

Studies on thermonuclease production by Domiati cheese microflora

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Summary

A total of 1005 isolates from Domiati cheese representative of thermophilic bacteria (431), Gram negative bacteria (270), yeast (159), faecal streptococci (131), micrococci (72) and *S. aureus* (32) were examined for their capability to produce thermonuclease (TNase). Only nine out of 131 isolated faecal streptococci, in addition to the 32 *S. aureus* isolates were TNase producers. Based on the observed differences in heat stability of TNase produced by isolated *S. aureus* and faecal streptococci, it could be recommended that heat treatment used with TNase test should be prolonged up to 20 min. Results also indicated that increasing salt content of milk beyond 10 % and up to 20 % obviously affected TNase production by *S. aureus* strains. TNase test, therefore, might not be used as an index for likely presence of *S. aureus* in case of Domiati cheese made from salted milk with more than 10 % sodium chloride.

Key words : Thermonuclease - *Staphylococcus aureus* - Domiati cheese.

Résumé

Etude de la production de thermonucléase par la microflore du fromage Domiati

Un total de 1 005 isolats obtenus à partir de fromage Domiati, représentant des bactéries thermophiles (431), des bactéries Gram négatives (270), des levures (159), des streptocoques fécaux (131), des microcoques (72) et *Staphylococcus aureus* (32) ont été examinés pour leur aptitude à produire de la thermonucléase (TNase). Seulement 9 des 131 isolats de streptocoques fécaux, en plus de la totalité des 32 isolats de *S. aureus*, se révèlent être producteurs de TNase.

En se basant sur les différences observées dans la stabilité thermique de la TNase produite par les isolats de *S. aureus* et par ceux de streptocoques fécaux, on pourrait recommander de prolonger jusqu'à 20 min le traitement thermique utilisé avec le test de la TNase.

Les résultats obtenus indiquent également que l'augmentation de la teneur en chlorure de sodium au-delà de 10 % et jusqu'à 20 % affecte incontestablement la production de TNase par les souches de *S. aureus*.

En conséquence, le test de la TNase ne devrait pas être utilisé pour la mise en évidence de la présence de *S. aureus* dans le cas du fromage Domiati fabriqué à partir de lait salé avec plus de 10 % de chlorure de sodium.

Mots clés : Thermonucléase - Microflore - *Staphylococcus aureus* - Fromage Domiati.

Introduction

MINOR and MARTH (1971) have discussed extensively staphylococci and staphylococcal food poisoning associated with cheese and other dairy products. Sanitary quality of such foods may be examined either by *S. aureus* counts or by dosing enterotoxins. Enterotoxin determination methods are too costly, laborious and time consuming and are carried only to justify suspect foods. On the other hand enumeration of *S. aureus* is used to indicate the likely presence of staphylococci in food.

The enterotoxigenicity of *S. aureus* is highly correlated with its potential production of TNase (CORDS and TATINI, 1933; LACHICA, 1980 and BATISH *et al.*, 1980). Therefore, TNase was recommended by several investigators (TATINI, 1980 and BATISH *et al.*, 1980) as an index for staphylococcal food poisoning. However, the limitation of such criteria is attributed to type of food and interfering strains having the ability to elaborate TNase. Meanwhile, many reports indicated that *S. aureus* is the only TNase producer in foods. However, THOMAS and NAMBUDEP (1974), PARK *et al.* (1980) and BATISH *et al.* (1982) stated that in addition to staphylococci, streptococci and bacilli have the ability to produce heat-stable nuclease.

There are few informations in the available literature about the resistance of the thermonuclease produced by different microorganisms isolated from cheese. Accordingly, the aim of the present study is to justify the use of TNase production as an indication of the frequently presence of *S. aureus* incriminated in food-poisoning by Domiati cheese which is a particular type of soft pickled cheese.

I. Materials and methods

Cheese samples

One hundred Domiati cheese samples were randomly collected from the market of Cairo. Of each sample, 20 g as homogenized and proper serial dilutions were prepared by using phosphate buffered distilled water to detect the main microflora of cheese.

Thermoduric bacteria

Tryptone Glucose Extract Agar medium (TGEA) was used for cultivation of thermoduric bacteria after heating the sample of cheese for 15 min at 80°C. An incubation period of 48 h at 30°C was used.

Gram-Negative bacteria

Crystal Violet Tetrazolium Agar medium (CVTA) was used for Gram-negative counts after an incubation period of 48 h at 30°C.

Faecal Streptococci group

The faecal streptococci group was grown on Thallus Acetate Tetrazolium Agar medium (TITG). TNase producing strains were identified to species level according to HARTMAN *et al.* (1966).

Micrococci and yeast

For cultivation of micrococci spp. the basal micrococci and staphylococci medium was used (NAGUIB, 1968), while *S. aureus* were cultivated on Baird-Parker medium. Typical colonies were tested according to BAIRD-PARKER (1963, 1965). Potato Dextrose agar (PDA) medium was used for yeast cultivation after acidification to pH 3.5 with citric acid and incubated at room temperature, for 5 days.

Isolation

Representative cultures were isolated from the above mentioned culture media, and purified twice. All isolated-cultures were tested for TNase production.

Thermonuclease assay

Thermostable nuclease activity was determined by the microslide method according to LACHICA *et al.* (1971). Brain heart infusion (BHI) broth cultures were heated in boiling water bath for 15 min.

Positive strains were tested for resistance of TNase to prolonged boiling periods up to 90 min.

Effect of NaCl concentration on nucleases production

Ten *S. aureus* cultures were examined for both nuclease and thermonuclease enzymes production in the presence of increasing NaCl concentration (10, 15 and 20 %). Nuclease was tested by method of LACHICA *et al.* (1971). Sterilized sodium chloride solutions were added to a double strength BHI medium in order to reach the proper salt concentrations.

Extraction and assay of TNase in cheese

The extraction procedure as described by CORDS and TATINI (1973) was used. TNase was detected according to LACHICA *et al.* (1971). Cheese was manufactured by using cheese-milk contained 8 %, 15 % and 20 % salt concentrations according to FAHMI and SHARARA (1950).

Determination of moisture content : total chlorides, and acidity of cheese

Cheese moisture was determined according to British standard methods (1952). Total chlorides and acidity of cheese samples were determined by the official methods according to A.O.A.C. (1960).

Production of TNase in cheese-milk with S. aureus cultures artificially inoculated

The ability of 15 isolated *S. aureus* strains to produce thermonuclease was determined. Cheese milk samples were inoculated with about 1×10^6 org/ml. Samples were examined every 30 up to 180 min.

II. Results and discussion

A total of 1005 isolates from Domiati cheese representative of thermophilic bacteria (341), Gram-negative bacteria (270), Yeast (159) and faecal streptococci (131) and *Micrococcus* spp (72) and *S. aureus* (32) were examined for their

TABLE I

Production of thermonuclease by the main groups of microorganisms present in Domiati cheese

Production de thermonucléase par les principaux groupes de micro-organismes présents dans le fromage Domiati

Organisms	No. of Total isolates	TNase Producing isolates	
		No.	%
Thermoduric bacteria	341	0	0.00
Gram-negative bacteria	270	0	0.00
Yeast	159	0	0.00
<i>Micrococcus spp.</i>	72	0	0.00
Faecal streptococci	131	9	6.87
<i>S. faecalis</i>	2	2	100.0
<i>S. faecalis</i> var. <i>liquifaciens</i>	7	7	100.0
<i>S. aureus</i>	32	32	100.0
Total	1 005	50	

TABLE II

Resistance of thermonuclease produced by S. aureus and enterococci isolates to prolonged boiling periods

Résistance de la thermonucléase produite par des isolats de S. aureus et d'entérocoques à différentes durées d'ébullition

TNase Producing organisms	No. of tested isolates	15	20	30	60	90 min
<i>S. aureus</i>	3	++	++	+	+	—
	4	++	++	+	±	—
	3	++	++	±	—	—
Enterococci	9	+	—	—	—	—

++ Strong.
+ Medium.

± Faint colour.
— Negative.

potential capability of producing TNase (table I). No strains of the thermoduric bacteria, Gram-negative bacteria, yeast or micrococci had the ability to produce TNase. Out of the 131 isolates of faecal streptococci, 9 were TNase producers.

They were found to belong to *S. faecalis* (2) strains and *S. faecalis* var. *liquefaciens* (7) strains. All the isolates of *S. aureus* (32 strains) were TNase producers. Similar conclusions were reported by THOMAS and NAMBUDEPEDI (1974), PARK *et al.* (1980), BATISH (1982) and EL-HINDAWY *et al.* (1982). Evaluation of thermonuclease resistance produced by different microorganisms isolated from Domiati cheese (*S. aureus* and enterococci) to prolonged boiling up to 90 min is shown in table II.

All tested *S. aureus* TNase resisted to boiling up to 20 min. However, faint pink colour reactions were observed with only 3 strains when their cultures were boiled up to 30 min before TNase examination. These thermonuclease produced by the above mentioned three strains of *S. aureus* failed to resist boiling up to 60 minutes. In addition, TNase produced by the other four strains of *S. aureus* resisted up to 60 min, increasing boiling time up to 90 min clearly suppressed TNase enzyme activity. Boiling up to 90 min completely inactive TNase produced by *S. aureus* strains.

Of all the tested enterococci, 9 produced TNase that survived boiling up to 15 min. No colour reaction was observed when enterococci cultures were boiled up to 20 min.

It could be concluded from these results that, boiling up to 20 min may be used to differentiate between thermonuclease produced by *S. aureus* strains and that produced by enterococci.

LACHICA (1971) recommended boiling up to 15 min for tested cultures as a method to differentiate between *S. aureus* and other group of microorganisms. However, it is obvious from the present study that the application of the typical method introduced by this author could not be easy to differentiate TNase produced by *S. aureus* and other microorganisms.

In view of the well known fact that enterococci are often in high counts in Domiati cheese (NAGUIB, 1965 ; NAGUIB, 1968), the increase of the boiling time to 20 min, as recommended may exclude thermonuclease enzyme produced by enterococci when Domiati cheese is examined. In view of the production of TNase by *S. aureus* and streptococci strains, this enzyme can not be used as a sole index for the presence of staphylococcal food poisoning, following the method of TNase detection recommended by LACHICA *et al.* (1971).

Table III includes a study about the time needed for the production of thermonuclease in artificially inoculated cheese milk with *S. aureus* strains isolated from market Domiati cheese. For the 15 isolates, after incubation period of 60 min no production of TNase was noticed by 12 isolates (80 %). After 150 min TNase was produced in all isolates.

The effect of increasing sodium chloride concentrations on nuclease produced by *S. aureus* isolated from Domiati cheese is shown in table IV. TNase and DNase produced by 10 *S. aureus* isolates were active in broth cultures containing up to 10 % salt concentration. TNase and DNase produced by most strains were not detected in cultures containing 15 % NaCl or more.

These results may lead to conclude that increasing salt content of broth up to 20 % clearly affect TNase production by *S. aureus* strains.

S. aureus detected in Domiati cheese made from salted milk with 8 % and 15 % after one week storage were 50×10^{10} and 79×10^6 org/g respectively

TABLE III

Production of thermonuclease in artificially inoculated cheese-milk with S. aureus strains isolated from market Domiati cheese*

Production de thermonucléase dans du lait artificiellement inoculé avec des souches de S. aureus isolées du fromage Domiati du commerce

Number of strains	Time of growth in milk at 37° C			
	60	90	120	150
3	—	+	+	+
9	—	—	+	+
3	—	—	—	+

* Initial count : 10⁶ organisms per ml of cheese milk.

TABLE IV

Effect of increasing NaCl concentration on nucleases produced by S. aureus strains isolated from Domiati cheese

Influence de l'augmentation de la concentration en NaCl sur les nucléases produites par des souches de S. aureus isolées du fromage Domiati

Number of isolate	NaCl concentration g/l			
	0	10	15	20
7	+	+	—	—
2	+	+	± (b)	—
1	+	+	+	+

(a) DNase and TNase.

(b) Faint colour.

(TOHAMY, 1983). Such high population of *S. aureus* could have the possibility to produce sufficient TNase to be detected in cheese. Therefore TNase activity in Domiati cheese during storage up to 90 days at room temperature was studied.

The TNase activity revealed positive results in cheese made from 8 % salted milk during pickling up to 90 days. However, no TNase activity was detected in cheese made from higher salted milk (table V). The detection of TNase activity in fresh cheese agrees with the data presented in table 4 and so confirms the ability of *S. aureus* to produce TNase in medium containing up to 10 % NaCl. Accordingly increasing salt/water ratio in cheese made from 15 % and 20 % salted milk to level of 15.2 % and 18.8 % (table V) is expected to inhibit the production of TNase. This fact is obvious in table V. Therefore it could be concluded that salt/water ratio in Domiati cheese at the extent of more than 10 % would inhibit the TNase activity of *S. aureus* in such cheese. The potential

TABLE V

Detection of TNase, salt/water ratio and acidity of Domiati cheese during storage at room temperature

Détection de TNase, teneur en sel et acidité du fromage Domiati pendant le stockage à température ambiante

Storage periods	TNase production			Salt/water ratio			Acidity		
	I	II	III	I	II	III	I	II	III
1	+	—	—	9.37	15.2	18.8	0.38	0.25	0.18
30	+	—	—	8.38	15.15	18.18	0.89	0.69	0.64
45	+	—	—	ND	ND	ND	ND	ND	ND
60	+	—	—	8.46	15.66	18.48	1.77	1.01	0.81
75	+	—	—	ND	ND	ND	ND	ND	ND
90				7.87	14.94	18.14	1.99	1.33	1.11

I Cheese made from 8 % salted milk.

II Cheese made from 15 % salted milk.

III Cheese made from 20 % salted milk.

ND Not detected.

increase in the acid content in the cheese, as a result of adding starter during manufacture would increase the salt/water ratio where the inhibitive action developed. However other interaction between the variables of the microbial and chemical properties of the cheese may not be excluded.

Contrary to most cheese varieties, TNase test might not be used as an index for likely presence of *S. aureus* in the case of Domiati cheese.

However this test may be applicable to the same cheese with low salt level i.e. cheese made from 10 % salted milk or less.

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