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Changes in oxidation-reduction potential during milk fermentation by wild lactic acid bacteria

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Oxidation-reduction potential (E_h) is a fundamental physicochemical property of lactic acid bacteria that determines the microenvironment during the cheese manufacture and ripening. For this reason the E_h is of growing interest in dairy research and the dairy industry. The objective of the study was to perform a comprehensive study on the reduction activity of wild lactic acid bacteria strains collected in different periods (from 1960 to 2012) from Italian dairy products. A total of 709 strains belonging to *Lactococcus lactis, Enterococcus durans, E. faecium, E. faecalis* and *Streptococcus thermophilus* species were studied for their reduction activity in milk. Kinetics of milk reduction were characterised by the minimum redox potential (E_{h7}) and time of reaching E_{h7} (t_{min}), the maximum difference between two measures (Δ_{max}) and the time at which these maximum differences occurred (t^*). Broad diversity in kinetic parameters was observed at both species and strain levels. *E. faecalis* and *L. lactis* resulted to be the most reducing species, while *S. thermophilus* was characterised by the lowest reducing power while the greatest heterogeneity was pointed out among *E. durans* and *E. faecium* strains. Considering the period of collection (1960–2012) we observed that the more recently isolated strains generally showed less reducing activity. This trend was particularly evident for the species *E. durans, E. faecium* and *L. lactis* while an opposite trend was observed in *E. faecalis* species. Data reported in this research provide new information for a deeper understanding of redox potential changes during milk fermentation due to bacterial growth. Gain knowledge of the redox potential of the LAB cultures could allow a better control and standardisation of cheesemaking process.

Keywords: E_h, redox potential, reducing activity, lactic acid bacteria.

Lactic acid bacteria (LAB) are nonpathogenic, food safe bacteria that are used in the production of many dairy products. These microorganisms may include added starter (such as *Lactococcus lactis* and *Streptococcus thermophilus*), and nonstarter lactic acid bacteria (NSLAB) that enter in cheese through milk or the processing environment. Enterococci, in particular *Enterococcus durans, E. faecium* and *E. faecalis*, occur as NSLAB in a variety of artisan cheeses produced in the southern Europe (Psoni et al. 2006; Morandi et al. 2011; Nieto-Arribas et al. 2011). During cheese ripening, the starter culture, along with the NSLAB promote a complex series of biochemical reactions which are vital for proper development of both flavour and texture (Beresford et al. 2001).

The biotechnological and safety characterisation of starter and NSLAB has been based on acidifying, proteolytic and lipolytic activities, however, in the recent years redox potential has stood out as a fundamental physicochemical property (Cachon et al. 2002).

The redox or oxidation-reduction potential (E_h), is a measure of the ability of a chemical/biochemical system to oxidise (lose electrons) or reduce (gain electrons). The E_h of milk at 25 °C is usually in a range from +250 to +350 mV, while in bacteria-ripened cheeses it has a negative E_h value (Topcu et al. 2008; Caldeo & Mc Sweeney, 2012). The exact mechanism by which the E_h of cheese is reduced is not clear but is almost certainly related to the fermentation of lactose to lactic acid by the starter during its growth and is probably related to the reduction of the

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small amounts of O_2 in milk to H_2O. Tachon et al. (2010) showed that elimination of oxygen is required for milk reduction and that NADH oxidase (NoxE) is mainly responsible for the rapid removal of oxygen from milk before the exponential growth phase. Moreover, also the electron transport chain also contributes slightly to oxygen consumption, especially during the stationary growth phase (Tachon et al. 2010). Because of these reactions, the ripened cheese is essentially an anaerobic system in which only facultatively or obligately anaerobic microorganisms can grow. Redox potential can be an important determinant of the types of microorganisms that can grow in cheese (Fox et al. 2000). The E_h affects the development and activity of the secondary microbiota in cheese and the viability of lactic acid bacteria (Ouvry et al. 2002; Bolduc et al. 2006). Oxidation-reduction potential could have also a great impact on flavour formation by LAB, and it has been assumed that a negative E_h is required for the development and stability of flavour in cheese (Kieronczyk et al. 2006). In recent years the redox potential is increasingly taken into account in food processes to improve both the microbiological and organoleptic quality of dairy products (Caldeo & Mc Sweeney, 2012; Abraham et al. 2013) but to our knowledge, few studies have described the reduction kinetics of LAB in milk (Cachon et al. 2002; Brasca et al. 2007). For this reason, the purpose of this work was to evaluate the reduction potential of 709 wild strains belonging to starter (*L. lactis* and *S. thermophilus*) and NSLAB species (*E. durans, E. faecium, E. faecalis*) collected in different periods (from 1960 to 2012) from Italian dairy products.

Materials and methods

Bacterial strains

A total of 709 strains belonging to *L. lactis, E. durans, E. faecium, E. faecalis* and *S. thermophilus* species were studied. Four strains were provided by different culture collections while the other 705 were isolated during the cheese-making of different dairy products (Table 1). Enterococci and lactococci strains were subcultured in de Mann Rogosa Sharpe (MRS) broth (Scharlau Microbiology, Barcelona, Spain) while streptococci in M17 broth (Scharlau Microbiology). All strains were incubated at 37 °C for 18 h according to Brasca et al. (2007).

pH and redox potential measurements

A multi-channel pH-Eh-meter (Acidification Monitoring System and Analyser AMSA, Star Ecotronics, Milan, Italy) was used to obtain the pH and E_h values in the milk during 24 h of incubation at 37 °C. Overnight strains were inoculated at a level of 1% in reconstituted sterile non-fat dry milk (10% w/v) (Sacco srl, Cadorago, Italy). Combined pH electrodes (InLab 51343050, Mettler-Toledo, Greifensee, Switzerland) were calibrated using standard solutions with pH 4·0 and 7·0 (Mettler-Toledo) before each analysis. The accuracy of E_h electrodes (InLab Redox, Metler Toledo) was checked using two redox solutions (240 and 470 mV; Hanna Instruments, Villafranca Padovana, Italy). The redox potential was calculated using the equation

$$E_h = E_m + E_r$$

where E_m is the recorded redox potential and E_r is the potential of the reference electrode, (Jacob 1970; Mc Sweeney et al. 2010). Temperature compensation was done using the following formula (Caldeo & Mc Sweeney, 2012):

$$E_r = +199 - 1{\cdot}01(T - 25°C)$$

Since the change of pH during the fermentation modify the E_h, the redox potential was expressed at pH 7 according to Abraham et al. (2013), using the following formula:

$$E_{h7} = E_h - [(7 - pH)^* a]$$

where α is the E_h – pH correlation factor and pH is the pH of the medium. This factor α was determined experimentally by measuring the variation of E_h in the reconstituted sterile non-fat dry milk with pH using acid and base solutions; a variation in E_h of 31 mV/pH unit at 37 °C was measured.

Two replicates for each bacterial strain were used to estimate the reduction activity, determining the minimum value of E_{h7} over 24 h (E_{h7min}, (mV)), and the time at which E_{h7min} (t_{min} (min)), the maximum difference between two measures (Δ_{max} (mV)) and the time at which these maximum differences occurred (t^*(h)) (Brasca et al. 2007).

Statistical analysis

Statistical analysis was performed (SAS, 2001) using CLUSTER procedure by average linkage method, considering the variables of redox potential measurements (E_{h7min}, t_{min}, Δ_{max} and t^*) of 5 species separately. The level of the tree defining disjoint clusters was set at 0·65 average distance. A preliminary variance analysis showed a quite similar coefficient of variation for those parameters, and it was not necessary transforming variables before performing the cluster analysis. A cluster analysis of all strains was not particularly clear. The results were expressed as mean (±SD) of measured parameters for each cluster.

Results

The milk utilised to evaluate the reduction activity of the strains had a pH of 6·2 ± 0·1 at 37 °C and the initial E_{h7} value of the inoculated milk was 182·8 ± 80·0 mV.

Evaluating the reducing capacity of LAB strains we observed a different behaviour among the various species but also among the strains belonging to the same species (Figs 1–3). In most of strains the reduction of milk can be described in

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Table 1. Origin of the 709 LAB strains examined in this study

Cheese	*E. durans*	*E. faecalis*	*E. faecium*	*L. lactis*	*S. thermophilus*	Total
Bitto	1	6	77	1	44	129
Caciocavallo Ragusano	1	1	2	–	–	4
Goat cheese	–	–	–	1	–	1
Emmenthal	–	–	2	–	–	2
Fatulì	3	4	–	21	–	28
Fontina	–	2	2	–	2	6
Formagèla Val Seriana	34	12	6	22	30	104
Formaggella del Luinese	4	7	6	12	3	32
Formaggella Val di Scalve	–	34	–	28	14	76
Formai de Mut	1	3	1	1	–	6
Grana Padano	–	3	4	–	–	7
Grasso d'Alpe	–	–	–	1	–	1
Provolone	–	–	2	–	–	2
Quartirolo	–	–	1	–	–	1
Scimudin	–	8	3	3	–	14
Semigrasso d'Alpe	–	–	–	1	–	1
Semuda	7	25	7	9	6	54
Silter	2	5	6	19	3	35
Soft cheese	3	7	2	–	–	12
Taleggio	–	–	3	–	–	3
Valtellina Casera	7	61	65	12	22	167
Raw milk	3	12	3	2	–	20
Bacterial collection†	–	3	1	–	–	4
Total	66	193	193	133	124	709

†American Type Culture Collection (USA): E. *faecalis* ATCC 8043, ATCC 27285 and ATCC 23655.
Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig (Germany): *E. faecium* DSM 20477.

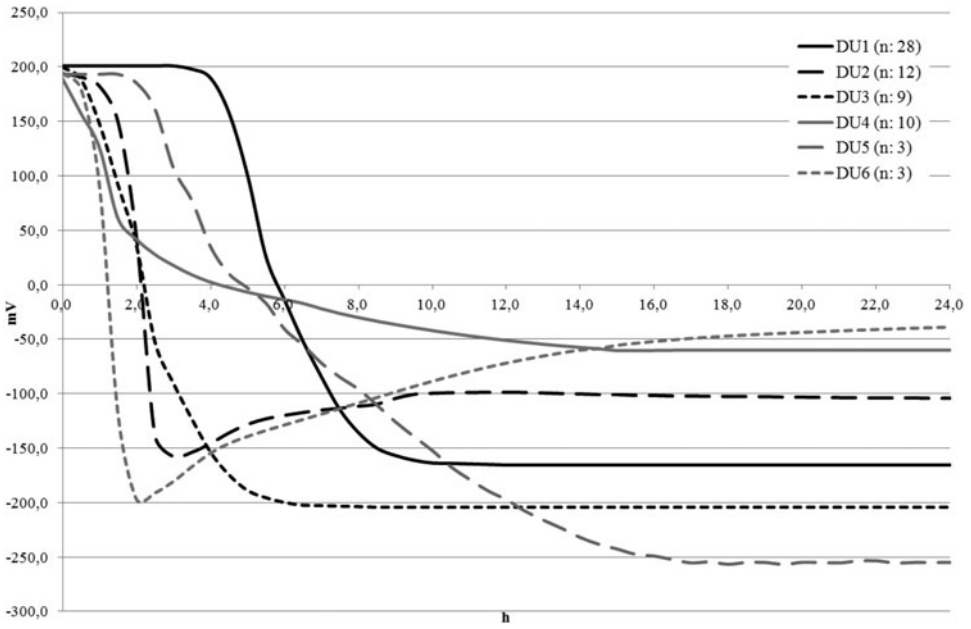


Fig. 1. Examples of development in E_{h7} detected in *Enterococcus durans* strains. In brackets, the number of strains for each clusters.

three phases: 1- the reduction lag-type phase when E_{h7} remained stable or decreased slowly; 2- the drop of E_{h7} to the minimum value (E_{h7min}) and 3- the stabilisation phase, E_{h7} either stabilised at the minimum value or increased slightly.

Enterococcus durans

Sixty-six *E. durans* have been evaluated for their reducing capability, and the strains belonging to this species were

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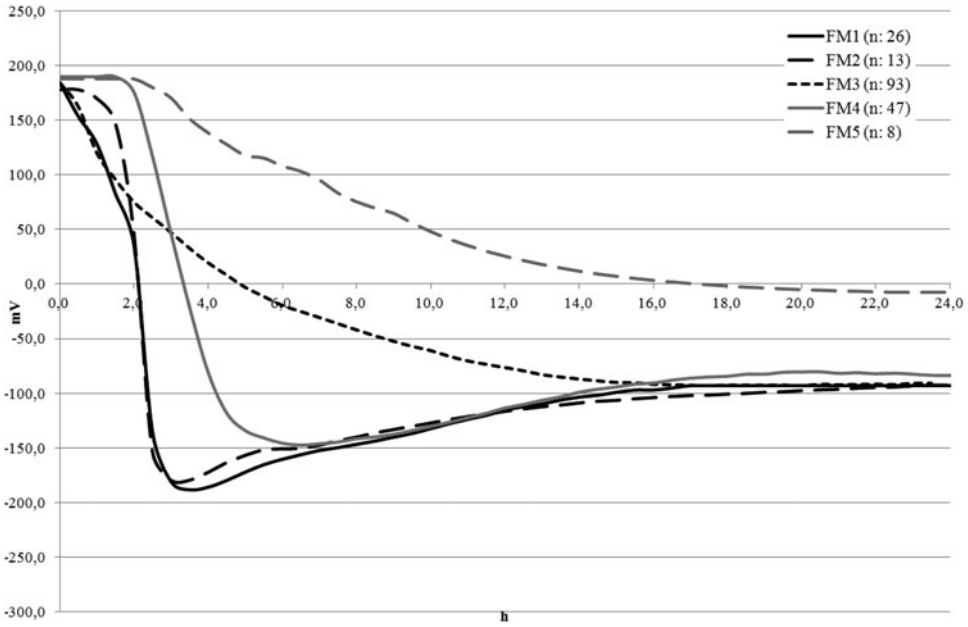


Fig. 2. Examples of development in E_{h7} detected in *Enterococcus faecium* strains. In brackets, the number of strains for each clusters.

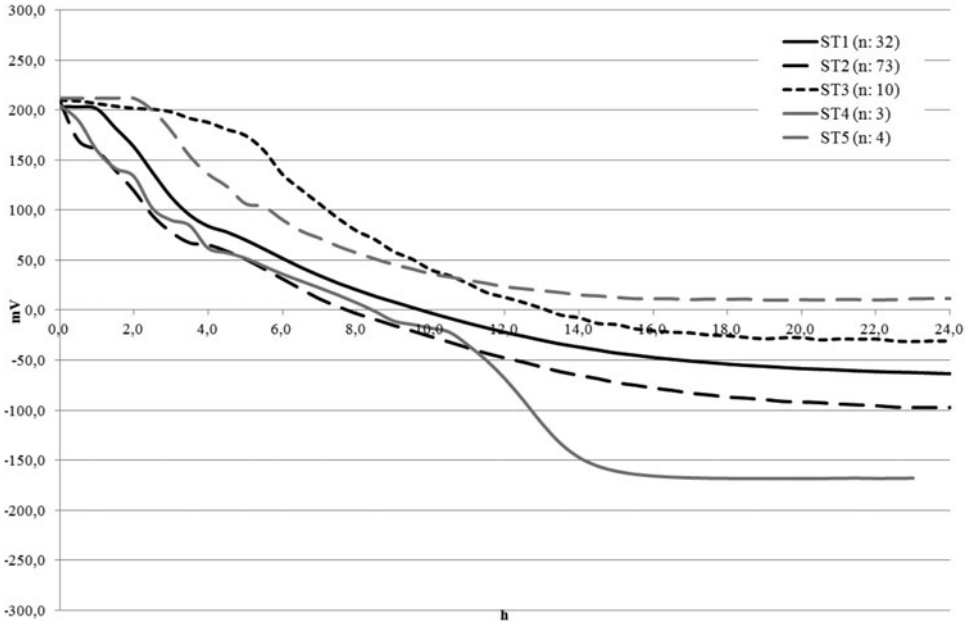


Fig. 3. Examples of development in E_{h7} detected in *Streptococcus thermophilus* strains. In brackets, the number of strains for each clusters.

characterised by a moderate reduction power (E_{h7min} −152·32 ± 56·15 mV; Table 2). Six clusters were observed inside this species and the majority of the strains fell in cluster DU1 (28 strains) followed by clusters DU2 (12 strains) and DU4 (10 strains) (Fig. 1).

The *E. durans* with higher reducing activity were grouped in cluster DU3 (E_{h7min} −214·93 ± 12·05 mV) and DU5 (E_{h7min} −253·22 ± 17·06 mV) while the strains belonging the cluster DU6 resulted to be the fastest to achieve the E_{h7min} (2·67 ± 0·58 h) and to reach the Δ_{max} (t*: 0·67 ± 0·29 h) (Table 2; Fig. 1). The strains clustered in DU4 displayed the lowest reduction power (E_{hmin} −65·42 ± 25·85 mV) and achieved the E_{h7min} after 19·70 h. One strain did not fall in any cluster (E_{h7min} −88·08 mV; t_{min}: 11 h).

Considering the data relative to the period of collection (1960–2012) we observed that the more recently isolated strains generally showed less reducing activity in fact the percentage of *E. durans* with a moderate-high reduction power

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Table 2. Kinetic parameters from the reduction rates of *E. durans*, *E. faecalis* and *E. faecium*, cultured in reconstituted skimmed milk according to cluster analyses (means values ± SD)

Specie	Parameters	Total	Clusters					
			DU1	DU2	DU3	DU4	DU5	DU6
E. durans	n. of strains	66	28	12	9	10	3	3
	E_{h7min} (mV)	−152·32 ± 56·15	−150·85 ± 21·11	−160·69 ± 18·70	−214·93 ± 12·05	−65·42 ± 25·85	−253·22 ± 17·06	−194·55 ± 7·17
	t_{min} (h)	10·98 ± 8·45	13·64 ± 8·10	3·33 ± 0·54	4·00 ± 1·97	19·70 ± 4·75	19·83 ± 2·57	2·67 ± 0·58
	Δ_{max} (mV)	103·57 ± 66·62	74·74 ± 29·36	170·68 ± 27·84	125·18 ± 26·50	31·50 ± 20·62	62·13 ± 11·76	260·07 ± 24·70
	t^* (h)	2·44 ± 3·87	2·73 ± 4·11	1·58 ± 0·47	0·67 ± 0·71	4·15 ± 5·66	5·33 ± 7·51	0·67 ± 0·29
		Total	FS1	FS2	FS3	FS4		
E. faecalis	n. of strains	193	81	48	45	19		
	E_{h7min} (mV)	−237·31 ± 30·22	−247·17 ± 18·00	−240·64 ± 25·93	−240·76 ± 15·43	−178·66 ± 42·87		
	t_{min} (h)	4·18 ± 3·54	2·60 ± 0·74	3·28 ± 1·86	5·08 ± 2·78	11·05 ± 6·17		
	Δ_{max} (mV)	279·22 ± 113·67	389·82 ± 37·82	273·22 ± 23·42	170·74 ± 31·94	79·79 ± 31·34		
	t^* (h)	0·88 ± 0·62	0·77 ± 0·48	0·98 ± 0·50	0·82 ± 0·43	1·22 ± 1·31		
		Total	FM1	FM2	FM3	FM4	FM5	
E. faecium	n. of strains	193	26	13	93	47	8	
	E_{h7min} (mV)	−123·73 ± 51·06	−181·49 ± 22·28	−178·74 ± 15·12	−91·63 ± 22·96	−153·48 ± 18·78	−6·89 ± 10·63	
	t_{min} (h)	11·82 ± 8·08	3·60 ± 1·00	3·23 ± 0·60	17·45 ± 6·37	7·01 ± 4·19	19·63 ± 6·71	
	Δ_{max} (mV)	84·14 ± 64·72	151·64 ± 17·44	218·88 ± 21·66	41·31 ± 15·26	84·07 ± 20·87	36·90 ± 15·32	
	t^* (h)	2·13 ± 1·52	1·90 ± 0·55	1·88 ± 0·55	2·22 ± 1·97	2·39 ± 1·02	1·06 ± 0·821	

Strains not clustered: 1 *E. durans* and 6 *E. faecium*.

decreases over the years, while the percentage of the lowest reduction strains increases remarkably (cluster DU4) (Table 3).

Enterococcus faecalis

E. faecalis resulted to be the species with the highest reduction power (−237·31 ± 30·22 mV) and the most rapid to reach the E_{h7min} (t_{min}: 4·18 ± 3·54 h). The strains tested were grouped in 4 clusters and the majority of *E. faecalis* fell into cluster FS1 (42%), characterised by the lowest E_{h7min} (−247·17 ± 18·00 mV). Considering the reduction curves, *E. faecalis* belonging to the clusters FS1, FS2 and FS3 showed a similar trend, differing in length of the first step (when E_{h7} remained stable or decreased slowly) and in the values of Δ_{max}, in fact the E_{h7min} were recorded after 2·60, 3·28 and 5·08 h and the values of Δ_{max} decreased from 389·82 (cluster FS1) to 170·74 mV (FS3) (Table 2).

The redox curves of *E. faecalis* strains were very similar to those detected for *E. durans* grouped in cluster DU6 (data not shown).

Nineteen strains (10%) grouped in the FS4 were characterised by a moderate reduction power (E_{h7min} −178·66 ± 42·87 mV) and achieved the E_{h7min} slower than the other strains (t_{min}: 11·05 h). With regard to the period of collection it can be noticed that the *E. faecalis* with the lowest reduction activity (cluster FS4) decreased from 21·2% (1960–1999) to 9·6% (2009–2012) (Table 3).

Enterococcus faecium

A notable heterogeneity in reduction capability was noticed within the *E. faecium* species (Fig. 2). Five clusters, characterised by different E_h parameters were observed. The highest reducing capacity was determined in *E. faecium* grouped in clusters FM1 and FM2; these strains displayed a moderate reduction power (−181·49 ± 22·28 and −178·74 ± 15·12 mV), and were the most rapid to achieve the E_{h7min} (t_{min}: 3·60 and 3·23 h) but showed different Δ_{max} values, 151·64 and 218·88 mV respectively (Table 2).

Ninety-three *E. faecium* (48%) fell in cluster FM3 and were characterised by a poor reduction power (−91·63 ± 22·96 mV). These strains reduced the E_h slowly reaching the E_{h7min} after 17·45 ± 6·37 h. The *E. faecium* clustered in FM4 (47 strains) showed a moderate reduction capability, with a similar E_h trend than the strains grouped in FM1and FM2, but different values of E_{h7min} (−153·48 ± 18·78 mV) and of Δ_{max}, (84·07 ± 20·87 mV).

The least reducing activity was detected in the 8 *E. faecium* belonging to clusters FM5 (E_{h7min} −6·89 ± 10·63 mV) and 7 out of the 8 strains being isolated from cheeses of Valtellina valley (Bitto and Valtellina Casera). Six strains with different reduction activity did not fall in into any of the clusters (e.g.: E_{h7min} from −234·12 to −27·82 mV). Considering the period of isolation we observed an increase in the percentage of *E. faecium* strains with a low reduction activity (clusters FM3) over the years, while the percentage of high reduction strains decreases (clusters FM1 and FM2) (Table 3).

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Table 3. Percentage distributions of LAB isolated in different periods (from 1960 to 2012) according to cluster analyses

Species	Years	Number of strains (%)	Clusters (%)						Strains not clusterd
			DU1	DU2	DU3	DU4	DU5	DU6	
E. durans	1960–1999	7 (100%)	–	28·6	28·6	–	14·2	28·6	
	2000–2008	36 (100%)	58·3	22·2	8·3	5·6	5·6	–	
	2009–2012	22 (100%)	31·8	9·1	18·2	36·4	–	4·5	1
			FS1	FS2	FS3	FS4			
E. faecalis	1960–1999	33 (100%)	36·3	36·4	6·1	21·2			
	2000–2008	35 (100%)	54·3	20·0	25·7	–			
	2009–2012	125 (100%)	40·0	23·2	27·2	9·6			
			FM1	FM2	FM3	FM4	FM5		
E. faecium	1960–1999	27 (100%)	25·9	11·1	25·9	29·6	7·5		1
	2000–2008	89 (100%)	12·4	8·9	50·6	23·6	4·5		2
	2009–2012	71 (100%)	10·7	2·7	54·7	24·0	7·9		3
			LC1	LC2	LC3	LC4			
L. lactis	1960–1999	16 (100%)	6·3	31·2	56·2	6·3			
	2000–2008	41 (100%)	34·1	39·0	17·1	9·8			
	2009–2012	76 (100%)	40·8	22·4	35·5	1·3			
			ST1	ST2	ST3	ST4	ST5		
S. thermophilus	1960–1999	14 (100%)	50·0	21·4	14·3	14·3	–		
	2000–2008	60 (100%)	24·6	57·4	9·8	1·6	4·9		
	2009–2012	48 (100%)	20·8	72·9	4·2	–	2·1		2

Lactococcus lactis

All *L. lactis* strains had a good reduction capacity (E_{h7min} −219·05 ± 22·44 mV), in fact the mean values of E_{h7min} of the 4 clusters were lower than −200 mV (Table 4). The reduction curves of the strains belonging to the cluster LC2, LC3 and LC4 showed a similar trend, characterised by a different reduction lag-type phase, a rapid decrease of E_{h7} to the minimum value (t_{min}: 3·17 ± 1·23, 4·73 ± 3·14 and 3·58 ± 1·46 h) and by the stabilisation phase (Table 4).

As observed for *E. faecalis* the redox curves of *L. lactis* were very similar to those detected for *E. durans* grouped in cluster DU6 (data not shown). *L. lactis* grouped in cluster LC1 showed a delay in reduction in comparing with the strains of the other the clusters (t_{min} 7·02 ± 4·07 h), due to a more graduate drop of E_{h7}.

Also with regard to this species, it was confirmed that the more recently isolated strains generally showed less reducing activity; in fact, the percentage of *L. lactis* which showed the least reduction activity (cluster LC1; E_{h7min} −210·41 ± 13·83) increased during the considered period (Table 3).

Streptococcus thermophilus

S. thermophilus resulted to be the species with the lowest reduction power (−81·12 ± 32·67 mV) and the slowest to reach the E_{h7min} (t_{min}: 22·07 ± 2·42 h). According to the cluster analysis, the majority of the strains fell in cluster ST2 (73 strains) followed by ST1 (32 strains), and ST3 (10 strains) (Table 4). One strain characterised by a low reduction power did not fall in any cluster (E_{h7min} 129·98 mV; t_{min}: 22·50 h). As showed in Fig. 3, the redox curves of these strains were characterised by low values of Δ_{max}, in fact the E_h decreased slowly reaching the E_{h7min} after more than 18 h. The most reducers *S. thermophilus* were grouped in ST4 and showed a moderate reduction power with a mean Δ_{max} of 50·03 ± 5·52 mV.

Considering the period of collection it is possible to notice a decrease in the percentage of strains with a moderate reduction activity (clusters ST4), while the percentage of *S. thermophilus* grouped in ST2 (E_{h7min} −96·63 ± 13·16 mV) increased (Table 3).

Discussion

The current study provides detailed information about the reducing capability of lactic acid cocci of dairy origin. The majority of wild strains were able to reduce the milk until negative values and a negative E_h is essential for the cheese quality and for the satisfactory development of flavour characteristics of fermented dairy products (Green & Manning, 1982).

Despite the demonstrated effects of the E_h on dairy product quality, LAB are still essentially characterised by their acidification activities and not by their abilities to reduce the E_h of milk (Cachon et al. 2002; Brasca et al. 2007). At the present time the mechanism involved in the decrease of E_h is not completely understood. Jeanson et al. (2009) showed that the E_h of milk dropped only after all of the dissolved oxygen was consumed by *L. lactis*. Successively Tachon et al. (2010) confirmed that the elimination of O_2 is a prerequisite for milk reduction and NADH oxidase (*nox*E) was the enzyme responsible of the rapid removal of oxygen from milk before the exponential bacterial growth phase. Recently Larsen et al. (2015) demonstrated

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Table 4. Kinetic parameters from the reduction rates of *L. lactis* and *S. thermophilus* cultured in reconstituted skimmed milk according to cluster analyses (means values ± SD)

Specie	Parameters	Clusters					
		Total	LC1	LC2	LC3	LC4	
L. lactis	No. of strains	133	46	38	43	6	
	E_{h7min} (mV)	−219·05 ± 22·44	−210·41 ± 13·83	−229·53 ± 21·36	−214·72 ± 23·87	−249·89 ± 24·31	
	t_{min} (h)	5·03 ± 3·43	7·02 ± 4·07	3·17 ± 1·23	4·73 ± 3·14	3·58 ± 1·46	
	Δ_{max} (mV)	213·09 ± 105·35	104·87 ± 25·80	331·74 ± 31·72	195·01 ± 34·49	421·00 ± 10·81	
	t^* (h)	1·21 ± 1·09	1·12 ± 1·30	1·16 ± 0·78	1·35 ± 1·15	1·17 ± 0·68	
		Total	ST1	ST2	ST3	ST4	ST5
St. thermophilus	No. of strains	124	32	73	10	3	4
	E_{h7min} (mV)	−81·12 ± 32·67	−63·41 ± 6·93	−96·63 ± 13·16	−38·53 ± 8·06	−165·62 ± 2·91	0·29 ± 12·44
	t_{min} (h)	22·07 ± 2·42	22·91 ± 1·01	21·79 ± 2·67	22·30 ± 2·74	19·17 ± 4·65	22·38 ± 1·93
	Δ_{max} (mV)	23·63 ± 8·07	22·12 ± 7·38	24·22 ± 6·80	18·20 ± 4·76	50·03 ± 5·52	18·50 ± 5·74
	t^* (h)	3·90 ± 2·97	3·86 ± 3·08	3·45 ± 1·77	5·15 ± 5·63	6·67 ± 6·43	4·75 ± 2·40

Strains not clustered: 2 *S. thermophilus*.

that the decrease in redox potential was highest at the time of oxygen depletion, followed by a more gradual decrease to the final redox values, indicating that both oxygen and biological factors are involved in the reduction process.

In this paper the variation in reduction potential of a considerable number of wild LAB strains present in raw milk cheeses (*E. durans, E. faecalis* and *E. faecium*) or used as starter cultures (*L. lactis* and *S. thermophilus*) was evaluated. It is worthy of note that *E. faecalis, L. lactis* and *S. thermophilus* are characterised by a well defined redox potential in milk in relation to E_{h7min}, while *E. durans* and *E. faecium* showed different main courses. These different behaviours among the species could be linked to the production of menaquinones (vitamin K_2), a vitamin involved in the reduction of E_h of milk, in fact *L. lactis* and *E. faecalis* are menaquinones producers, while *S. thermophilus*, does not synthesises these compounds (Morishita et al. 1999; Tachon et al. 2010).

The lower reduction power detected in *S. thermophilus* species could be linked to a possible no-growth rate of these strains in milk. This hypothesis was not supported by the pH values achieved by the strains at the end of incubation (4·37 ± 0·22, data not shown) and by a study in which was demonstrated that *S. thermophilus* satisfies its amino acid requirement thanks to its efficient biosynthetic capacities (Iyer et al. 2010).

A great heterogeneity in reduction capability was observed in *E. faecium* species, in particular, 54% of strains showed a low reduction power while 46% of *E. faecium* were high-moderate reducers. These data integrate the findings of Brasca et al. (2007) who reported that *E. faecium* was one of the most reducing species among LAB tested. As proposed by Tachon et al. (2010) the different reduction activity inside the bacterial species could be due to activation/inactivation of the *nox*E gene responsible for the rapid removal of oxygen from milk at start of fermentation.

Selective pressures from the environment, and the cheesemaking technology itself, lead to the build-up of biotypes with different characteristics. A pattern in the reduction potential of the strains isolated throughout the years was pointed out: in the considered time span (1960–2012) it is possible to observe an increase in the percentage of strains with lower reduction activity while the number of good reducer strains decreases. This was particularly evident for the species *E. durans, E. faecium* and *L. lactis* while the strains of *E. faecalis* across the same time span showed the opposed property.

Many studies focused the importance to follow the evolution of redox potential during cheese-making, but few studies provided a deeper insight on the contribution of different LAB species to changes in the cheese oxidation-reduction potential and very little information is available on NSLAB reduction activity in raw milk cheeses. Data reported in this research supply new information for the starter and adjunct cultures selection for a better control of the kinetics of reduction in the cheese microenvironment during manufacture and ripening improving the cheese-making processes and the sensory characteristics of dairy products.

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