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ORIGINAL PAPER

Bioconversion of ovine *scotta* into lactic acid with pure and mixed cultures of lactic acid bacteria

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Abstract *Scotta* is the main by-product in the making of ricotta cheese. It is widely produced in southern Europe and particularly in Italy where it represents a serious environmental pollutant due to its high lactose content. With the aim of evaluating whether *scotta* bioconversion into lactic acid can be considered as an alternative to its disposal, besides providing it with an added value, here the growth, fermentative performances, and lactic acid productions of pure and mixed cultures of *Lactobacillus casei*, *Lactobacillus helveticus*, and *Streptococcus thermophilus* were evaluated on ovine *scotta*-based media, without and with the addition of nutritional supplements. The outcomes indicate that ovine *scotta* can be utilized for the biotechnological production of lactic acid with yields up to 92%, comparable to those obtained on cheese-whey. Indeed, the addition of nutritional supplements generally improves the fermentative performances of lactic acid bacteria leading to about 2 g l^{-1} h^{-1} of lactic acid. Moreover, the use of mixed cultures for *scotta* bioconversion reduces the need for nutritional supplements, with no detrimental effects on the productive parameters compared to pure cultures. Finally, by using *L. casei* and *S. thermophilus* in pure and mixed cultures, up to 99% optically pure L-lactic acid can be obtained.

Keywords *Scotta* · Whey · Lactic acid bacteria · Lactose bioconversion · Lactic acid

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Introduction

Lactic acid is naturally produced by lactic acid bacteria (LAB) in fermented dairy goods, such as yogurt and cheese, and it is used as a flavoring or pH regulator in bakery products, beverages, meat products, and confectionery. It is widely used by the pharmaceutical, biomaterial, detergent, leather, and textile industries, and over the past few years it has received increasing attention as a building block for biodegradable plastics such as polylactic acid and polylactic-*co*-glycolic acid [15]. In particular, lactic acid production, which reached about 150,000 t/year in 2002 [24], is growing to cover the needs of the biodegradable plastic market. According to Shen et al. [26], the bioplastic industry is expected to grow on average by 19% per year from 2007 to 2020. Lactic acid exists as two enantiomeric forms: L-lactic acid and D-lactic acid, which can be produced either by chemical synthesis or by fermentation. Chemical synthesis is based on raw materials from the bulk chemical industry and results in mixtures of L- and D-lactic acid. In contrast, the biotechnological production of lactic acid may lead to the desired pure enantiomeric form and requires lower temperatures and energy consumption in comparison with chemical approaches [12]. Therefore, 90% of the worldwide lactic acid production derives from the fermentation of agricultural or agro-industrial wastes by LAB [24]. Among these, lactose-rich by-products of dairy

industries, such as cheese-whey and *scotta*, represent attractive substrates for LAB-mediated lactic acid production, due to their abundance and content in nutrients essential for microbial growth. Accordingly, lactic acid production on cheese-whey-based media has already been reported [6, 7, 17, 25], also with the addition of nutritional supplements to improve the lactose bioconversion and the lactic acid productivity [3, 9, 10]. On the contrary, the use of *scotta* has only been explored for the production of bio-ethanol by yeasts [22, 23].

Scotta is the main by-product of ricotta cheese production and is widely produced in southern Europe, with 1 million tons per year being produced in Italy alone [22]. It is obtained after the flocculation of whey proteins and their separation as ricotta cheese induced by thermal treatment of cheese-whey at 85–90°C for about 20 min. *Scotta* from bovine whey contains proteins (0.15–0.22%), salts (1.0–1.13%), and lactose (4.8–5.0%), and has a biological oxygen demand of 50 g l^{-1} and a chemical oxygen demand of 80 g l^{-1} [22]. *Scotta* from ovine milk is generally characterized by a higher protein content [14, 19]. Thus, similar to what is observed for cheese-whey, the disposal of *scotta* represents a serious environmental problem and its biological treatment is an economically demanding step for the dairy industries, particularly for cheese-makers [17]. On the other hand, *scotta* may be considered as a source of lactose and other nutrients with possible biotechnological applications. For all of these reasons, the use of *scotta* as a raw material for alternative processes is recommended by the European Commission [4]. In fact, the bioconversion of *scotta* into valuable products, besides representing an appealing approach to the reduction of its environmental impact, would allow the exploitation and valorization of this by-product.

In this context, we report the evaluation of ovine *scotta* without and with addition of nutritional supplements as a substrate for the biotechnological production of lactic acid by pure and mixed cultures of LAB.

Materials and methods

Strains

The *Lactobacillus casei* LC1 (LC), *Lactobacillus helveticus* PCC76 (LH), and *Streptococcus thermophilus* PCC282 (ST) strains were used. Strain LC belongs to the cultures collection deposited at the Cartif Technology Centre (Spain), while LH and ST are the property of Porto Conte Ricerche srl (Italy). The bacterial strains were stored at −80°C, with LC and LH in De Man, Rogosa and Sharpe broth medium (MRS, Merck, Germany), and ST in M17 broth medium (Oxoid, UK), with 20% glycerol added. LH and ST were cultured at 42°C and LC at 37°C.

Table 1 Composition of the ovine *scotta*

Components	Mean content[a] (%)
Total solids	6.86 ± 0.14
Lactose	4.98 ± 0.36
Protein	1.05 ± 0.04
Fat	0.32 ± 0.10
Ash	0.54 ± 0.02

[a] Means of data obtained during the whole cheese-factory production season

Culture media

Ovine *scotta* utilized to prepare the two different cultural media was provided by a local dairy farm (F.lli Pinna Industria Casearia s.p.a, Sardinia, Italy), with the composition reported in Table 1. The culture media used consisted of pure *scotta* (SC_{medium}) and pure *scotta* added with 3 g l^{-1} yeast extract and 0.03 g l^{-1} $MnSO_4 \cdot H_2O$ ($SCYE_{medium}$).

Inoculum preparation

After thawing on ice, the LAB were sub-cultured twice, as follows: 1 ml of each bacterial culture was inoculated into 10 ml of the suitable medium (MRS or M17, depending on the strain), incubated at the appropriate temperature (37 or 42°C) for 24 h, and used to inoculate 100 ml of the same medium. After 20 h the pure bacterial cultures were harvested by centrifugation (8 min at 4,300 × *g* at 10°C) and used to inoculate 1 l of SC_{medium} or $SCYE_{medium}$. Mixed cultures were obtained by mixing 50 ml of each pure culture, and using this to inoculate 1 l of SC_{medium} or $SCYE_{medium}$. The mixed cultures tested were: Mix1, LC × LH; Mix2, LH × ST; and Mix3, LC × ST.

Batch fermentation

Fermentations were carried out in 3-l dish-bottomed glass bioreactors (Applikon, Holland) equipped with two Rushton impellers, a gel-fitted pH sensor, and a Pt100 temperature sensor. The temperature and pH were controlled by an ADI 1030 Bio-Controller, with stirring at 30 rpm controlled by an ADI 1032 Stirrer Speed Controller. The data were recorded with the BioXpert software, version 1.16 (Applikon, Holland). The pH was buffered to 5.5 by the addition of 10 M KOH throughout the fermentation process. Growth and fermentation kinetics were monitored for 20 h. Samples were collected at 0, 5, 10, 15, and 20 h after inoculation. Viable plate counts were carried out in anaerobic jars for each sampling time, on MRS agar after 48 h incubation at 37°C for LC, and at 42°C for LH. ST was counted on M17 agar after 48 h incubation at 42°C.

Analytical methods

L(−) and D(+)-lactic acid, lactose, glucose, and galactose were purchased from Sigma–Aldrich. HPLC analysis was made by a Thermo® HPLC apparatus equipped with UV6000LP (for lactic acid detection, 225 nm) and a Sedex55 light-scattering detector (for lactose, galactose, and glucose). Fermentation samples were centrifuged (6,800 × *g* for 40 min at 10°C) and filtered (0.2-µm syringe filter) before analysis. Lactic acid and the sugars were efficiently separated with an Aminex HPX-87H column (300 mm × 7.8 mm, Bio-Rad, USA) held at 62°C. A 0.02% trifluoroacetic acid aqueous solution was used as the mobile phase with 0.7 ml min^{-1} flow. The light-scattering detector was held at 45°C under a constant flow of N_2 (2.5 bar). The concentrations were calculated by comparison with calibration curves obtained with the areas measured for standard solutions of pure compounds. Using the HPLC system described above, L- and D-lactic acid were efficiently separated at room temperature with a Chirex® 3126 chiral D-penicillamine column (150 mm × 4.6 mm, Phenomenex, USA) using 1 mM $CuSO_4$ as mobile phase (1 ml min^{-1}).

Experimental design and statistical analysis

Fermentations were performed according to a completely randomized design with two factors and three replicates. Factors were "substrate" (SC_{medium} or $SCYE_{medium}$) and "microbial culture" (LC, LH, ST, Mix1, Mix2, Mix3). Data were subjected to one-way analysis of variance (ANOVA) using Statgraphics Plus, version 5.1 (Statistical Graphics Corp.) and means were separated using the Duncan's multiple range test to detect significant differences ($p < 0.05$).

Results and discussion

Fermentative performances and lactic acid production of pure cultures of lactic acid bacteria

To assess whether ovine *scotta* can be efficiently used as a valuable substrate for lactic acid production, the growth and fermentative performances of the LC, LH, and ST strains were evaluated in SC_{medium} and $SCYE_{medium}$. As shown in Fig. 1, LC and LH produced the highest and lowest number of colony-forming units (CFU), respectively in the two media. ST, besides showing a steeper exponential growth phase compared to LC and LH in both media, significantly increased the number of CFU in $SCYE_{medium}$. Therefore, it was the most receptive to the availability of vitamins, amino acids and trace elements contained in the nutritional supplements [2]. On the contrary, in the second half of the fermentation process, LH decreased the number of CFU in the presence of nutritional supplements (Fig. 1b).

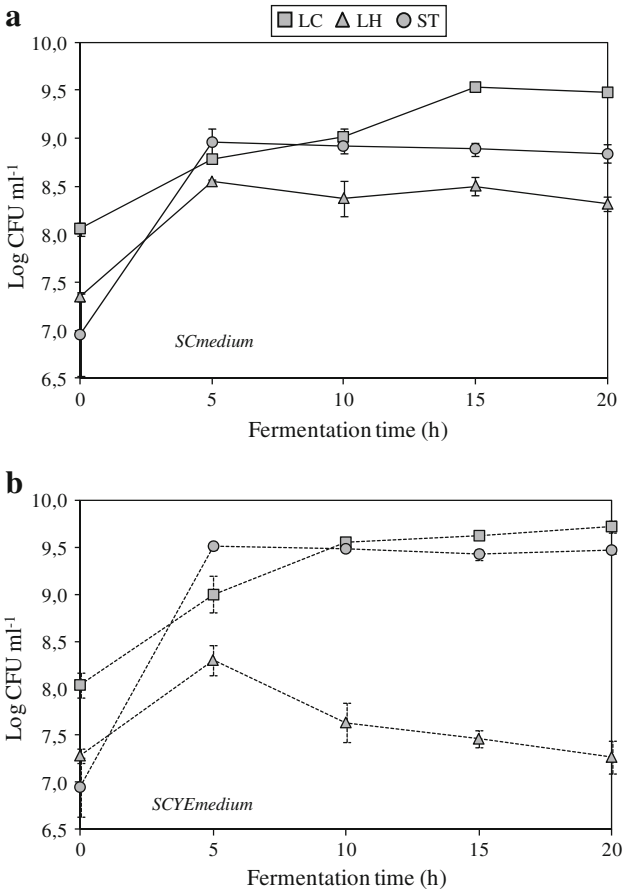

Fig. 1 Growth of pure LAB cultures in *scotta*-based media. Colony forming units (CFU) in SC_{medium} (**a**) and in $SCYE_{medium}$ (**b**). Data are means ± standard deviations of at least three independent experiments. Where not visible, the standard deviation *bars* lie within the *symbols*

None of the strains completely consumed the lactose in SC_{medium} after 20 h of fermentation, with LC showing the worst performance in this medium (Fig. 2a). Conversely, and in agreement with what has been shown for cheese-whey-based media [1, 3, 6, 7], the addition of the nutritional supplements (3 g l^{-1} yeast extract and 0.03 g l^{-1} $MnSO_4 \cdot H_2O$) resulted in increased lactose consumption (Fig. 2a). In particular, ST and LH completely consumed the lactose in 10 and 20 h, respectively, while LC, although showing significantly improved lactose consumption, left 10.73 g l^{-1} residual lactose at 20 h.

Interestingly, lactose consumption shown by LC on $SCYE_{medium}$ was similar to those of ST and LH on SC_{medium} (Fig. 2a). Thus, LC showed poor fermentative performances, with respect to the other strains, even in the presence of the nutritional supplements (Fig. 2a). Moreover, since LC showed in both media viable counts comparable

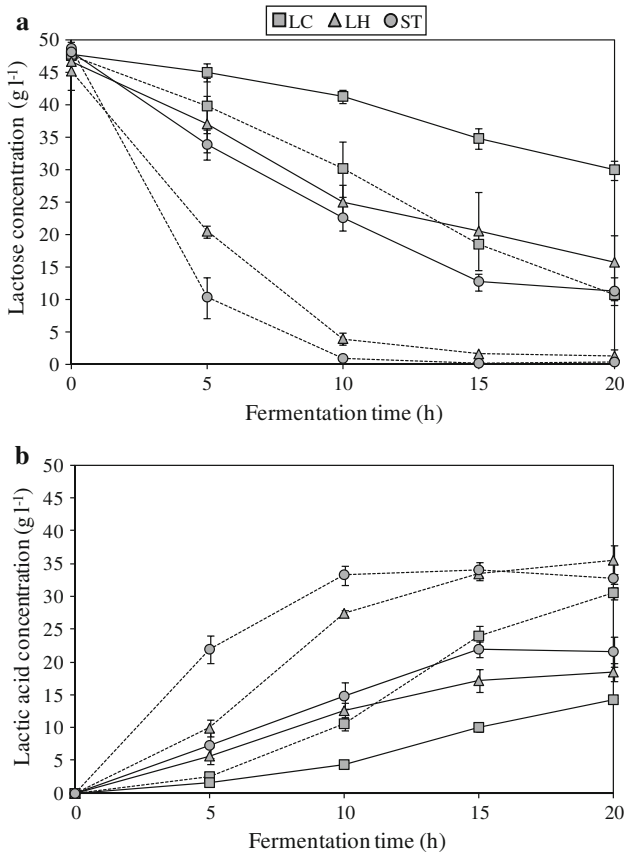

Fig. 2 Lactose consumption (**a**) and lactic acid production (**b**) of pure LAB cultures in *scotta*-based media. *Solid lines* represent SC_{medium}; *dashed lines* represent $SCYE_{medium}$. Data are means ± standard deviations of at least three independent experiments. Where not visible, the standard deviations lie within the *symbols*

or higher to that of ST and LH, its lower lactose consumption could not be ascribed to a poor growth.

Differences among strains were also seen for lactic acid production during fermentation (Fig. 2b). In agreement with their more rapid lactose consumption in both of the media, LH and ST produced greater amounts of lactic acid during the fermentation process, as compared to LC. However, while the total amounts of lactic acid produced by the three strains were higher in $SCYE_{medium}$ compared to SC_{medium}, at the end of the fermentation in $SCYE_{medium}$ the lactic acid produced across the three strains was comparable (Fig. 2b).

Thus, in agreement with what has been reported for whey permeate [6] and other cheese-whey-based media [2, 9, 10], over the 20-h incubation period, the three strains showed significantly increased lactic acid productivity and yield (Table 2) when *scotta* was added with nutritional supplements ($SCYE_{medium}$). In particular, LC, which was the least productive in SC_{medium} (Table 2), doubled its lactic acid productivity in $SCYE_{medium}$, reaching a value comparable to that of ST (Table 2). In agreement with Hickey et al. [8] LC, although leaving residual lactose in both SC_{medium} and $SCYE_{medium}$, completely consumed the glucose (data not shown) and galactose (Table 2) in both of these media. Moreover, it was the most efficient for lactose conversion and showed the highest lactic acid yield (92%) in $SCYE_{medium}$. This last was comparable to that obtained by Fitzpatrick et al. [6] after 24 h of fermentation in whey permeate. Thus, the higher concentration of residual lactose left by LC at the end of the fermentation was the consequence of a more efficient utilization of the monosaccharides deriving from lactose hydrolysis. In contrast, LH and ST although completely hydrolyzing lactose, left considerable amounts of residual galactose in both of these media (Table 2). As for LH, the availability of the nutritional supplements increased galactose consumption and resulted in significant increases in lactic acid productivity and yield in spite of the decrease in the number of CFU. Thus, as already reported by Lee [13], growth and lactic acid production were uncoupled in LH. On the contrary, ST did not increase galactose consumption in $SCYE_{medium}$ (Table 2). This was in agreement with an impaired ability to use galactose, as has been reported for the *S. thermophilus* species [11, 18].

Fermentative performances and lactic acid production of mixed cultures of lactic acid bacteria on SC_{medium}

To evaluate any possible synergistic effect of LAB on ovine *scotta* bioconversion, SC_{medium} was inoculated with the following mixed cultures: Mix1 (LC × LH), Mix2 (LH × ST), and Mix3 (LC × ST).

Significant differences in viable counts were seen among mixed cultures within the first 10 h of growth, with Mix2 (LH × ST) showing the best performance as expected based on the growth parameters of LH and ST in pure cultures (Fig. 3a). The mixed cultures also showed significant differences in lactose consumption and lactic acid production (Fig. 4). Mix2 showed complete lactose consumption at 20 h while Mix1 and Mix3 showed 3.42 and 6.67 g l^{-1} residual lactose, respectively, at this sampling time (Fig. 4a). Accordingly, the lactic acid production of Mix2 was significantly higher as compared to those of Mix1 and Mix3 during the whole fermentation process. Interestingly, in spite of a poor galactose consumption, Mix2 confirmed its strong supremacy in terms of lactic acid productivity and yield (Table 2). On the contrary, Mix1 (LC × LH) and Mix3 (LC × ST), that left lower amounts of residual galactose, as expected on the basis of the contribution of LC to the fermentation process, showed significantly lower lactic acid productivity and yield as compared to Mix2 (LH × ST). Thus, an efficient galactose utilization was not sufficient to ensure better fermentative performances for Mix1 and Mix3.

Table 2 Fermentative parameters of the LAB over the 20-h incubations in pure and mixed cultures

LAB	Medium	Lactic acid productivity (g l^{-1} h^{-1})	Lactic acid yield* (%)	Residual galactose (g l^{-1})	L-lactic acid optical purity (%)
LC	SC	0.715 ± 0.02^{f}	86.48 ± 7.02^{c}	0.00 ± 0.00^{e}	91.42 ± 2.31^{c}
	SCYE	1.526 ± 0.08^{cd}	92.37 ± 3.51^{ab}	0.00 ± 0.00^{e}	95.28 ± 1.51^{d}
LH	SC	0.925 ± 0.12^{e}	52.19 ± 1.46^{f}	10.36 ± 2.23^{b}	1.62 ± 0.50^{g}
	SCYE	1.779 ± 0.19^{b}	85.66 ± 3.87^{c}	5.79 ± 0.28^{c}	2.26 ± 0.53^{g}
ST	SC	1.080 ± 0.04^{e}	64.08 ± 2.99^{e}	13.66 ± 0.01^{a}	99.99 ± 0.00^{a}
	SCYE	1.633 ± 0.07^{bc}	71.73 ± 2.56^{d}	14.77 ± 2.35^{a}	99.99 ± 0.00^{a}
Mix1(LC × LH)	SC	1.339 ± 0.08^{d}	68.72 ± 2.24^{e}	5.90 ± 1.03^{c}	23.31 ± 3.90^{f}
	SCYE	2.072 ± 0.04^{a}	97.09 ± 1.49^{a}	2.59 ± 0.45^{d}	23.29 ± 2.38^{f}
Mix2(LH × ST)	SC	1.643 ± 0.13^{bc}	78.45 ± 2.68^{d}	12.74 ± 0.55^{a}	60.66 ± 1.93^{d}
	SCYE	1.997 ± 0.15^{a}	88.41 ± 2.46^{bc}	2.62 ± 0.66^{d}	38.78 ± 0.47^{e}
Mix3(LC × ST)	SC	1.088 ± 0.08^{e}	53.19 ± 3.36^{f}	9.58 ± 0.98^{b}	95.44 ± 0.35^{b}
	SCYE	1.977 ± 0.06^{a}	85.27 ± 2.02^{c}	3.59 ± 0.01^{cd}	95.98 ± 0.45^{b}

Data are means ± standard deviations of at least three independent experiments

Different letters within each column indicate significantly different values ($p < 0.05$)

* Yield = g of lactic acid produced/g of lactose consumed × 100

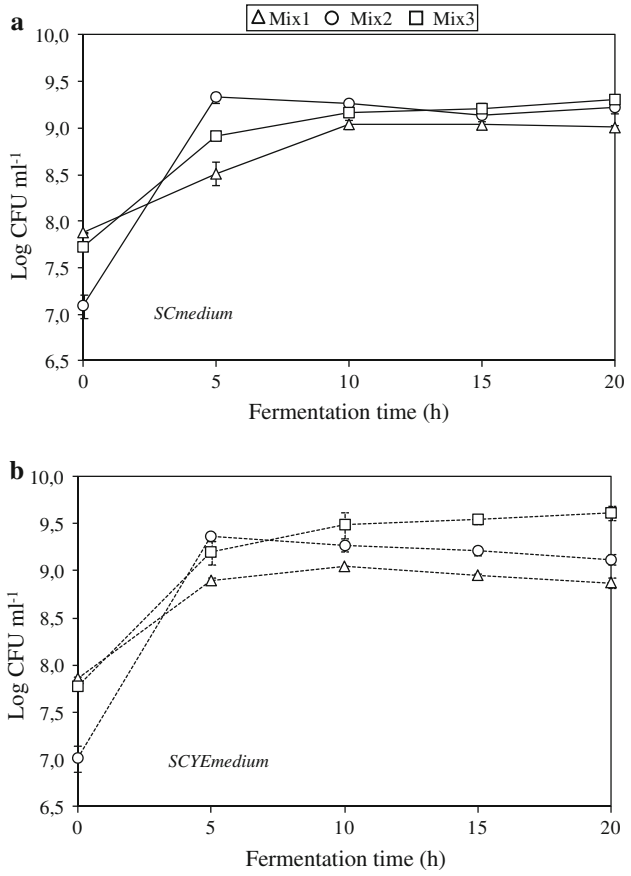

Fig. 3 Growth of mixed LAB cultures in *scotta*-based media. Colony-forming units (CFU) in SC_{medium} (**a**) and in $SCYE_{medium}$ (**b**). Data are means ± standard deviations of at least three independent experiments. Where not visible, the standard deviation *bars* lie within the *symbols*

The advantage of mixed cultures over single cultures, in terms of lactic acid production, has been demonstrated before in MRS media [13] and whey media [18, 20, 21]. Accordingly, with the exception of Mix3 (LC × ST), where the productivity was comparable to that of ST alone in SC_{medium}, the mixed cultures showed better lactic acid productivities than the pure (mono-strain) cultures (Table 2). This was possibly due to the synergistic effects of LAB in lactose and protein utilization [13, 18].

Fermentative performances and lactic acid production of mixed cultures of lactic acid bacteria on $SCYE_{medium}$

To evaluate the effect of the addition of yeast extract and manganese salts on ovine *scotta* bioconversion, Mix1, Mix2, and Mix3 were inoculated in $SCYE_{medium}$. The availability of the nutritional supplements did not show any effect on Mix1 and Mix2 viable counts, while Mix3 showed a significant increase in viable counts in the second half of the fermentation process (Fig. 3b). Lactose consumption was faster in $SCYE_{medium}$ (Fig. 4a). In particular, Mix2 (LH × ST) and Mix3 (LC × ST) completed their lactose consumption within 10 h, while Mix1 (LC × LH) needed 5 h more. This result was consistent with both the increase in lactose consumption rate shown by ST and LH in response to the availability of nutritional supplements, and the slow utilization of lactose shown by LC (Fig. 2). Lactic acid production increased in the presence of nutritional supplements (Fig. 4b) and reached its maximum at the end of the fermentation, for all mixed cultures. Thus, the complete

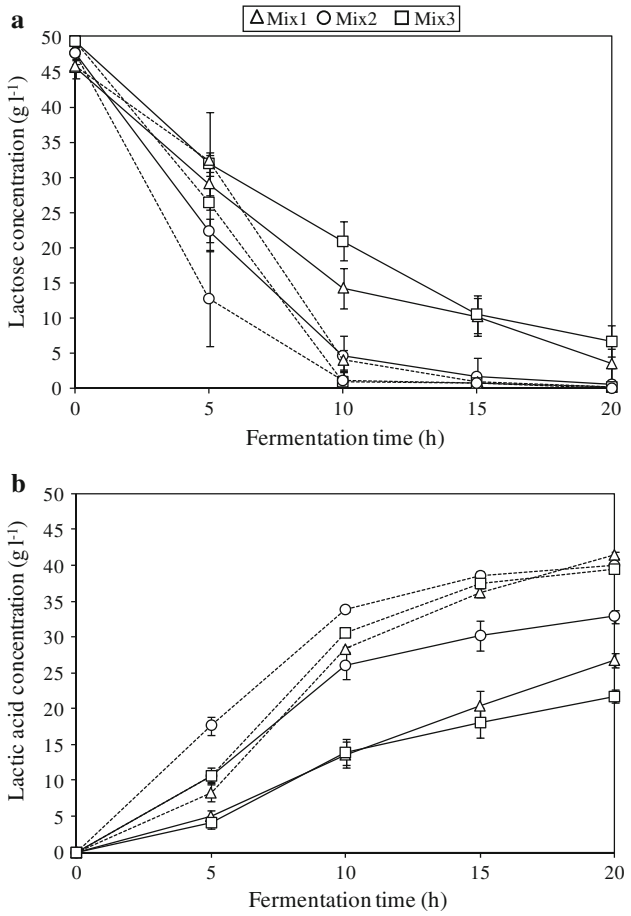

Fig. 4 Lactose consumption (**a**) and lactic acid production (**b**) of mixed LAB cultures in *scotta*-based media. *Solid lines* represent SC_{medium}; *dashed lines* represent $SCYE_{medium}$. Data are means ± standard deviations of at least three independent experiments. Where not visible, the standard deviations lie within the *symbols*

transformation of glucose and galactose into lactic acid was delayed with respect to lactose hydrolysis [27].

For what concerns lactic acid productivity (Table 2), Mix1 (LC × LH) and Mix3 (LC × ST) showed 54 and 83% increases, respectively, while Mix2 (LH × ST) showed the lowest increase (21%). The limited effect of the availability of the nutritional supplements on the fermentative parameters of Mix2 was compatible with the hypothesis of a strong synergy between strains LH and ST for the fermentation of ovine *scotta*. Accordingly, Mix2 showed better performances in SC_{medium} as compared to the other two mixes. Moreover, all mixed cultures increased significantly lactic acid yield and galactose consumption (Table 2). However, contrary to that observed in SC_{medium}, lactic acid production and productivity and residual galactose were comparable for all of the mixed cultures. Thus, the availability of nutritional supplements resulted in an equalization of the productive parameters.

Lactic acid optical purity

In agreement with previous reports [17], while LC and ST produced >90% L-lactic acid, LH produced about 98% D-lactic acid (Table 2). Consequently, as Mix1(LC × LH) and Mix2 (LH × ST) both contained LH, they produced a mixture of L-lactic acid and D-lactic acid, while Mix3 (LC × ST) yielded nearly pure L-lactic acid. Indeed, the relative amount of the stereoisomers produced by Mix1 and Mix2 was strictly dependent on the contribution of the two strains involved in lactic acid production. Accordingly, in spite of the presence of the L-lactic acid producer LC, Mix1 produced low relative levels of L-lactic acid, which was possibly due to the predominance of LH in the fermentation of lactose (Fig. 2). Similarly, for Mix2, the major role played by ST in lactose bioconversion resulted in a higher relative amount of L-lactic acid in SC_{medium}. Interestingly, Mix2 showed a significant increase in the relative levels of D-lactic acid in the $SCYE_{medium}$, which was due to the observed increase in the fermentation activity of the D-lactic acid producer LH in the presence of nutritional supplements (Table 2). Finally, Mix3 (LC × ST) produced more than 95% L-lactic acid on both media.

Since the market price of pure L-lactic acid is higher than that of mixtures of the two enantiomers [5, 16, 28], this result highlights the importance of microorganisms selection for *scotta* bioconversion.

Conclusions

Despite its large availability and low market price, *scotta* is poorly utilized as a substrate for biotechnological processes. Moreover, due to its high lactose and protein contents, it is regarded as a serious environmental pollutant. Therefore, *scotta* bioconversion into valuable products, besides providing it with an added value, can be considered as an alternative to its disposal. To the best of our knowledge, the results presented here show for the first time that ovine *scotta* can be used as a fermentation substrate for the production of lactic acid, with results comparable to those provided by cheese-whey-based media [20, 21, 28]. As already shown for cheese-whey, the addition of nutritional supplement increases the fermentative performances and lactic acid productions of lactic acid bacteria both in pure and mixed cultures. However, the use of mixed cultures reduces the need for nutritional supplements for ovine *scotta* bioconversion with no detrimental effects on the productive parameters, as compared to the pure cultures on $SCYE_{medium}$. Moreover, by using the LC or the ST strains, in pure or mixed cultures, it is possible to obtain optically pure L-lactic acid that represents a product with more value added as compared to the D-form.

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References

1. Amrane A, Prigent Y (1993) Influence of media composition on lactic acid production. Biotechnol Lett 36:461–464
2. Büyükkileci AO, Harsa S (2004) Batch production of L(+) lactic acid from whey by *Lactobacillus casei* (NRRL B-441). J Chem Technol Biotechnol 79:1036–1040
3. Chiarini L, Mara L, Tabacchioni S (1992) Influence of growth supplements on lactic acid production in whey ultrafiltrate by *Lactobacillus helveticus*. Appl Microbiol Biotechnol 15:239–244
4. Commission Regulation (EC) No 79/2005, of 19 January 2005 implementing Regulation (EC) No 1774/2002 of the European Parliament and of the Council as regards the use of milk, milk-based products and milk-derived products, defined as Category 3 material in that Regulation. Off J Eur Union L 16:46–50
5. Datta R, Henry M (2006) Lactic acid: recent advances in products, processes and technologies—a review. J Chem Technol Biotechnol 81:1119–1129
6. Fitzpatrick JJ, Ahrens M, Smith S (2001) Effect of manganese on *Lactobacillus casei* fermentation to produce lactic acid from whey permeate. Process Biochem 36:671–675
7. Fitzpatrick JJ, Murphy C, Mota FM, Pauli T (2003) Impurity and cost considerations for nutrient supplementation of whey permeate fermentations to produce lactic acid for biodegradable plastics. Int Dairy J 13:575–580
8. Hickey MW, Hillier AJ, Jago GR (1986) Transport and metabolism of lactose, glucose, and galactose in homofermentative Lactobacilli. Appl Environ Microbiol 51:825–831
9. Hofvendahl K, Hahn-Hagerdal B (2000) Factors affecting the fermentative lactic acid production from renewable resources. Enzyme Microbial Technol 26:87–107
10. Hujanen M, Linko YY (1996) Effect of temperature and various nitrogen sources on L (+)-lactic acid production by *Lactobacillus casei*. Appl Microbiol Biotechnol 45:307–313
11. Iyer R, Tomar SK, Maheswari TU, Singh R (2010) *Streptococcus thermophilus* strains: multifunctional lactic acid bacteria. Int Dairy J 20:133–141
12. John RP, Nampoothiri KM, Pandey A (2007) Fermentative production of lactic acid from biomass: an overview on process developments and future perspectives. Appl Microbiol Biotechnol 74:524–534
13. Lee KB (2005) Comparison of fermentative capacities of Lactobacilli in single and mixed culture in industrial media. Process Biochem 40:1559–1564
14. Mucchetti G, Carminati D, Pirisi A (2002) Fresh ricotta cheese from cow's and ewe milk. Observations on production techniques and the product. Il Latte 27:154–166
15. Nampoothiri KM, Nair NR, John RP (2010) An overview of the recent developments in polylactide (PLA) research. Bioresour Technol 101:8493–8501
16. Paster M, Pellegrino JL, Carole TM (2003) Industrial bioproducts: today and tomorrow. Energetics, Incorporated, Columbia, Maryland for the US Department of Energy, Office of Energy Efficiency and Renewable Energy, Office of the Biomass Program, Washington, DC
17. Panesar PS, Kennedy JF, Gandhi DN, Bunko K (2007) Bio-utilisation of whey for lactic acid production. Food Chem 105:1–14
18. Pescuma M, Hébert EM, Mozzi F, Font de Valdez G (2008) Whey fermentation by thermophilic lactic acid bacteria: evolution of carbohydrates and protein content. Food Microbiol 25:442–451
19. Pintado ME, Lopes da Silva JA, Malcata FX (1999) Comparative characterization of whey protein concentrates from ovine, caprine and bovine breeds. Lebensm Wiss Technol 32:231–237
20. Plessas S, Bosnea L, Psarianos C, Koutinas AA, Marchant R, Banat IM (2008) Lactic acid production by mixed cultures of *Kluyveromyces marxianus*, *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Lactobacillus helveticus*. Bioresour Technol 99:5951–5955
21. Roukas T, Kotzekidou P (1998) Lactic acid production from deproteinized whey by mixed cultures of free and co-immobilized *Lactobacillus casei* and *Lactococcus lactis* cells using fedbatch culture. Enzyme Microbial Technol 22:199–204
22. Sansonetti S, Curcio S, Calabrò V, Iorio G (2009) Bio-ethanol production of *ricotta* cheese-whey as an effective alternative non-vegetable source. Biomass Bioenerg 33:1687–1692
23. Sansonetti S, Curcio S, Calabrò V, Iorio G (2010) Optimization of ricotta cheese-whey (RCW) fermentation by response surface methodology. Bioresour Technol 101:9156–9162
24. Sauer M, Porro D, Mattanovich D, Branduardi P (2008) Microbial production of organic acids: expanding the markets. Trends Biotechnol 26:100–108
25. Schepers AW, Thibault J, Lacroix C (2002) *Lactobacillus helveticus* growth and lactic acid production during pH-controlled batch cultures in whey permeate/yeast extract medium. Part I. multiple factor kinetic analysis. Enzyme Microbial Technol 30:176–186
26. Shen L, Haufe J, Patel MK (2009) Scenarios for markets of bio-based plastics. In: Product overview and market projection of emerging bio-based plastics. Utrecht University. www.europeanbioplastics.org/wpcontent/uploads/2011/03/publications/PROBIP2009_Final_June_2009.pdf. Accessed 19 May 2011
27. Tejayadi S, Cheryan M (1995) Lactic acid from cheese-whey permeate. Productivity and economics of a continuous membrane bioreactor. Appl Microbiol Biotechnol 43:242–248
28. Wee YJ, Kim JN, Ryu HW (2006) Biotechnological production of lactic acid and its recent applications. Food Technol Biotechnol 44:163–172