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Detection and isolation of lactic acid bacteria and its use as local starters in Syrian Akawi cheese processing

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Abstract

Lactic Acid bacteria can be considered as the most important bacteria in the dairy products due to its vital effects and its portion of the total flora. The main objective of this study was to isolate and classify the Lactic Acid bacteria from Syrian Akawi cheese samples. 27 isolates were isolated from 30 samples collected from Syrian cities, country side and urban markets in 2011. These bacterial isolates were identified using the API Biochemical test. The dominant bacteria was *Enterococcus* genus with 40.7%, followed by *Lactobacillus* 37.1%, *Lactococcus* 14.8% and *Leuconostoc* 7.4%. In order to form three starters to be used in Akawi cheese processing, many strains were used and these were *Lc. lactis* ssp. *lactis, Lc. lactis* ssp. *cremoris*, *Lb. bulgaricus, Lb. paracasei,* and *E. faecium*. Samples sensory evolution including physical appearance, colour, impurities, taste, smell and texture were carried out. The results showed that the best conventional samples were with starters made from the strains *Lc. lactis* ssp. *cremoris, Lb. bulgaricus* and *E. faecium*, and in lower level the strains *Lc. lactis* ssp. *lactis, Lb. bulgaricus* and *Lb. paracasei*. and last the strains *Lc. lactis* ssp. *lactis, Lb. bulgaricus* and *E. faecium.*

Introduction

Cheese is considerable and suitable feed medium for microorganism's growth and construction and configuration elements. the microorganisms presence has great importance in cheese, and in particular Lactic Acid bacteria. A Lactic Acid bacterium produces high proportion of lactic acid from lactose fermentation as well as flavor materials (Corfield *et al.*, 2001; Settanni and Corsetti, 2008). These bacteria includes various species with different physiological characteristics (Hernandez *et al*., 2005), Gram-positive, aerobic, anaerobic and non-animated except *Lactobacillus agilis* and *Lactobacillus ghanensis* (Nielsen *et al*., 2007) and *Lactobacillus capillatus* (Chao *et al*., 2008), non-spore, non-producing catalase enzyme (this enzyme releases H_2O and O_2 from H_2O_2), and has a low content of guanine and cytosine. Morphologically, these bacteria species are existed as single or double coccus and bacillus in short and long strings (Kandler and Weiss, 1986), as well as the fermentation of carbohydrate is undergone anaerobic conditions (Salminen and von Wright, 1998). Lactic acid bacteria species have many roles in cheese manufacture, some species produce high levels of lactic acid by fermenting lactose, which called (SLAB- Starter Lactic Acid Bacteria), other species contribute in ripening stage and known as (NSLAB- Non Starter Lactic Acid bacteria) (Antonsson *et al*., 2003).

In general, lactic acid bacteria possess a complicated protein enzyme system due to amino acid requirement (McSweeney, 2004). The bacteria obtain free amino acid from alkazin protein and peptides which contribute directly in cheese basic test and indirectly in cheese flavor (Fox and Wallace, 1997). In cheese manufacture, adding defined strains of *Lactobacillus* to milk produces free amino acids in high levels causing increase in cheese flavor (Lane and Fox, 1996; Ur-Rehman *et al*., 2000).

Remarkably adding *Lb. paracasei* to starters used in the manufacturing of Italian cheese causes similar increase of free amino acids content compactable to the results of market starters (Poveda *et al.*, 2004), the usage of *Lb. casei* subsp. *rhamnosus* and *Lc. lactis* effectively supports proteolysis during low-fat Italian cheese ripening (Michaelidou *et al.*, 2003). Non starter lactic acid bacteria *Lb. paracasei* and *Lb. plantarum* have synergistic action with *Lactococcus* (Ortigosa *et al*., 2006), therefore the various effects

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of lactic acid bacteria depend on starter type (Skeie *et al*., 2008).

Lactic acid bacteria may have many additional functions such as, producing bio peptides by proteolysis process, producing antibacterial substances that prevent unpreferable microorganisms activity (Siragusa *et al*., 2007; Izquierdo *et al*., 2009; Wang *et al*., 2010), producing acetaldehyde – De acetyl, as a flavor component (Fox *et al*., 1993), increasing pH to be unsuitable to unpreferable and corrupted bacteria (Nascimento *et al.*, 2008).

Many studies carried out about Turkish white cheese manufactured from raw milk, in order to isolate lactic acid bacteria strains, the studies proved that the existence of lactococcus was slightly higher than lactobacillus and then gradually decreased during the ripening stage of lactococcus, as in yogurt, because of the final product acidity which helped the bacteria tolerant acidity lactobacilli to dominate and lactococcus to disappear in the final product (Karakus *et al*., 1992; Hayaloglu *et al*., 2002; Manolopoulou *et al.*, 2003).

Abou Younes *et al.* (2007) studied about traditional white cheese lactic acid bacteria and revealed that the dominant lactococcus was *Enterococcus* type 51.7 and a low proportion of isolated lactobacilli (1.02%) from the total isolation. The research objectives were to isolate the lactic acid bacteria from Akawi cheese and identified biochemical characteristics and verify the possibility to use these bacteria as local starters in cheese processing as well as to substitute foreign starters.

Materials and Methods

Sampling

Thirty samples of Syrian Akawi cheese, made of cow's milk in traditional way, were collected randomly from many places in Damascus and Damascus Countryside District in 2011.

Organisms and culture conditions

Lactic acid bacteria was isolated from the cheese samples as follows: Decimal dilutions were prepared by adding 20 g from tested Akawi cheese samples inside sealed and sterilized flasks containing 180 ml of diluted solution (tryptone and salt).

To isolate *Lactococcus* we use M17 medium which contain generally glucophosphate, lactose and B12 Vitamin, after sterilization a distribution carried out by using petri dishes which contain 1 ml suitable decimal dilution and incubated at 45ºC for 72 hr and at 37ºC for 48 hr in order to distinguish between high thermophiles *Lactococcus* and moderate thermophiles *Lactococcus*.

Also we use Rogosa medium with B vitamins group by sterilizing and distributing in petri dishes of 1 ml suitable decimal dilution and incubated at 37ºC and 45ºC for 72 hr anaerobically. Typical colonies grown at M17 were chosen and grown in M17 broth and re - cultured by planning at M17 and incubated for 24 hr, at a temperature suitable for first degree isolation.

Typical colonies from Rogosa were chosen and grown in MRS broth and re-cultured on Rogosa and incubated for 24 hr, at a temperature suitable for first degree isolation. In order to detect the culture field purity and obtain single culture field to define morphological characteristics for the culture and study bacteria's biochemical properties (Caridi, 2003; Guessas and Kihal, 2004; Nespolo and Brandelli, 2010; Kunduhoglu *et al*., 2012).

Defined strains biochemical tests were carried out as microscopic test, gram's dye, spores formation as well as some other biochemical tests summarized as follows (Folkertsma and Fox, 1992; International Dairy Fedaration, 1995; Piraino *et al*., 2008): growth at 10ºC and 45ºC, growth at various concentration of NaCl (4% and 6.5%), milk fermentation to define the fermentation type (homo, hetero), gas producing during glucose fermentation, cetrates as only source of carbon, study Caseanolysis and lipolysis activity, acidity producing, glucose - lactose and sucrose fermentation on TSI (Tri Sugar Iron) medium, acetoine producing from glucose by Voges - Proskauer test.

API 20 Strep technique were used to distinguish isolated lactococcus types from M17,and *API* 50 CHL technique to distinguish isolated lactobacillus types from Rogosa, this techniques from BioMérieux company - France including some biochemical tests allow to study carbohydrates metabolism of Lactic Acid bacteria's types.

Akawi cheese manufacture by local isolated starters

Three samples were made by adding isolated Lactic Acid bacteria as mixed starters, every starter consist of many strains. Akawi cheese was manufactured as following: a good qualities cow's milk contains 3% fat were used. The milk were pasteurized at 72ºC for 15S and cooled to 37ºC. The starter was added as yogurt with 0.5% of the total quantities and incubated at 37ºC/30 min. Then calcium chloride was added in the form of salty solution at 2.5 g/10 liters of milk, and 1.5 g of animal rennet/100 liters of milk were added and Incubated the mix on 37ºC for 45 minutes. Coagulation was cut manually and the curds rests until pH = 5.2 (about

Table 1. Isolated strains from rural Akawi cheese physical and Physiologic properties

Type		Bacillus	Coccus	Total
Isolation number		10	17	27
Gram dye		+	+	
Catalase		-	-	
CO_2 produce		4	10	14
Acetone from glucose produce		7	11	18
Citrates Consume		3	11	14
Temperature growth	10ºC	6	17	23
	45ºC	5	11	16
NaCl concentrate growth	4%	10	15	25
	6.5%	3	13	16
Lipolysis		5	8	13
caseanolysis		5	10	15
Carbohydrate fermentation	Glucose	7	17	24
	Sucrose	4	7	11
	Lactose	10	17	27
Acidity percentage	less than 0.5%	3	5	8
	More than 0.5%	7	12	19
Milk Fermentation	Homofermentative	4	6	10
	Heterofermentative	6	11	17

2 hr), the cheese curd was drained in a cheese cloth and lightly pressed. The curd was preserved in salted solution 16%.

Sensory evaluation

Sensory evaluation carried out after Akawi cheese manufacture is completed. Sensory evaluation include physical appearance color, impurities, smell, texture and taste, by expert individuals depending on Hedonic Scale where every property given 5 degrees (5 = excellent, 4 = very good, 3 = good, 2 = amid, 1 = acceptable) (Lawless and Heymann, 1999).

Results and Discussions

Study the properties Isolated Lactic Acid bacteria from Akawi cheese

Twenty seven isolates from 30 samples of Akawi cheese (traditionally made) were carried out. all isolates were gram positive and catalase, oxidase negative. The physiologic and physical properties were studied for all the isolates, and results are shown in (Table 1). The diagnosis results of Lactic Acid bacteria are shown in (Table 2): coccus bacteria represent 63% from total isolates and bacillus bacteria represent 37% from total isolates which mean that the coccus are dominant, and that agree with various researchers (Karakus *et al*., 1992; Hayaloglu *et al*., 2002; Manolopoulou *et al*., 2003).

Lactic Acid bacteria classification according to *API* offered that the coccus bacteria were presented in three types *Enterococcus* 64.7% and the dominants were *E. avium* 72.7% and *E. faecium* 27.3%. *Lactococus* 23.5% and the dominants *Lc. lactis* ssp. lactis 50%, *Lc. lactis* ssp. *cremoris* 50%, *Leuconostoc* spp. represent 11.8%. High level presence of Enterococcus during the ripping period was due to wide range tolerant of environmental conditions as

Table 2. The distribution of percentages of the isolated Lactic Acid bacteria

Microscope bacteria	%	Genus	% Total Isolates	%	Type	%
Coccus	63	Enterococcus	40.7	64.7	*E. avium*	72.7
					E. faecium	27.3
		Lactoccocus	14.8	23.5	*Lc. lactis ssp lactis*	50
					Lc. lactis ssp cremoris	50
		Leuconostoc	7.4	11.8	-	-
Bacillus	37	Lactobacillus	11.8	100	*Lb. plantarum*	40
					Lb. paracasei	30
					Lb.bulgaricus	10
					Lb. pentosus	10
					Lb. brevis	10

Table 3. Proportions of strains and Akawi cheese starters

Starter	Strains	Proportions
First	*Lc. lactis ssp lactis*	1
	Lb.bulgaricus	1
	E. faecium	1
Second	*Lc. lactis ssp cremoris*	1
	Lb.bulgaricus	1
	E. faecium	1
Third	*Lc. lactis ssp lactis*	1
	Lb.bulgaricus	1
	Lb.paracasei	1

low temperature and high concentration of salty and acidity, and that agree with (Garg and Mital, 1991; Zarate *et al*., 1997).

The bacillus bacteria were represented in one genus *Lactobacillus* represented as *Lb. plantarum* 40%, *Lb. paracasei* 30, *Lb. bulgaricus* 10, *Lb. pentosus* 10% and *Lb. brevis* 10%. According to microscopic tests the distribution of Lactic Acid bacteria isolates from 30 samples of Akawi cheese shown in (Table 2). The results of this study are agree with rural white cheese (traditionally made) (Abou Younes *et al*., 2007), those results offer that dominant coccus bacteria were type *Enterococcus* 51.7% and low percentage of bacillus isolated represented 1.02%.

Akawi cheese industrialization by using isolated lactic acid bacteria starters

Various strains of the following bacteria types: *Lc. lactis* ssp. *lactis, Lc. lactis* ssp. *cremoris, Lb. bulgaricus, Lb. paracasei* and *E. faecium* were manipulated in formation of three starters to made Akawi cheese (Table 3). The starters were prepared by grown of every strain separately and then the three strains mixed together in proportion 1:1:1. The strains were chosen according to lipoproteolysis ability and CO_2 producing inability. Three samples of Akawi cheese were manufactured by using The starters in (Table 3), after 15 days of storage in salty solution 16% and preservation at 5ºC temperature, non casein nitrogen (NCN) and non protein nitrogen (NPN) were

Table 4. Dissolved nitrogen g/1000 g cheese in Akawi cheese samples

Sample	15 days /salty solution 16% temperature 5ºC /	
	NCN	NPN
Sample from the first starter	2.64 ±0.01[a]	0.75±0.02[a]
Sample from the second starter	4.97 ±0.01[b]	2.14 ±0.01[b]
Sample from the third starter	4.15 ±0.01[c]	1.89 ±0.02[c]

*The different letters in one column refer to considerable differences between samples at confidence level 0.05

determined, the nitrogen was evaluated by Kjeldahl technique and (Table 4) shows the results.

The results refer to considerable differences between the three samples and the second starter samples gave highest level of proteolysis after 15 days of storage in salty solution 16% and 5ºC, where the level of non casein nitrogen reached to 4.97 g nitrogen/1000 g cheese, and level of non protein nitrogen reached to 2.14 g nitrogen/1000 g cheese, the samples made by the first starter gave lowest proteolysis level, NCN level reached to 2.64 g nitrogen/1000 g cheese and NPN level reached to 0.75 g nitrogen/1000 g cheese, while the NCN arrived 4.15 g/1000 g ,and NPN were 1.89 g/1000 g in the cheese samples made by the third starter .

Sensory evaluation results for manufactured Akawi cheese

Results are shown in Table 5. The sensory evaluation did not show considerable differences between the three samples in color, texture and impurities that is normal may be due to same milk resources and milk filtration before the manufacture and same manufacture stages (same time pressure gave same texture). On other side there were a considerable differences between the samples in taste and smell, the samples gave best evaluated Physical properties by using starters *Lc. lactis* ssp. *cremoris*, *Lb. bulgaricus* and *E. faecium,* and in less degree by using starters *Lc. lactis* ssp. *lactis, Lb. bulgaricus* and *Lb. paracasei,* and in less acceptable degree by using starters *Lc. lactis* ssp. *lactis, Lb. bulgaricus*, *E. faecium.*

From all above we observed that the most acceptable cheese were the samples manufactured by second starter because the high proteolysis activity of *Lc. lactis* ssp. cremoris bacteria existence this activity gave high level of free amino acids and increasing the degree of flavor and taste of cheese, those results are agree with Dagdemir *et al.* (2003) and Goncu and Alpkent (2005). The cheese samples made by the third starter had Less acceptance than

Table 5. Manufactured Akawi cheese sensory evaluation results

Sample	Physical Appearance		Taste	Smell	Texture	Total
	Color	Impurities				
1	3.11±0.31[a]	3.21±0.46[a]	2.86±0.53[a]	3.14±0.23[a]	2.93±0.92[a]	15.25
2	3.99±0.26[a]	3.79±0.50[a]	4.43±0.44[b]	4.12±0.33[b]	3.64±0.93[a]	19.97
3	3.71±0.42[a]	3.71±0.61[a]	3.50±0.52[ab]	3.64±0.36[ab]	3.21±0.80[a]	17.77

*the different letters in one column refer to considerable differences between samples at confidence level 0.05

the second due to existence of *Lb. paracasei* bacteria as well as the high level of free amino acids and free faty acids which contributed directly or indirectly in cheese flavor, this result is agree with (Ur-Rehman *et al.*, 2000; Awad *et al*., 2007).

Conclusions

The coccus isolated bacteria belong to the genus *Lactoccocus*, including *Lc. lactis* ssp. *Lactis* and *Lc. lactis* ssp. *cremoris*, and the *Enterococcus* genus including *E. avium* and *E. faecium,* where the third genus were *Leuconostoc* spp. The bacillus isolated bacteria belong to the genus *Lactobacillus* were distributed to the following types *Lb. plantarum* (40%), *Lb. paracasei* (30%), *Lb. bulgaricus* (10 %), *Lb. pentosus* (10 %), *Lb. brevis* (10%). The second starter isolated and used to manufacture Acawi cheese (*Lc. lactis* ssp. *cremoris, Lb. bulgaricus* and *E. faecium*) show the best of sensory aspects.

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