.21
Co. Var.%	11.74	27.06	6.52	23.33	43.20	19.39	4.58	9.72	39.13

Bacterial cells compactness

Compactness measure expressed how compact were the features of the microorganism. *Lb. acidophilus*, *Lb. casei* and *Lb. paracasei*, grown in both media, showed close compactness values. Low standard deviations were found which indicated measurements efficacy. RSM as a growing medium affected positively the compactness of the cells except for *Lb. casei* that the increase in compactness was insignificant. RSM lowered the compression on cells walls either by the adsorbed water to the surface of the cells or by the extra nutrients it has.

Bacterial cells roughness

In order to quantify the surface irregularities of the bacterial cells, surface topography roughness was characterized and quantified for each strain and imaged at different sample positions." Fig. 2" showed that the surfaces of the cells were practically non smooth, with irregularities. All the parameters are based on two-dimensional standards that were extended to three dimensions.

The roughness average, *Sa*, is defined as:

$$S_a = \frac{1}{MN} \sum_{k=0}^{M-1} \sum_{i=0}^{N-1} |z(x_k, y_i)|$$

The software tool can be used to recognize local variation in cells surface roughness, and particularly in local average cells roughness (Sa). Fig. 3." showed decreases in cells surface average roughness (Sa) as a result of using RSM in growing of the bacterial cells over that of broth medium. The same trend was followed for surfaces kurtosis (Sku). The smoothness caused by growing in RSM could be due to cells surfaces expansion which reduced their cavities. The Surface Kurtosis**, *Sku***, describes the "peakedness" of the surface topography. Roughness values depend strongly on measurement conditions especially scan range and sample density. Analysis of variance for bacterial cells roughness (Sa) and (Sku) showed highly significance for bacterial strains, growing medium and their interaction (P<0.001) with a correlation of 0.877 and 0.868, respectively.

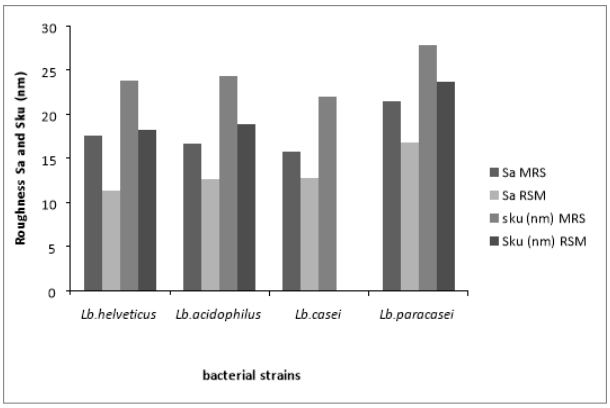

Figure 3. Average cells surface roughness (Sa) and cells surface kurtosis *(Sku,* nm) of *lactobacillus* strains grown in both MRS broth and RSM.

Bacterial cells hardness

The hardness analysis utilities are based on tests where the imprint created by an indenter is analyzed and quantified. The indentation process and some of the parameters used for hardness analysis can be measured by the load unload curve measured during the indentation test. Hardness of the strains cells varied as a result of strains variation, type of medium used for growing and the interaction between the pervious factors.

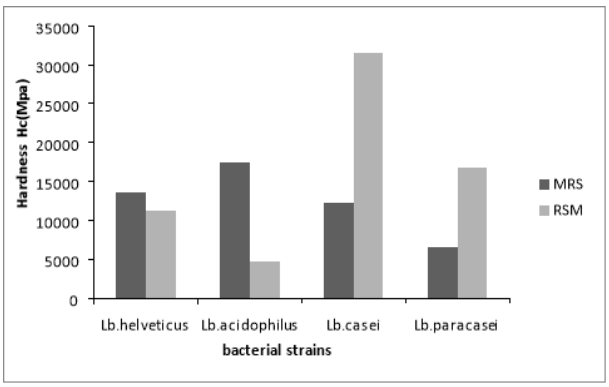

Figure 4. Average cells hardness (MPa) for *lactobacillus* strains grown in both MRS broth and RSM.

As for *Lb. casei*, the RSM increased the hardness of the cells dramatically, followed by *Lb. paracasei*. On the other hand, it decreased harness for *Lb. helveticus*764N and *Lb. acidophilus*791N over that of cells grown in broth medium as shown in "fig.4." Analysis of variance F test for bacterial cells hardness (Mpa) significantly influenced by bacterial strain, growing medium and their interaction ($P<0.001$). Within treatments, all bacterial cells differed significantly (LSD value = 2.259 at $\alpha= 0.050$).

Bacterial cells' wall thickness

Cells wall thickness of lactobacillus strains was calculated as the difference of outer and inner bacterial cell diameter. *Lb. paracasei* showed the highest maximum cells wall thickness, in addition to the maximum average (0.820 µm) when grown in RSM. The thinnest cells wall was reported for *Lb. helveticus* 764N grown in RSM medium. Statistical insignificant differences were found within treatments between *Lb. helveticus* 764N and *Lb. paracasei* in cells wall thickness as an impact of growing medium type when compared by LSD value = 0.1607 at $\alpha = 0.050$. Analysis of variance of cells wall thickness value supported the results maintained by LSD test for the insignificance of the growing medium type. However, bacterial cells strain ($P<0.05$) and the interaction between bacterial cells strain and growing medium affected the cells wall thickness significantly ($P<0.001$).

Bacterial cells aseptic ratio

Aseptic ratio is defined as length over breadth. From this definition the aseptic ratio will always be greater than or equal to 1.0. Aseptic ratio of both a circle and square shapes are 1.0, whereas other shapes will have a value more or less than 1.0.

Table 2: Mean, standard deviation (SD), coefficient of variation (Co. Var.) of morphological and geometrical parameters of LAB grown in RSM (Selective broth medium).

Bacterial strains	Mean ± SD								
	Breadth (µm)	Length (µm)	Elongation (µm)	Perimeter (µm)	Area (µm2)	Aseptic ratio (0-1)	Solidity (0-1)	Compactness (0-1)	Wall Thickness (µm)
Lb.helveticus	0.40±0.03	1.93±0.49	0.78±0.04	4.64±1.27	0.49±0.13	4.79±1.5	0.81±0.91	0.40±0.04	0.097±0.02
Lb.acidophilus	0.93±0.10	1.96±2.34	0.64±0.16	7.53±0.10	2.14±1.80	3.14±1.70	0.87±0. 58	0.55±0.12	0.377±0.06
Lb.casei	0.65±0.08	2.17±2.82	0.69±0.06	5.30±2.52	1.05±0.53	3.32±1.55	0.85±0.76	0.52±0.51	0.230±0.15
Lb.paracasei	0.82±0.53	1.25±0.70	0.70±0.16	6.76±2.15	1.61±0.48	3.41±0.66	0.07±0.15	0.08±0.06	0.820±0.26
Co. Var.%	11.74	27.06	6.52	23.33	43.20	19.39	4.58	9.72	39.13

Table (2) showed that *Lb. helveticus* grown in RSM recorded the highest aseptic ratio between length and breadth of the cells as it was higher than 5 times as much as their cells breadth grown in RSM (5.467) while, *Lb. paracasei* grown in MRS broth recorded the least ratio (1.187). All the aseptic ratios measured were higher than 1. The aseptic ratio increased for both *Lb. acidophilus* and *Lb. paracasei* grown in RSM. On the contrary, it was reduced for the other two strains *Lb. helveticus* and *Lb. casei*. In spite of that, neither the increase nor the decrease was significant as a growing medium influence ($P>0.05$). The only significant influence statistically found was for bacterial strain ($P<0.001$) with a Coefficient of Variation = 19.39%. Additionally, insignificant differences were found within treatments for *Lb. acidophilus* and *Lb. paracasei* grown in RSM and *Lb. casei* grown in broth medium (LSD value = 0.7925 at $\alpha= 0.050$).

RHEOLOGICAL AND TECHNOLOGICAL PROPERTIES OF LAB

Viscosity, shear stress and torque of milk at pH 4.6

At complete clotting (pH 4.6), *Lb. helveticus* showed the minimum values for viscosity (221.17 cP), shear stress (21.06 D/cm^2), torque (23.03 %) and clotting time (11 hrs). *Lb. acidophilus* had higher viscosity (340.86 cP), shear stress (23.0 D/cm^2), and torque (29.033%) than both *Lb. helveticus* and *Lb. casei* strains.

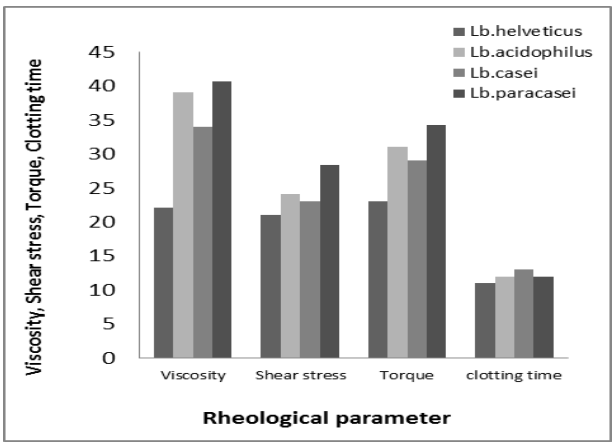

Figure 5. Average viscosity (V), shear stress (SS) and torque (T) values of milk at pH 4.6.

Maximum clotting time (13 hrs) was recorded for *Lb. casei*. Moreover, *Lb. paracasei* showed the maximum values for viscosity (407.02 cP), shear stress (28.37 D/cm^2) and torque (34.3%) with 12 hrs clotting time.

Viscosity, shear stress and torque during milk clotting process

Viscosity of *Lb. helveticus* increased almost 21 times at incubation period of 10 to 11 hrs which agreed with the results obtained by[14]. The viscosity steep time for *Lb. helveticus*, *Lb. acidophilus*, *Lb. casei* and *Lb. paracasei* was after10, 8, 10 and 6 hrs of incubation, respectively "Figs.6".

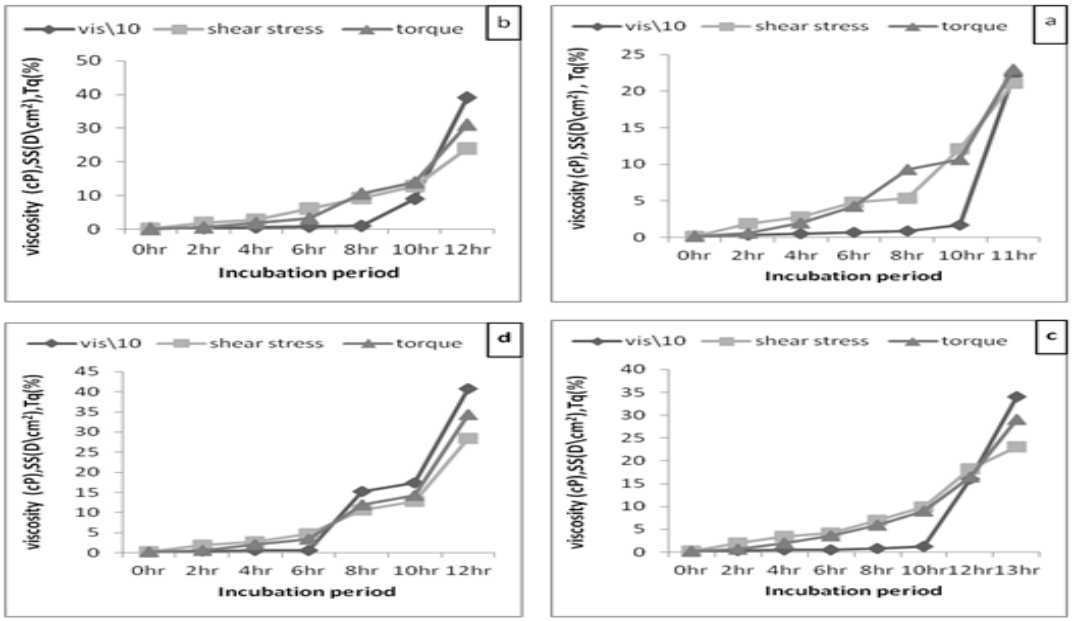

Figure 6. Average viscosity/10 (cP), shear stress*10 (D/cm^2) and torque (%) of RSM through clotting process by *Lb. helveticus (a), Lb. acidophilus (b), Lb. casei (c)* and *Lb. paracasei (d).*

The complete clotting time reached the maximum for *Lb. casei* (13 hrs). The final viscosity (at pH 4.6) for *Lb. acidophilus* and *Lb. paracasei* were close (391.5 and 407.02 cP, respectively). The maximum shear stress was reached at 11, 12, 13 and 12 hrs of incubation for *Lb. helveticus*, *Lb. acidophilus*, *Lb. casei* and *Lb. paracasei*, respectively. Steep rise in shear stress observed at the same times as viscosity (after 10, 8, 10 and 6 hrs of incubation) for *Lb. helveticus*, *Lb. acidophilus*, *Lb.casei* and *Lb. paracasei*, respectively. The minimum percent for torque was recorded by *Lb. helveticus* (23.03%) and the maximum value was obtained with *Lb. paracasei* (34.3%) which was almost 1.5 times as much as *Lb. helveticus* value.

Acid production and pH profiles

The fastest strain reached the iso-electric point (pH 4.6) was *Lb. helveticus* (11 hrs, "Fig.7a".

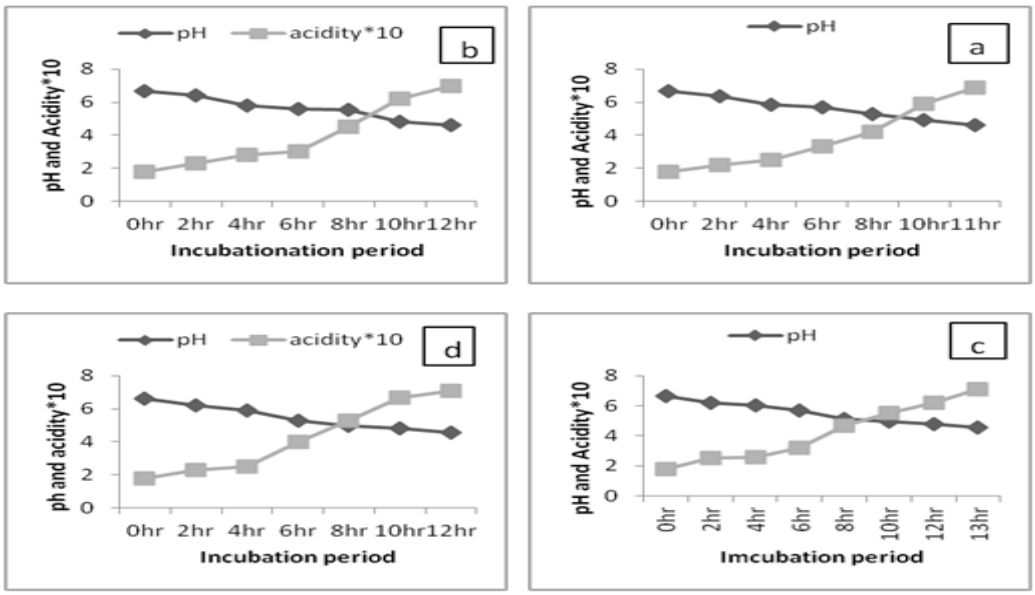

Figure 7. Average acid production and pH profiles of milk through clotting process by *Lb. helveticus (a), Lb. acidophilus (b), Lb. casei (c), and Lb. paracasei (d)*

Lb. casei took 13 hrs "Fig.7 c" which agreed with that obtained by.[15-16] The same trend was found with acidity produced by both organisms.[17] Average acid production and pH profile of milk through clotting process by *Lb. helveticus* 744N (a), *Lb. acidophilus* (b), *Lb.casei* 780N (c) and *Lb.paracasei* (d) were shown in "Fig. 7".

CONCLUSION

Great accuracy, repetition and fidelity image analysis technique together with SEM could be a good tool for measuring many morphological shape and size parameters, for the viable bacterial cells which can be used for characterization of rod LAB, but growth conditions and growth phase must be determined as effective factors and standard conditions. Additionally, rheological and technological properties could be useful aid for characterizing many bacterial species.

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