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INVESTIGATIONS INTO THE USAGE OF THE MINERAL ALGINITE FERMENTED WITH *LACTOBACILLUS PARACASEI* FOR COSMETIC PURPOSES

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A remarkable interplay between the skin and the fermentation of lactic acid bacteria (LAB) occurs. The lactate and amino acids in the supernatant of this bacteria help to hydrate the skin. The fermentation broth of lactic acid bacteria, generally referred as "lactic acid bacteria ferment" according to International Nomenclature of Cosmetic Ingredients (INCI), has been used to make a variety of cosmetic components. The goal of this study was to evaluate new approaches to assess ferment filtrates (also known as cell-free fermentation broths) that could be used in cosmeceuticals. Despite years of research on the production of lactic acid, aesthetic evaluations have not yet been performed. First, the *Lactobacillus paracasei* strain NCAIM B.01525 was employed in our research. Furthermore, a fermentation broth was produced containing the Hungaricum mineral alginite and the impact of hydration on human skin tested. The influence of alginite on the fermentation of LAB was also examined. According to the results of the trials, although alginite might double and triple biomass and specific growth rates, respectively, it cannot facilitate hydration of the skin. These results might contribute to the development of more widely accessible, environmentally-friendly cosmetic components in the future.

Keywords: human skin, dermatoscope, moisturizing effect

1. Introduction

The use of cosmetics dates back 7,000 years to Ancient Egypt and were later used in the Roman Empire, for example, according to some legends, Cleopatra bathed in goats' milk to preserve the youthfulness of her skin. Later, milk baths became popular in the English royal court, for instance, Catherine Parr, the last queen of the House of Tudor, and later Elizabeth I, Queen of England, regularly used a milk bath to preserve their beauty. In today's modern world, using natural ingredients in cosmetics has come to the fore again. Although our laboratory has been dealing with lactic acid producers for many years, to date, no measurements have been made in terms of cosmetics.

The structure of the skin and the composition of the stratum corneum (SC) are shown in *Fig. 1*, including the natural moisturizing factor (NMF). The skin consists of two main layers, namely the dermis and epidermis. The epidermis is further divided into two main layers, that is, the viable epidermis and the stratum corneum (SC), which is the outermost layer mainly consisting of dead cells. The essential function of the SC is to act as a barrier, preventing dehydration caused by water loss from the body. The SC contains 30% NMF, which consists of 40% amino acids such as serine, glycine and alanine as well as 12% lactate, which is capable of retaining water in the stratum corneum [2].

On the other hand, lactic acid bacteria (LAB) are generally defined as Gram-positive, non-spore-forming, catalase-negative, aerotolerant, acid-tolerant, nutrient-demanding and strictly fermentative organisms that lack cytochromes as well as produce lactic acid as the main end product of carbohydrate metabolism [3]. LAB contains cell wall-bound proteinase that initiates the transformation of extracellular proteins into oligopeptides [4]. The protease activity as well as

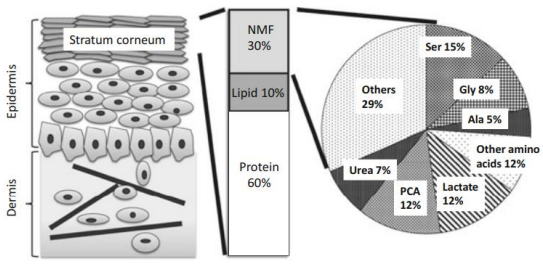

Figure 1. Composition of the skin and its natural moisturizing factor (NMF), where PCA is pyrrolidone carboxylic acid [1]

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catabolic production of proteins and peptides enable LAB to produce amino acids in the fermentation broth [5]. Since many amino acids are contained in various LAB-fermented foods such as cheese [6], sausage [7] and even Japanese sake [8], a LAB-fermented ingredient contains large amounts of lactic acid and amino acids, which together form the NMF. Therefore, these ingredients exert a hydration effect when they are applied on the skin. Furthermore, a particular combination of a substrate and a strain of LAB could provide another beneficial functional impact on the skin.

Our goal was to investigate how the mineral alginite affects fermentation and moisturization of the skin. Another aim of this study was to screen for new methods to evaluate the ferment filtrate, i.e. cell-free broth, in terms of usage in cosmeceuticals.

2. Experimental

2.1. Fermentation

The planned experiment concerning the production of lactobacillus ferment filtrate was performed using a strain of *Lactobacillus paracasei* from the National Collection of Agricultural and Industrial Microorganisms (NCAIM, Hungary), namely NCAIM Lactobacillus paracasei B.01525.

The fermentations were conducted in 250 ml shake flasks and 10 ml BacTrac impedance tubes (SY-LAB, Austria - *Fig. 2*) at 37°C. Only the shake flasks were shaken at 150 rpm. The fermentations could be monitored online using a SY-LAB BacTrac 4100 microbiological analyzer due to changes in the impedance of the medium (M%) and on the surface (E%) of the electrodes by following the same method previously reported by Áron Németh [9]. The measurements were performed using the BacTrac analyzer and the results displayed by BacMonitor Y 1.39Er software. Since the BacTrac program only shows curves and does not give the corresponding points, these points had to be extracted in a different way. The curves were saved as QRP files, which were converted into JPEG format using SmartQRP software. The JPEG files had already been recognised by DigitizeIt, which made it easy to determine the points associated with the curves. The points were copied into a Microsoft Excel spreadsheet and curves were fitted to the points using SigmaPlot 2001 software for Windows version 7.0. The generalized logistic equation by Verhulst-Pearl was used [10].

The nutrient solution was the following Marie-Rogosa-Sharpe medium: peptone (10.0 g/l), yeast extract (5.0 g/l), beef extract (10.0 g/l), glucose (20.0 g/l), KH_2PO_4 (2.0 g/l), sodium acetate (5.0 g/l), magnesium sulfate (0.2 g/l), manganese sulfate (0.05 g/l), Tween 80 (1.08 g/l) and ammonium citrate (2.0 g/l). The fermentations with alginite were supplemented with 10g/l of alginite.

During the fermentation, the changes in glucose and lactic acid concentrations were monitored by a Waters

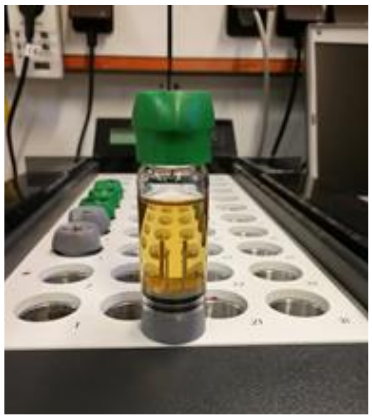

Figure 2. BacTrac and its vials with electrodes

Breeze HPLC system consisting of a Bio-Rad Aminex HPX-87H Column, Waters 717 Plus Autosampler and Waters 2414 RI detector. After appropriate dilution steps, the samples were mixed with a 0.2 µm-pore-size mixed ester syringe filter (ViaLab Magyarország Kft.).

The dry matter was measured as follows: the contents of the BacTrac tubes, that is, 10 ml of fermentation broth, were loaded into Falcon Conical Centrifuge Tubes and centrifuged at 6000 rpm for 10 mins. using a Hermle Z200A centrifuge. The supernatant was then decanted before the cells were suspended in distilled water and centrifuged once more. The supernatant was decanted again and the biomass poured into the crystallization cup with 2-3 ml of distilled water before being dried relatively quickly using our Sartorius MA35 moisture analyzer.

2.2. Determination of moisture content

The short-term/immediate hydration effect of the ferment filtrates was determined by taking triplicate measurements using a dermatoscope (*Fig. 3*). On the forearm of the subject, three 1 cm^2 areas were marked out onto which 20 µl of fermented juice was pipetted. After 5 mins., these areas were wiped with a dry hand towel and then the hydration on that part of the subject's skin was measured at given intervals with the installed Corneometer (capacitance) sensor. In order to establish a basis for comparison, the hydration of the subject's skin

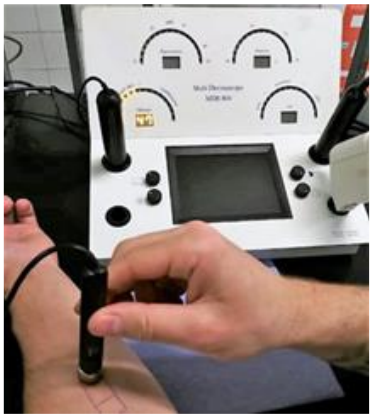

Figure 3. Measuring with a dermatoscope

was recorded before measurements were taken and the values displayed here corrected according to this value.

3. Results and Discussion

3.1. BacTrac results

The effect of alginite on the kinetics of LAB fermentation was also investigated. The fermentations were monitored by BacTrac online by replicating both setups three times, that is, in the presence and and absence of alginite, supplemented with a non-inoculated (blank) reference. The reference BacTrac tube contained the medium MRS and 10 g/l of non-inoculated alginite. The replicates were compared by excluding outliers from further processing and their

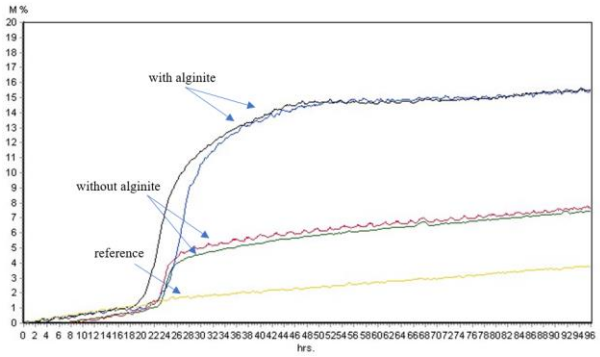

Figure 4. Lactobacillus paracasei fermentations in BacTrac tubes

curves are presented in *Fig. 4*. The resulting curves clearly show the impedance of *Lactobacillus paracasei* fermentations in the presence of alginite during the declining growth phase is twice as high as that of non-alginite fermentations. Furthermore, the specific growth rate was also determined to quantitatively compare the effect of alginite on fermentation kinetics as is presented in *Fig. 5*.

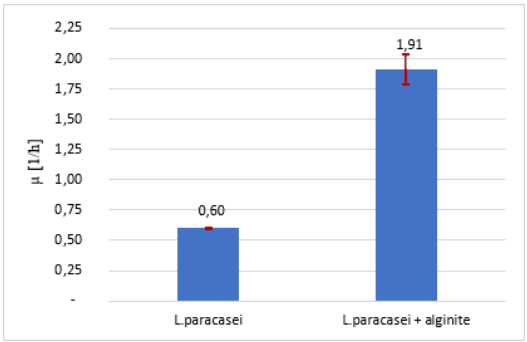

Figure 5. Specific growth rates of *L. paracasei* fermentations

The specific growth rates also differ significantly between the normal and alginite fermentations, namely 0.6 and 1.9 l/h, respectively (*Fig. 5*). Therefore, the specific growth rate of fermentation with alginite is three times higher than without.

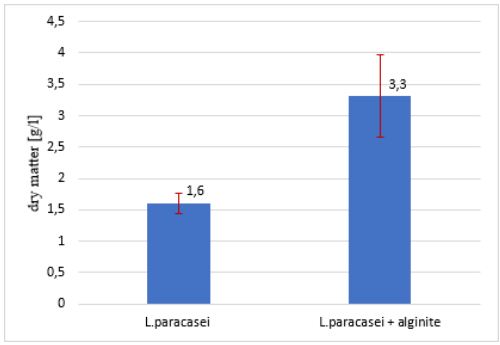

Figure 6. Concentration of dry matter in the *L. paracasei* fermentations

The data obtained from dry matter measurements also indicate that alginite fermentations achieved higher yields (*Fig. 6*).

3.2. Skin hydration

Since a lactic acid-producing strain was investigated, firstly the moisturizing effect of solutions of different lactic acid concentrations, namely 5, 10, 15 and 20 g/l of lactic acid in distilled water, was determined by a dermatoscope. How skin hydration changes over time once the given concentration of the lactic acid solution had been reduced is represented in *Fig. 7*. It is clearly visible that the level of hydration rapidly decreases over time but eventually stabilizes after ca. 35 mins.

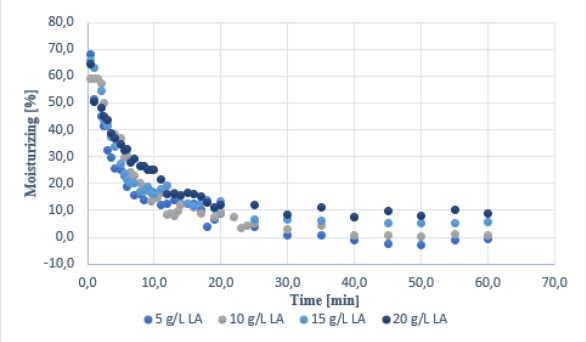

Figure 7. The moisturizing effect of the lactic acid (LA) solutions

Since the initial phase is difficult to quantify, the steady-state values after 30, 35, 40 and 45 minutes were used before being plotted against concentration as presented in *Fig. 8*. The trend is clear, that is, as the concentration of lactic acid increases, so does the moisturizing effect. The effect of the 5g/l lactic acid solution is so minimal that it has a relatively mild dehydration effect on the skin. A trendline was fitted to the points (R^2>0.99) and from the resulting equation, the hydration effect of the fermentation broth was predicted based on its lactic acid content.

While our technique for measuring the moisturizing effect is first described and applied here, the lactic acid sting test (LAST), which also measures capacitance

using a Corneometer (similarly to our dermatoscope's Corneometer) is highly comparable to it [11]-[13].

The only two differences are that the cited authors applied the ferment filtrates to the facial skin, to nasolabial folds to be exact, where the hydration effect was 6 times higher in contrast to on the forearm in our study, and that they only reported the measured level of hydration after 10 mins., which according to our time profile varies rapidly over time suggesting it may be more reliable.

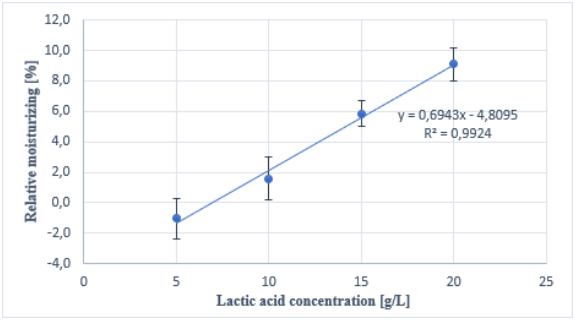

Figure 8. Steady-state moisturizing effects of LA solutions

Overall, a correlation with regard to the lactic acid solutions is observed between our results and those from the cited studies.

The effect on skin hydration of the *Lactobacillus paracasei* fermented broths was also measured and plotted against time (*Fig. 9*). A comparison concerning the steady-state values of the fermentation samples in the presence and absence of alginite is presented in *Fig. 10*. According to the results, the samples containing alginite were less hydrating than the cell-free fermentation broth. This may be due to the higher concentrations of lactic

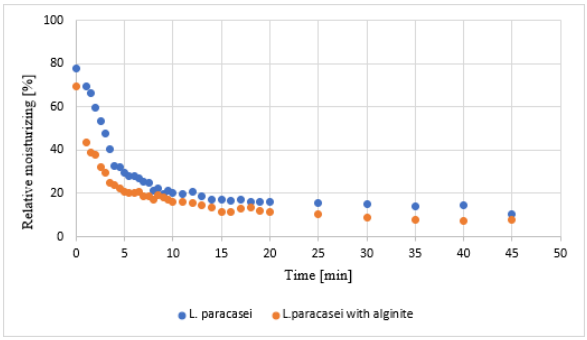

Figure 9. A comparison of the moisturizing effect of *Lactobacillus paracasei* ferment filtrates

acid produced in the alginite-free fermentation broth, namely 23.8 g/l and 28.8 g/l, respectively. By applying the equation in *Fig. 5*, hydration values of 15.2 and 11.7% were predicted for the alginite-free and alginite-containing fermentation broths, respectively, based on these lactic acid concentrations. Although the measured results in both cases are slightly lower, the difference is insignificant.

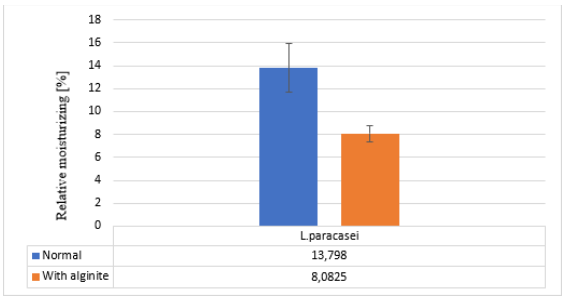

Figure 10. Lactobacillus paracasei ferment filtrates moisturizing comparison in steady state

4. Conclusion

The current study investigated the effects of the mineral alginite on the fermentation of Lactobacillus paracasei and its moisturizing effects on the skin. This study also aimed to test novel approaches for evaluating ferment filtrates (also known as cell-free fermentation broths) for use in cosmeceuticals. According to studies on the process, alginite increases the specific growth rate and dry matter content during fermentation. While the latter doubled, from 1.6 to 3.3 g/l to be exact, the former tripled from 0.60 to 1.91 l/h. Based on these encouraging findings, it was predicted that the hydration effect of the filtrate in the presence of alginite would outweigh that without. However, dermatoscopic measurements provided evidence to the contrary. The measured hydration levels of the alginite-free and alginite-containing fermentation broths were 13.9 and 8.1%, respectively.

Our future studies will focus on the effects of fermented broths containing alginite on additional aesthetic aspects such as antioxidants, skin whitening or the inhibition of the enzyme hyaluronidase.

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