

IDENTIFICATION AND CHARACTERIZATION OF ANTIBIOTIC SUSCEPTIBILITY OF ENTEROCOCCI ISOLATED FROM TERRINCHO CHEESE



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NFIF 2003
NON-FLAVORING
INGREDIENTS AND FOODS
8-11 April 2003, Copenhagen, Denmark

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INTRODUCTION

Enterococcus are ubiquitous microorganisms, which are found in the normal intestinal flora of many organisms including humans, as well as in the soil, in the natural water and on vegetables. They may cause food contamination, and play important roles in such dairy products as cheese. Enterococci occur and grow in a variety of cheeses, especially artisanal cheeses produced in Southern Europe (Freitas *et al.*, 1999); in some cases, they are the predominant microorganisms in the fully ripened product (Sarantinopoulos *et al.*, 2001); in many cheese varieties the growth of enterococci contributes to ripening and development of the final product flavour (Franz *et al.*, 1999). The beneficial roles of enterococci in cheese and their useful biotechnological traits (bacteriocin production and probiotic characteristics) has led to inclusion of enterococcal strains in certain starter cultures (Franz *et al.*, 1999; Giraffa, 2002). Several enterococcal species possess the ability to grow under adverse conditions, and are resistant to some antibiotics - which raises a public health concern and constrain application in the Food Industry.

The main purpose of this study was to identify enterococcal strains isolated from a traditional Portuguese cheese (Terrincho Cheese) and determine their susceptibility to some antibiotics.

MATERIALS AND METHODS

Origin of bacterial isolates

A total of 73 bacteria isolated from samples of various dairy products: milk, whey, curd and cheese were studied.

The cheese samples were taken at different stages of ripening: 0, 1, 4, 7, 14, 28, 45 and 60 days.

Phenotypic characterization

conventional tests

all isolates were initially tested for:

- ⇒ colony morphology in KAAA
- ⇒ Gram staining
- ⇒ catalase production
- ⇒ growth in BHI at 10°C and 45°C
- ⇒ growth in BHI with 6.5% NaCl
- ⇒ growth in BHI at pH 9.6
- ⇒ production of a yellow pigment in TSA
- ⇒ aesculin hydrolysis in KAAA
- ⇒ haemolysis in blood agar

the API 20 Strep chemotaxonomical system was also used



Susceptibility to antimicrobial agents

Disk-diffusion method



Bacterial culture grown in M17 agar 37°C/24h

Saline (0.9% NaCl) suspension of the bacterial culture to be tested (turbidity 0.5 McFarland)



Incubation 37°C/18-24h
Measurement of the diameter of the zone of growth inhibition around each disk (mm)



The plates are allowed to dry
The swab is streaked in various directions over the surface of Mueller Hinton agar, to obtain uniform growth
Determination if the strain is resistant, intermediate or susceptible to the antibiotics tested, using a standard table of antibiotic susceptibilities

Antibiotics tested

- ✓ Amoxicillin
- ✓ Oxacillin
- ✓ Penicillin G
- ✓ Piperacillin
- ✓ Gentamicin
- ✓ Streptomycin
- ✓ Vancomycin

RESULTS AND DISCUSSION

All isolates (catalase-negative, Gram-positive cocci) grew at 10°C, at 45°C, in the presence of 6.5% NaCl and at pH 9.6, as shown in Table 1. None of the strains exhibited haemolytic activity in blood agar, and only four strains isolated from cheese, three isolated from whey and one isolated from milk showed production of a yellow pigment in TSA. These 8 isolates were further identified as *Enterococcus faecium*.

Table 1 – Results of the conventional assay tests of the bacterial isolates from various dairy products

Product	Nº of Isolates	Conventional tests						
		Catalase production	Growth at 10°C	Growth at 45°C	Growth in 6.5% NaCl	Growth at pH 9.6	Aesculin hydrolysis	Haemolysis
Cheese	60	-	+	+	+	+	+	-
Whey	6	-	+	+	+	+	+	-
Curd	4	-	+	+	+	+	+	-
Milk	3	-	+	+	+	+	+	-

+: positive test, -: negative test

From the 73 bacteria isolated from dairy products, 33 were identified as *Enterococcus faecium* (45.2%), 29 as *Enterococcus durans* (39.7%), 2 as *Enterococcus faecalis* (2.74%) and 9 (12.3%) produced a doubtful result (see Table 2).

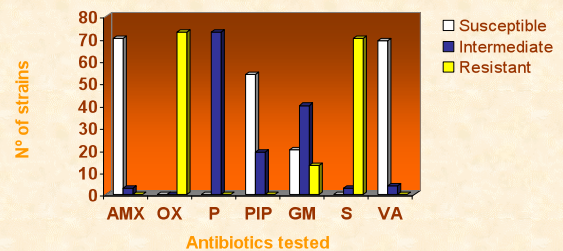
Table 2 – Results of the identification of the bacterial isolates using the API 20 Strep assay kit

Product	Number of strains			
	<i>E. faecium</i>	<i>E. durans</i>	<i>E. faecalis</i>	N.I.
Cheese	26	25	2	7
Whey	4	1	0	1
Curd	1	3	0	0
Milk	3	0	0	1
TOTAL	33	29	2	9

N.I. – not identified

Among the 73 isolates studied, the majority was sensible to amoxicillin, by the disk-diffusion method. All isolates were found to be oxacillin resistant, as expected since this resistance is intrinsic in enterococci. Gentamicin resistance occurred in 13 strains, and the Streptomycin resistance was observed in 70 strains (see figure 1). This resistance to aminoglycosides is not significant since the concentrations of the antibiotics assayed were not high.

Almost all strains were susceptible to vancomycin. Hence, the resistance to vancomycin, which is a common trait for enterococci of nosocomial origin, is not present among these dairy isolates.



AMX: amoxicillin, OX: oxacillin, P: penicillin, PIP: piperacillin, GM: gentamicin, S: streptomycin and VA: vancomycin

Figure 1 – Antibiotic resistance profiles of the strains

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ACKNOWLEDGMENTS

Financial support for the first author was provided by a Ph.D. fellowship (SFRH/BD/7000/2001) granted by the Portuguese government through program PRAXIS XXI (Fundação para a Ciência e Tecnologia, Portugal). Financial support for experimental work was provided by project POCTI/1999/AGR/36165 ENTEROCOCOS: deepening the knowledge on the role of enterococci, from manufacture through maturation of traditional cheeses funded by Fundação para a Ciência e Tecnologia, Program POCTI.