Characterization and selection of *Lactococcus lactis* strains from ewe's milk as potential cheese starter cultures



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INTRODUCTION

Raw milk and traditional dairy products are a source of indigenous strains that have great potential for developing and protecting the recognizable sensory quality of local products. *Lactococcus lactis* is one of the best described lactic acid bacteria and is commonly used as a commercial starter culture in the dairy industry. The use of indigenous strains of this bacterium in cheese production can affect the quality and safety, as well as the development of specific sensory characteristics of the product. Therefore, the aim of this study was to select lactic acid bacteria strains from Pag raw ewe's milk and test their technological and biochemical properties under controlled laboratory conditions.



MATERIALS AND METHODS



The lactic acid bacteria were isolated from sheep milk using MRS and M17 agar and identified by MALDI-TOF mass spectrometry. Out of the 18 identified strains of *Lactococcus lactis* subsp. *lactis*, five (M1-M5) were selected for further characterization. Strains were tested for growth in M17 broth at different temperatures (10°C, 30°C, 45 °C), pH values (6.5, 5.5, 4.5, 3.5, 2.5), and salt concentrations (4.5%, 5.5%, 6.5%). Cell growth was measured by plating on M17 agar and absorbance (OD₆₀₀). The acidification capacity of the strains was measured in MRS broth using the titration method. In addition, the lipolytic, proteolytic and antimicrobial activities of the isolates were tested, as well as their ability to produce gas by glucose degradation. Data analysis was performed using Statistica 13.5 software. The significance of differences between groups was evaluated using analysis of variance at the 0.05 level.

RESULTS

- none of the isolates produced gas by glucose degradation
- all isolates show the same proteolytic activity and a high level of acidifying ability
- lipolytic and antimicrobial activity of all isolates were absent
- at different temperature conditions, pH values and salt concentrations, all isolates grew but with significant differences (p<0.05), with *Lc. lactis* subsp. *lactis* M1 showing the best technological performance



| | рН | log CFU/ml | OD ₆₀₀ | |
|----|-----|---------------------------------|-------------------|--|
| M1 | | 2,0±0 ^{a,b} | 0,160 | |
| M2 | 2,5 | 1,5±0,70 ^{c,d} | 0,087 | |
| M3 | | <1,0±0 ^{a,c} | 0,077 | |
| M4 | | 1,0±0 | 0,080 | |
| M5 | | <1,0±0 ^{b,d} | 0,074 | |
| M1 | | 4,0±0 | 0,120 | |
| M2 | 3,5 | 1,0±0 | 0,089 | |
| M3 | | 3,95±0,07 | 0,103 | |
| M4 | | 1,0±0 | 0,102 | |
| M5 | | 3,8±0,21 | 0,100 | |
| M1 | 4,5 | 8,0±0 ^{a,b,c} | 0,480 | |
| M2 | | 1,0±0 ^{a,d,e,f} | 0,091 | |
| M3 | | 4,88±0,16 ^{b,d,g} | 0,095 | |
| M4 | | 8,6±0 ^{e,g,h} | 0,780 | |
| M5 | | 5,23±0,33 ^{c,f,h} | 0,097 | |
| M1 | | 7,5±0,70 | 0,932 | |
| M2 | 5,5 | 6,0±1,4 | 0,719 | |
| M3 | | 7,34±0,48 | 0,887 | |
| M4 | | 7,5±0,70 | 1,284 | |
| M5 | | 7,19±0,70 | 0,880 | |
| M1 | | 9,5±0,70 | 1,575 | |
| M2 | 6,5 | 9,5±0,70 | 1,566 | |
| M3 | | 8,8±0,28 | 1,112 | |
| M4 | | 9,3±0 | 1,433 | |
| M5 | | 9,12±0,49 | 1,152 | |

| | T °C | log CFU/ml | OD ₆₀₀ | % NaCl | log CFU/ml | OD ₆₀₀ |
|----|-------|------------------------|--------------------------|--------|----------------------------|--------------------------|
| M1 | 10 °C | 4,5±0,70 | 0,114 | 4,5 % | 8,5±0,70 | 1,574 |
| M2 | | 2,5±0,70 | 0,104 | | 9,0±0 | 1,131 |
| M3 | | 3,5±0,70 | 0,112 | | 8,5±0,70 | 1,602 |
| M4 | | 3,75±0,21 | 0,111 | | 9,3±0 | 1,224 |
| M5 | | 4,42±0,82 | 0,102 | | 8,5±0,70 | 1,590 |
| M1 | 30 °C | 9,0±0 | 1,885 | 5,5% | 7,5±0,70 | 0,873 |
| M2 | | 9,0±0 | 1,785 | | 6,0±1,4 | 0,202 |
| M3 | | 9,0±0 | 1,843 | | 7,34±0,48 | 0,350 |
| M4 | | 9,3±0,7 | 1,852 | | 7,5±0,70 | 0,155 |
| M5 | | 9,69±0 | 1,867 | | 7,19±0,70 | 0,188 |
| M1 | 45 °C | 6,0±0 ^a | 0,219 | 6,5% | 5,5±0,70 ^{a,b,c} | 0,127 |
| M2 | | 6,0±0 ^b | 0,198 | | 3,0±0 ^{a,d} | 0,109 |
| M3 | | 3,77±1,4 | 0,150 | | 4,99±0,43 ^{b.d,e} | 0,122 |
| M4 | | 2,0±0 ^{a,b,c} | 0,107 | | 3,0±0 ^{c,e} | 0,104 |
| M5 | | 4,69±0° | 0,151 | | 4,5±0,70 | 0,120 |

^{a, b, c, d, e} values in the same column marked with the same letter of statistical data differ significantly at the level of p<0.05

^{a, b, c, d, e, f, g, h} values in the same column of a certain category marked with the same letter of statistical data differ significantly at the level of p<0.05



DISCUSSION AND CONCLUSION

There are no major differences between these five isolates so according to their biochemical and technological properties it can be concluded that all the selected strains of *Lc. lactis* subsp. *lactis* can be used as potential cheese starter cultures. Considering the above differences of isolates between their growth ability in different conditions, the final selection of strains should be based on the technological process and organoleptic characteristics of the desired cheese variety.

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