Biodiversity of Lactococci in Flavour Formation for Dairy Products Innovation

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ABSTRACT

Biodiversity of 37 *Lactococcus lactis* strains, included 31 strains isolated from various environment (dairy and non dairy ecosystems) so-called "wild" strains and 6 industrial strains, in flavour production capacities was investigated in milk cultures. Organoleplic evaluation revealed that many strains produced different flavours distinct from those produced by industrial strains. Most dairy wild strains showed proteolytic activity and were good producers of lactic acids in contrast with non dairy wild strains. Some strains produce bacteriocin-like compound and exopolysaccharides. GC-MS analysis showed a variation between strains for formation of flavour compounds which correspond to the sensory descriptions. The major volatile compounds produced by strains were derived from amino acids. Since various LAB differ in amino acid converting abilities (leading to flavour components) and these activities are linked to the ability to synthesize amino acids, the amino acids-forming capacities of strains were determined. The results showed that the wild strains had much larger potential to synthesize amino acids as compared to industrial strains. Some strains were selected and applied in Domiati cheese. A good correlation between organoleplic description and GC-MS volatile compounds of cheese samples and milk cultures prepared with the same strain was found. The natural biodiversity found within the strains reflect their functional diversity and offers good possibilities for flavour diversification and product innovation. This knowledge with genome data which will become available for various LAB allows prediction of flavour forming capacity and leading to the design of improved tailor-made industrial cultures with attractive or selected flavour.

Key Words: Lactococcus lactis, natural biodiversity, wild lactic acid bacteria, flavour formation, sensory evaluation, GC-MS analysis, volatile compounds,* Domiati *cheese.

INTRODUCTION

Natural biodiversity exists within the group of LAB used in milk fermentations; this offers many opportunities for exploitation aiming at changes in product characteristics, such as flavour. Some important (metabolic) characteristics are nutritional requirements, temperature sensitivity, bacteriocin production (Boutibonnes *et al*., 1995, Allison *et al*., 1998, Hyronimus *et al*., 2000). In addition to the chromosomal DNA, most LAB carry plasmid DNA, which may also code for several for fermentation relevant characteristics, such as lactose and citrate metabolism, cell envelope proteinase and antibiotic resistances (Libudzisz *et al*., 1991, Perreten, 1996). *Lactococcus* is the main species of commercial interest and is the most frequent used organisms in cheese manufacture. Their anabolism, abilities to produce metabolites like vitamins and amino acids are very limited and therefore, these substrates are preferentially taken up from the environment. The essential amino acids is found to vary, *L. lactis* subsp. *cremoris* have more requirements than those belonging to *L. lactis* subsp. lactis (Otto, 1981, Keefe *et al*., 1995). Lactococci are generally associated with the milk environment (Sandine *et al*., 1972) but they can be isolated from other sources such as artisanal manufacture of fermented dairy product without the application of industrially prepared starter cultures and from non-dairy environments which are generally referred to as "wild" lactococci (Cogan *et al*., 1997). The pool of this bacteria contained many *L. lactis* strains which differ in a number of properties from the strains commonly present in industrial starters (Klijin *et al*., 1995, Ayad *et al*., 1999).

The flavour in fermented dairy products (e.g., cheese) is a result of a series of biochemical processes in which the starter cultures play a key role. Three main pathways can be identified; the conversion of lactose (glycolysis), fat (lipolysis) and casein (proteolysis). The enzymes involved in these pathways are derived from the starter cultures used

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in these fermentations, although also endogenous milk enzymes and/or added enzymes (rennet) can play a role (Urbach, 1993, Visser, 1993, Fox, *et al*., 1996). In the case of the lactose fermentation by L. *lactis*, the conversion leads to the formation of lactate, but a fraction of the intermediate pyruvate can alternatively be converted into flavour compounds such as diacetyl, acetoin, acetaldehyde and ethanol or acetic acid. Lipolysis results in the formation of free fatty acids, which may act as precursors for other flavour compounds such as methylketones, alcohols and lactones. The degradation of casein is the main pathway leading to flavour formation. Proteolysis and peptidolysis is a prerequist to generate free amino acids and the balance between these two activities is important in order to generate the desired (substrate) amino acids and to prevent accumulation of bitter-tasting peptides (Kranenburg *et al*., 2002). The volatile flavour components, which predominantly determine the overall flavour, are subsequently derived from the activity of enzymes converting amino acids (Yvon *et al*., 1998, Smit *et al*., 2000, Yvon & Rijnen 2001). In the LAB strains the physiological functions of these enzymes are most likely the biosynthesis of amino acids. Based on this, a strong regulation is anticipated and found for these enzymes. This characteristic is very important for practical applications, since it means that the activity of starter cultures can be influenced by the cultivation conditions and leading to natural biodiversity (Smit *et al*., 2002, Engles *et al*., 2003).

Domiati cheese is the most popular soft white pickled cheese in Egypt which can be consumed fresh or after pickling in salted whey or a brine solution for up to 2-4 months (Abd El-Salam *et al*., 1976, Abou-Donia, 1986). It is made from buffalo's or cow's milk or mixture of them; cheese process was described by Fahmi & Sharara (1950) and recently reviewed by Abou-Donia (2007). The salt concentration used in the manufacture (5 to 14%) depends on the season and the ripening conditions.

Nowadays consumer demands a large variation in flavour of dairy product, this has led to a request for novel strains, which can be achieved either by genetic modification of known strains or by exploring the biodiversity within natural strains from various ecological systems. The introduction of new microbial strains in cheese making is a powerful tool to change product characteristics, in Gouda and Proosdij cheeses produced with non-dairy LAB (wild strains) large differences in flavour formation were observed (Ayad *et al*., 2003a).

The aim of this work was to study the natural biodiversity of a large numbers of *Lactococcus lactis* strains isolated from various dairy and non dairy ecosystems in flavour production capacities and function properties. Moreover, to exploit these strains for flavour diversification and Domiati cheese innovation.

MATERIAL AND METHODS

Bacterial strains and growth conditions

Thirty seven *Lactococcus lactis* strains used in this study (21, dairy wild strains (DWS), 10 non-dairy wild strains (NDWS) and 6 industrial strains) of which their source of isolation are listed in Table (1). The strains were obtained from the culture collection of NIZO Food research, Ede, The Netherlands (B, code numbers) and from Ayad, (2004) (E, code numbers). The strains were cultivated in M17 medium (Oxoid, Hampshire, UK) and were stored in litmus milk with 0.1% yeast extract at - 40°C.

Flavour production in milk cultures

Individual strains were pre-grown for 16 h at 30°C in sterilised milk containing 0.1 % yeast extract, 1% of each culture was added to 100 mL skimmed UHT milk. Sensory evaluation was carried out after incubation at 30°C for 48 h. by 7 to 10 graders.

Characteristics and amino acids requirements of strains

Acidification activity, the ability to hydrolyse casein and the ability to grow at various temperatures (10, 30 and 40°C) and in the presence of 3, 4 and 5% NaCl, were tested. Antimicrobial activity was determined in agar well-diffusion assay against two target organisms either *Micrococcus flavus* NIZOB423 or *L. lactis* subsp. *cremoris* NIZO SK110, as described by Ayad *et al*, (2000). Strains were tested for exopolysaccharides (EPS) production using the inoculated loop method (Knoshaug *et al*, 2000). The amino acids requirements of strains were determined using the single omission technique (Cocaign-Boosquet *et al*, 1995) in a chemically defined medium as described by Otto *et al*, (1983) and modified by Poolman & Konings (1988).

Cheese making and analysis

Domiati cheese was made as described by Fahmi & Sharara (1950) from a mixture of fresh cow's and buffalo's milk (1:1), the milk was heated to

65°C for 30 min. and cooled to 35°C. Then 0.02% calcium chloride and 5% salt was added before renneting. The milk was divided into seven equal parts; the first one was used as control and the second one was inoculated with 2% of industrial strain B697. The other parts were inoculated with 2% E13, B1152, B1153, B1156 and B1157, as a single strain. Individual strains were pre-grown for 16 hr at 30°C in low-fat milk with 0.1% yeast extract and the culture was inoculated directly into processed milk via direct vat inoculation (DVI). Inoculated milks were held for 45 min before the addition of rennet (powder, Chr. Hansen's Denmark). The obtained cheese was pickled in their whey and kept at 10°C for 3 months. The sensory evaluation was carried out according to Ayad *et al*., (2003b) and the averages data with standard deviations were determined. Compositional analysis for fat, salt, pH, titratable acidity and moisture of cheeses were performed according to IDF Standards (1997, 1979, 1989 and 1982, respectively). Total nitrogen (TN) and soluble nitrogen (SN) was determined according to Noomen, (1977). All analysis were carried out in duplicate

Analysis of volatile compounds

Volatile compounds formed by the cultures in milk and in cheese were identified using purge-and-trap thermal desorption cold-trap (TDCT) gas chromatography mass spectrometry (GC-MS) (Neeter & De Jong 1992). Ten ml of the milk cultures was used directly and 20 ml of cheese slurry, obtained by homogenization of a mixture of cheese and double-distilled water (1:2 w/v) was prepared and used immediately after the preparation. The conditions for the chromatographic separation and mass spectrometry have been used as described by (Engels *et al*, 1997).

RESULTS AND DISCUSSION

Flavour production

Thirty seven *Lactococcus lactis* strains; 6 industrial strains, 21 DWS and 10 NDWS were screened for their flavour-producing capacity in milk (Table 1). There was a diversity in sensory evaluation was found between strains in milk cultures. Ten wild strains (9 DWS and 1 NDWS) appeared to produce flavours looks similar to those produced by the industrial strains; e.g., yoghurt, butter milk-like, creamy, sour and slightly sweet. The majority of wild strains (12 DWS and 9 NDWS) exhibited more pronounced sensory characteristics, specific (unusual/ new) flavours as compared to industrial starter strains. Descriptors such as yeasty, farm cheese-like, chocolate, malty, fatty acid, grass, sharp, diacetyl, esters, fruity, Laban Rayeb-like, herbs, sulphur etc. were mentioned by the sensory panel (Table 1 and Fig. 1). These results agree with Weerkamp *et al*., (1996) and Ayad *et al*., (2000), they found that wild lactococci strains produced specific flavours distinct from those produced by commercial strains.

Based on sensory evaluation and chemical analysis of dairy product (e.g. cheese), various groups of volatile compounds have been identified as being responsible for the final taste and aroma and listed in the database (Urbach, 1995, Nijssen *et al*., 1996). Description of some important key-flavours and their correspond metabolism are given in Table 2 (Badings, 1984, Griffith & Hammond 1989). Most of these flavours were found and mentioned by the sensory panel in the present study.

The production of volatile compounds during growth of some selected strains in milk was examined. Eight strains; E13, E16, B1152, B1157 (DWS) and B1153, B1156 (NDWS) and B697 and B14 (industrial strains) representative for the broad range of different flavours were selected for GC-MS analysis. As examples of variation in flavour production capacities of *L. lactis* strains, for the formation of a number of flavour components is shown in Fig. (2). Milk cultures prepared with strains E13, B1152, B1157 contained high levels of 2-methylbutanol and 3-methylbutanol and aldehydes, 2-methylpropanal, 3-methylbutanal in comparison with B697 and B14. Methylalcohols and methylaldehydes are most likely derived from the branched-chain amino acids leucine, isoleucine and valine (Molimard & Spinnler, 1996). Branched-chain alcohols give rise to a slightly sweet, fresh flavour. Methylaldehydes developed in raw milk by the metabolic activity of *L. lactis* subsp. *lactis biovar maltigenes* have been recognized as off-flavours in Cheddar cheese (Morgan, 1976). On the other hand, 3-methylbutanal has been found as major volatile compounds during ripening of Proosdij and Parmesan cheese, which are responsible for a spicy, cocoa flavour (Barbieri *et al*., 1994). Chocolate flavour was encountered during the organoleptic evaluation of milk incubated with E13, E16, B1152, B1157 and B1153, and was not with

Table 1: Flavour profile and some characteristics of strains

Strain	Subsp	Source[a]	Flavour description[b]	Acidification Activity (unit°N)[c]	Proteolytic activity[d]	Aitimicrobial activity
Industrial:						
B14	*lactis*	Commercial starter	Mild yoghurt, flat	50	+	-
B20	*lactis*	Commercial starter	Yoghurt, creamy, slightly sweet	52	+	-
B22	*lactis*	Commercial starter	Yoghurt, flat, acid (1)	49	+	-
B64	*cremoris*	Commercial starter	Mild yoghurt, slightly sour	40	+	-
B442	*cremoris*	Commercial starter	Yoghurt, acid (1)	41	+	-
B697	*cremoris*	Commercial starter	Yoghurt, sweet (1), butter milk, sour (1)	45	+	-
Dairy wild strains:						
E1	*lactis*	Raw cow milk (Eg)	Yoghurt-lik, creamy (1)	23	-	+
E2	*lactis*	Raw buffalo milk (Eg)	Flate, fermented milk-like	26	-	+
E4	*lactis*	Raw cow milk (Eg)	Sweet (2), creamy, flat	28	±	+
E7	*lactis*	Kariesh cheese (Eg)	Farm cheese-like (2), sharp	27	±	+
E9	*lactis*	Raw buffalo milk (Eg)	Mild yoghurt, sour (2)	39	+	+
E11	*lactis*	Raw buffalo milk (Eg)	Sweet (1), yoghurt-like	26	-	+
E13	*lactis*	Raw buffalo milk (Eg)	Chocolate (1), sour, yeasty (1), bitter (1)	24	-	-
E14	*lactis*	Kariesh cheese (Eg)	Farm cheese-like (1), sweet, creamy	25	-	+
E15	*cremoris*	Raw buffalo milk (Eg)	Mild yoghurt, sour (1), coarse	38	+	-
E16	*lactis*	Raw cow milk (Eg)	Malty (1), chocolate (1), fatty acids	22	-	-
E17	*lactis*	Raw buffalo milk (Eg)	Acid (1), grass, bitter (1), chocolate (1)	31	+	-
E18	*cremoris*	Kariesh cheese (Eg)	Yoghurt, acid (1), coarse	21	+	-
FAAU 1M[e]	*lactis*	Ras cheese (Eg)	Yeasty, sharp, acid (3), cheese-like	35	+	+
FAAU 6M[e]	*cremoris*	Ras cheese (Eg)	Diacetyl, ester, fruity (1)	30	+	-
FAAU67L[e]	*lactis*	Ras cheese (Eg)	Sweet (1), Laban Rayeb-like	32	+	+
B1152	*lactis*	Raw cow milk (Ne)	Cocoa, sour, ester, herbs	50	+	+
B1155	*lactis*	Raw cow milk (Ne)	Yoghurt, creamy (1), smooth structure	22	-	-
B1157	*cremoris*	Raw sheep milk (Sp)	Fruity, creamy, sour (1), flowery,chocolate	21	-	-
B1158	*lactis*	Raw goat milk (Fr)	Chocolate (1), sour, yeasty, bitter (1)	24	-	-
B1162	*lactis*	Raw goat milk (Fr)	Sour (1), malty (1), bitter (1), grass	33	+	-
B1167	*lactis*	Fermented milk (It)	Sweet, chocolate (1), malty	24	±	-
Non-dairy wid strains:						
B26	*lactis*	Chinese radish seed	Yoghurt-like, creamy (1)	25	-	+
B1153	*cremoris*	Milk machin (Ne)	Slightly chocolate, mild yoghurt, creamy	22	-	+
B1154	*lactis*	Soil (Ne)	Fruity, acid (1), faint, chocolate (1),sweet	22	-	+
B1156	*lactis*	Grass (Ne)	Fruity, sweet (1), sour (1), faint	22	-	+
B1159	*lactis*	Milk machin (Ne)	Sculpture, sharp smell, sweet (1)	24	-	+
B1171	*lactis*	Silage (Ne)	Slightly chocolate, fresh yoghurt, sweet	23	-	-
B1172	*lactis*	Silage (Ne)	Fruity, flat, sweet, creamy	22	-	-
B1173	*lactis*	Silage (Ne)	Fruity, sweet, flowery, dry grass	21	-	-
B1174	*lactis*	Silage (Ne)	Yoghurt, chocolate (1),fatty acids,creamy	23	-	-
B1175	*cremoris*	Soil (Ne)	Mild yoghurt, sour (1), coarse	21	-	-

[a] (Eg), Egypt; (Ne), The Netherlands; (Sp), Spain; (Fr), France; (It), Italy.
[b] Flavour intensity on scale from (1-4): 1: slightly, 2: moderate, 3: strong, 4: very strong.
[c] The acidity is expresed as degree N (the number of mL 0.1 N NaOH to neutralize 100 mL of milk).
[d] +, proteolytic; -, not proteolytic; ±, weakly proteolytic.
[e] Data from Ayad *et al*., 2006.

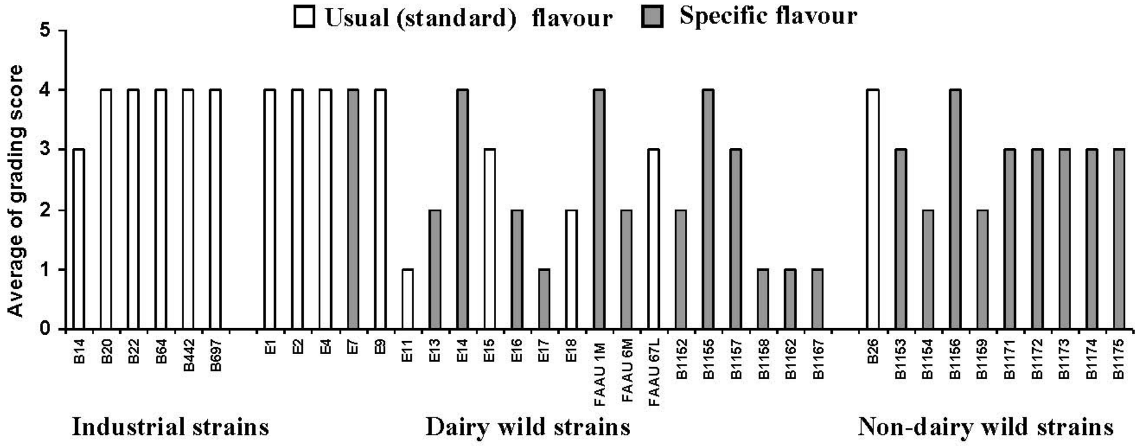

Fig. 1: Diversity in sensory evaluation of strains in milk culture. The grade on scale ranged from 1 to 4: 1, bad; 2, sufficient; 3, good; 4, very good. The results are means with standard deviation ranged from 0.1 to 0.5

B1156 and industrial strains (Table 1). The concentration of the different ketones also varied between the different samples. Diacetyl comes from citrate conversion and is responsible for a creamy flavour (Welsh *et al*., 1989). Some differences in levels of ethylesters were also encountered. These compounds, are formed by an enzymic or chemical reaction of fatty acids with primary alcohols, give a fruity and sweet character to cheese (Table 2).

Characteristics and amino acids requirements of strains

The strains were tested for various technological properties which are important for cheese-making (Table 1). About 56% of DWS and all NDWS showed low acidification activity. Fifty seven percent of DWS were able to hydrolyse milk proteins, while all NDWS showed no proteolytic activity. The results also showed that wild strains differ in a number of properties from the strains commonly present in industrial strains. All wild strains (subsp. *cremoris* and *lactis*) were able to grow at 40°C and in the presence of 5% NaCl in contrast to the industrial starters. Member of the subsp. *cremoris* are not able to grow under these condition (Salama *et al*., 1991, Cogan *et al*., 1997). These results agree with previous findings and seems to indicate that the phenotypical characteristics of *L. lactis* subsp. *cremoris* strains is confined to industrial starter cultures (Klijn *et al*., 1995, Weerkamp *et al*., 1996). The ability of the wild strains to grow at 40°C and in the presence of 5% NaCl could be functional for application in cheeses which are contain relatively high salt concentrations and cooked to high temperatures. One DWS B1155 was EPS (slim)-producer, this result confirmed the sensory evaluation (Table 1) since the milk culture prepared with this strain was described as smooth structure. The EPS-forming LAB have been used in the dairy industry as a natural biothickner to enhance the rheological quality of low fat cheese and in fermented milks (Hassan *et al*., 2004). Thirteen strains (8 DWS and 5 NDWS) appeared to have antimicrobial activity against the indicator organisms. Since many LAB are able to produce bacteriocins or bacteriocin-like substances (Jack *et al*., 1995), these antimicrobial activities are likely to be a consequence of bacteriocin production. Genetic studies using RAPD classification confirmed these results and revealed that wild lactococci had profiles different from those of reference (industrial) strains (Corroler *et al*., 1998). It is thus possible that natural habitant, including raw milk harbour lactococici with potential application in producing fermented dairy products.

Lactococci require various amino acids for growth because of their limited biosynthetic capacity. The requirement of a certain amino acid can result either from the absence of functional genes for specific biosynthetic reactions or from specific regulatory mechanisms (Chopin, 1993). The numbers of essential amino acids is strain dependent and vary from 6 for *L. lactis* subsp. *lactis* up to 14 for *L. lactis* subsp. *cremoris* strains (Mittchell *et al*., 1941, Reiter & Oram, 1962). Since several wild strains produced relatively high levels of primary alcohols and branched aldehydes in the milk

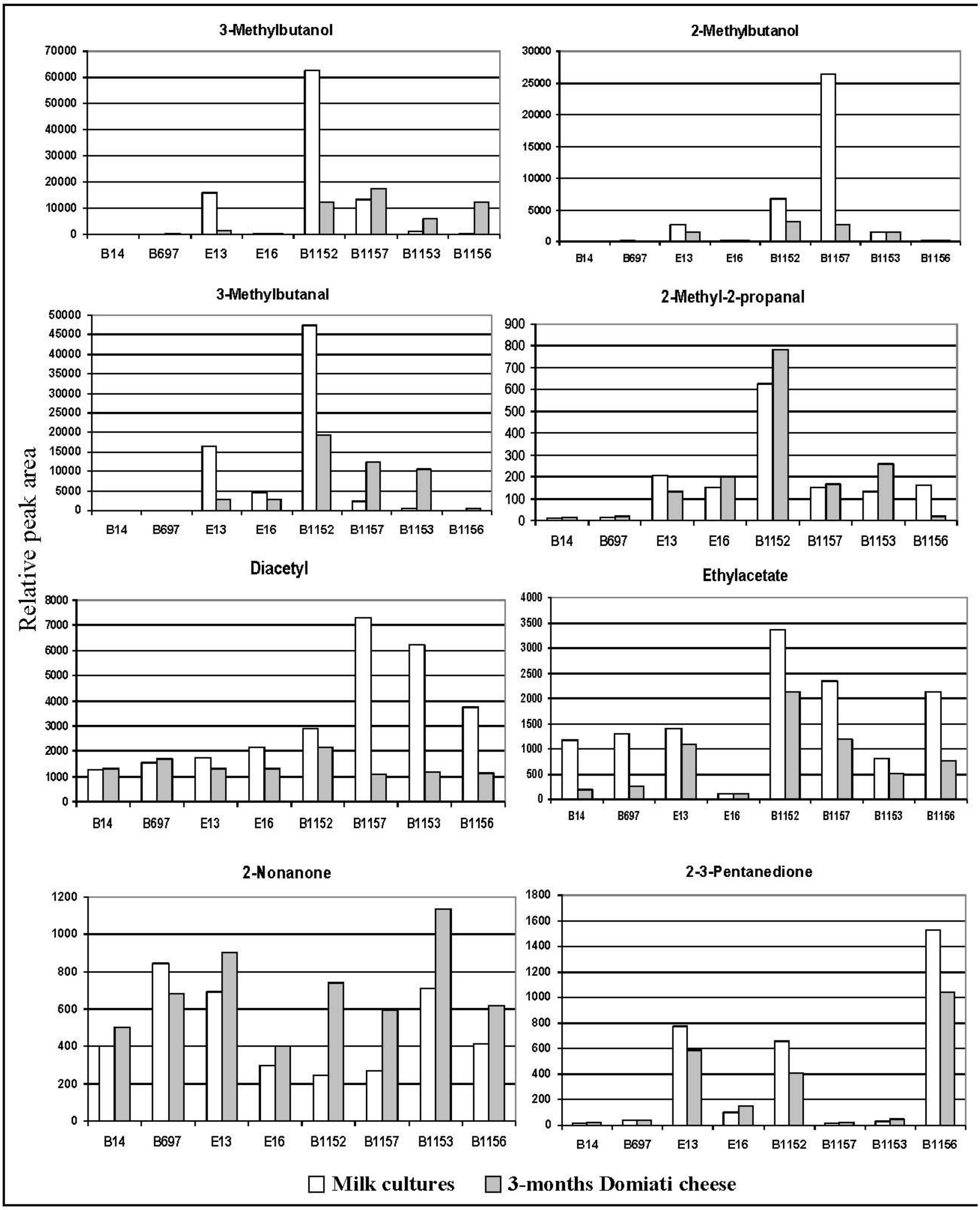

Fig. 2: Diversity in flavour production capacities in *Lactococcus lactis* strains in milk cultures and in Domiati Cheese

Table 2: Description of some important key-flavours and their correspond metabolism[a]

Description	Flavour compound	Metabolism
Chocolate-like, malty, banana	2-Methylpropanal	Amino acids (Ileu)
Malty, cheese	3-Methylbutanal	Amino acids (Leu)
Fresh cheese, alcoholic	3-Methylbutanol	Amino acids (Leu)
Cheese, sweet, rancid	3-Methylbutyric acid	Amino acids (Leu)
Sweet, cheese, strong, acid, butter	Butyric acid	Fat
Sour milk, cheese	Proponic acid	Sugar
Fruity, buttery	Ethylbutanoate Ethyl-3-methyl-butanoate	Combined pathway
Fruity, sweet, yeasty	Ethylesters	Combined pathway
Buttery, strong	Diacetyl	Sugar
Yoghurt, green	Acetaldehyde	Sugar

[a]From Badings (1984), Griffith & Hammond (1989).

cultures, which originate from amino acid degradation, wild strains may have different amino acid requirements than industrial strains. The amino acid requirements of 6 selected strains were compared with the two industrial strains (Table 3). The results showed that industrial strains, B14 subsp. *lactis* and B697 subsp. *cremoris* required 6-9 amino acids; glutamate, leucine, valine, methionine, histidine, proline, phenylalanine, isoleucine and serine, for growth. These results are in agreement with previous studies (Ayad *et al*., 1999). Both dairy and non-dairy wild strains required less amino acid than the industrial strains. The subsp. *lactis* strains E13, E16, B1152 and B1156 required only two amino acids; glutamate and valine. While subsp. *cremoris* strains required from 3 to 4 amino acids for their growth, glutamate, valine, leucine for B1152, glutamate, valine, leucine and methionine for B1157. Wild strains are more dependent on their own synthesis of amino acids compared to industrial strains and probably harbour more active amino convertases. Since these enzymes play a key role in the formation of amino acids derived flavour components, this pool of strains is interesting with respect to novel flavour formation in the manufacture of fermented dairy products.

Cheese making and analysis

Domiati cheese was made with lactococci strains; E13, B1152, B1157 (DWS), B1153, B1156 (NDWS), B697 (industrial strain) and without starter (control). The values of pH, titratable acidity, moisture, fat, salt and soluble nitrogen are summarized in Table (4). There was no apparent difference in cheese composition between control cheese and other cheeses, as the levels are within margins for composition of Domiati cheese (Abou-Donia, 1986, Egyptian Standard for Domiati Cheese, 2005). During ripening the titratable acidity of all cheese samples increased while the pH values decreased and cheese samples made with lactococci strains had higher pH values than control. Moisture content of cheese decreased and fat content increased along the storage period. The addition of strains to cheese milk had no effect on moisture, fat and salt content. The results showed that SN and SN/TN increased in all cheese samples with added lactococci strains as compared to the control till the

Table 3: Amino acids essential to industrial and wild strains

	Industrial		wild strains					
	subsp *lactis*	*subsp cremoris*	subsp *lactis*				subsp *cremoris*	
	B14	B697	E13	E16	B1152	B1156	B1153	B1157
Number of essential amino acids	6	9	2	2	2	2	3	4

Table 4: Chemical composition of cheese during storage at 10°C

Samples	pH	Titratable acidity %	Moisture	Fat (%)	Salt (%)	SN (%)	SN/TN (%)
Control :							
Fresh	6.48	0.31	62.25	18.3	5.1	0.19	8.14
3 months	5.3	0.63	59.51	22.3	3.2	0.59	24.08
B697:							
Fresh	6.25	0.42	62.1	18.2	5.2	0.24	9.88
3 months	4.65	1.32	59.64	22.1	3.1	0.68	26.77
E13:							
Fresh	6.34	0.4	62.21	18.1	5.1	0.22	9.32
3 months	4.73	1.1	60	22	3	0.65	26
B1152:							
Fresh	6.27	0.43	62.3	18.3	5.2	0.25	10.25
3 months	4.66	1.31	60.01	22.2	3	0.69	27.27
B1157:							
Fresh	6.35	0.38	62.3	18.1	5.1	0.2	8.51
3 months	4.8	0.95	59.8	22.1	3.2	0.65	25.9
B1153:							
Fresh	6.36	0.37	62	18.2	5.2	0.21	8.89
3months	4.78	0.97	59.37	22.3	3.1	0.64	25.7
B1156:							
Fresh	6.33	0.4	62.24	18.2	5.1	0.21	8.86
3 months	4.75	1.0	59.43	22.4	3	0.66	26.27

S.N.: Soluble Nitrogen. T.N.: Total Nitrogen.

end of ripening, El-Abd, *et al*., (2003) and Salama, (2004) reported similar results. The rate of increase was more in cheese made with B697 and B1152 as compared with the others which was may be due to its proteolytic activity.

The sensory results showed that the presence of lactococci strains raised the score of resulting Domiati cheese than control cheese (Table 5 and Fig. 3). All cheese samples had good texture characteristics and appearance, not noticeably different from the control cheese. The wild strains produced flavours in cheeses distinct from that produced by industrial strain. The flavours mentioned by the sensory panel are in agreement with those encountered in milk culture prepared with the same strain (Table 1). These results indicate that wild strains are able to produce specific flavour characteristics in Domiati cheese.

The volatile compounds produced in 3-months old cheeses were identified using GC-MS. Many different compounds were detected and characterized in the cheeses; each culture produced a typical pattern of volatile compounds which matched with the sensory flavour descriptions Fig. (2). As an example, the GC-MS aroma profiles of cheese prepared with industrial strain (B697), DWS (B1152) and NDWS (B1156) are presented in Fig. (4). Cheeses manufactured with B1152, B1153 and B1157 contained high levels of methylaldehydes (2-methylbutanal, 3-methylbutanal and 2-methyl-propanal) which linked to the chocolate/cacao and malty flavours in these cheeses. These compounds have been recognized as key flavour compounds in some cheese types (Bosset & Gauch, 1993). Some cheeses, considered to show fruity, sweet and yeasty flavours (Table 5), contained different levels of ethylesters (ethylacetate, ethylbutanoate and 3-methylbutylacetate). These results are in accordance with the sensory evaluations. Taken all together the results indicated that the use of wild lactococci strains as starters for the development of new (specific) flavours looks promising. It was found that the use of selected wild LAB strains in cheese resulted in an increase in the key flavour production, the intensity of the cheese flavour and lead to tailor the flavour of cheese (Ayad *et al*., 2003a).

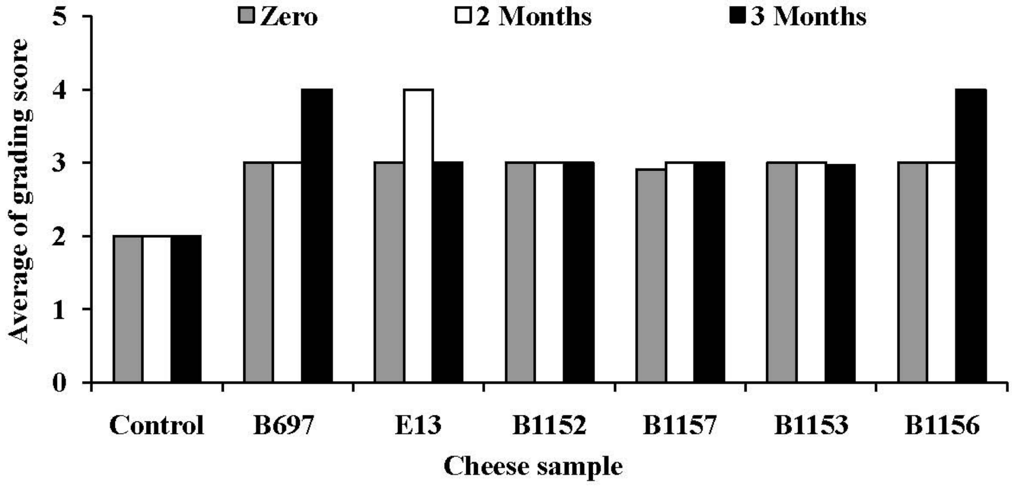

Fig. 3: Flavour of Domiati cheese during ripening time. The grade on scale ranged from 1 to 4: 1, bad; 2, sufficient; 3, good; 4, very good .The results are means with standard deviation ranged from 0.1 to 0.6.

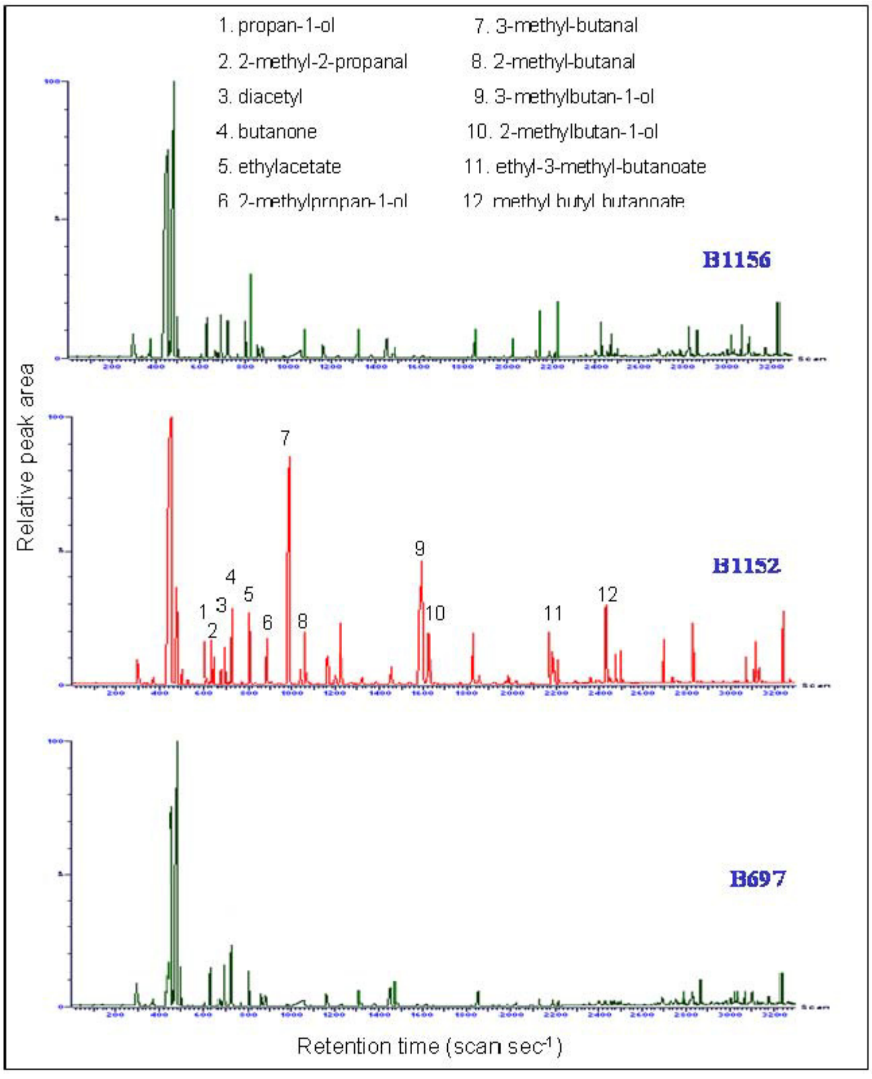

Fig. 4: GC-MS aroma profiles of volatile compounds purged from 3-months old Domiati cheese prepared with industrial strain (B697), DWS (B1152) and NDWS (B1156)

Table 5: Sensory evaluation of 3-months Domiati cheeses prepared with lactococci strains

Cheese sample	Grade[a]	Description of Flavour (intensity)[b]	Overall Grade (100)
Control	2±0.6	flat flavour	78±2.5
B697	4±0.5	Creamy (1), sour (1)	85±3.2
E13	3±0.2	Malty (1), farm cheese-like, fatty acids	84±4.1
B1152	3±0.5	Chocolsate (1), fruity (1), new flavour	81±2.5
B1157	3±0.4	Fruity (1), yeasty (1), chocolate (1)	83±3.4
B1153	3±0.3	Malty (1), sharp (2), strong	80±5.1
B1156	4±0.6	Fruity (1), sweet (1), sharp	86±4.5

[a]Grade of flavour (1-4): 1: bad, 2: sufficient, 3: good, 4: very good.

[b]Intensity (1-4): 1: slightly, 2: moderate, 3: strong, 4: very strong.

CONCLUSION

The results of the present study showed that the flavour forming abilities vary considerably within the species of *Lactococcus lactis*, various strains isolated from dairy and non-dairy environments have the ability to produce flavour components distinct from industrial cultures. This might be explained by the presence of specific amino-acid-converting enzymes present in these strains and/or differences in their regulation. This hypothesis is supported by the observation that several wild lactococci strains required only a few amino acids for growth, indicating that these strains harbour more amino acids converting enzymes, since these enzymes are primarily involved in synthesis of amino acids, rather than degradation. In conclusion the large natural biodiversity that is found within lactococci strains offer good possibilities for flavour diversification for consumer demands.

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دراسة الإختلافات الحيوية لسلالات lactococci فى تكوين النكهة لتحديث المنتجات اللبنية

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يهدف هذا البحث إلى دراسة الإختلافات الحيوية لعدد ٣٧ سلالة من *Lactococcus lactis* تشمل ٣١ سلالة معزولة من مصادر مختلفة (لبنية وغير لبنية) والتى تسمى "wild" وعدد ٦ سلالات صناعية (تجارية) وذلك لقدرتها على تكوين النكهة فى اللبن. أوضحت نتائج تقييم الخواص العضوية والحسية أن بعض السلالات أنتجت طعوماً مختلفة عن الطعوم المتكونة بواسطة السلالات الصناعية. وأن معظم السلالات المعزولة من مصادر لبنية لها نشاط بروتيوليتي وكذا لها مقدرة على إنتاج جيد لحمض اللاكتيك بالمقارنة بالسلالات المعزولة من مصادر غير لبنية. وكانت بعض السلالات قادرة على إنتاج مركبات مضادة للنمو البكتيرى وسكريات عديدة خارجية. كما أوضحت نتائج التحليل بجهاز كروموتوجرافيا الغاز- الطيف الكتلي (GC-MS) أن هناك إختلافات بين السلالات فى تكوين مركبات النكهة والتى تتمشى مع نتائج التقييم الحسى وغالبيتها مشتقة من الأحماض الأمينية. بما أن العديد من سلالات بكتريا حمض اللاكتيك مختلفة فى مقدرتها لتحول الأحماض الأمينية إلى مشتقاتها (المسئولة عن تكوين مركبات النكهة) وهذا النشاط مرتبط بالمقدرة على إنتاج أو تكوين الأحماض الأمينية لذا تم إختبار مقدرة السلالات لإنتاج الأحماض الأمينية. وأوضحت النتائج أن للسلالات مقدرة عالية على تكوين الأحماض الأمينية بالمقارنة بالسلالات المرجعية. وتم إنتخاب بعض السلالات لتطبيقها فى الجبن الدمياطى وكان هناك تطابق فى نتائج التقيم الحسى ومركبات النكهة الناتجة المتعرف عليها بجهاز GC-MS لعينات الجبن والمزارع اللبنية المحضرة بنفس السلالة. هذه الإختلافات الطبيعية التى وجدت بين السلالات تعكس اختلافاً فى وظائفها وتسمح بإمكانية جيدة لطعوم مختلفة وتحديث للمنتجات اللبنية. هذه المعلومات مع توافر المعلومات الوراثية التى هى متاحة الآن للعديد من LAB تسمح بتحميل المقدرة على تكوين الطعوم وتوفير مزارع بادئات صناعية محسنة لإنتاج أطعمة مرغوبة.