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Antibiotic susceptibility of *Lactobacillus rhamnosus* strains isolated from Parmigiano Reggiano cheese

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Abstract – This work aimed to evaluate the antibiotic susceptibility of 63 *Lactobacillus rhamnosus* strains isolated from Parmigiano Reggiano cheese, of *Lactobacillus* GG and of the type strain *L. rhamnosus* DSM 20021. Antimicrobial susceptibility was determined by the disc diffusion method on 41 antibiotics. Inhibition zone diameter was carefully measured and the results (the mean of four determinations) were expressed in terms of resistance or susceptibility. All the strains isolated from cheese showed resistance to six antibiotics (cefixime, vancomycin, neomycin, enoxacin, pefloxacin and sulphamethoxazole plus trimethoprim). The strain DSM 20021^T was resistant to nine antibiotics (the previous six plus cephalexin, bacitracin and lincomycin), while the commercial strain *L.* GG showed resistance to eighteen antibiotics. A high strain-specific resistance to different antibiotics was ascertained in *Lactobacillus rhamnosus* isolated from cheese. The results obtained in this study confirm that antibiotic resistance is a very important feature in the selection of potentially probiotic lactic acid bacteria.

***Lactobacillus rhamnosus* / lactic acid bacteria / antibiotic susceptibility / cheese / probiotic**

Résumé – Sensibilité aux antibiotiques de souches de *Lactobacillus rhamnosus* isolées du fromage Parmigiano Reggiano. Le but de cette étude était l'évaluation de la sensibilité aux antibiotiques de 63 souches de *Lactobacillus rhamnosus* isolées du fromage Parmigiano Reggiano, de la souche *Lactobacillus* GG et de la souche type de *L. rhamnosus* DSM 20021. La sensibilité ou la résistance à 41 antibiotiques de ces 65 souches de *L. rhamnosus* a été déterminée avec le test de diffusion de disque en gélose. Les diamètres des zones d'inhibition, mesurés avec précision, ont permis d'individualiser pour chaque antibiotique testé les souches résistantes et celles sensibles. Toutes les souches isolées du fromage se sont avérées résistantes à six antibiotiques (céfixime, vancomycine, néomycine, enoxacine, péfloxacine et triméthoprime + sulfaméthoxazole). La souche type DSM 20021^T était résistante à neuf antibiotiques (les six précédents plus cefalexine, bacitracine et lincomycine), tandis que la souche commerciale *L.* GG a montré une résistance à 18 antibiotiques. Une résistance souche-spécifique à différents antibiotiques a été montrée pour les souches de *Lactobacillus rhamnosus* isolées du fromage. Les résultats obtenus dans cette étude ont confirmé que la résistance aux antibiotiques est une caractéristique très importante pour sélectionner les bactéries lactiques potentiellement probiotiques.

***Lactobacillus rhamnosus* / bactérie lactique / sensibilité aux antibiotiques / fromage / probiotique**

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1. INTRODUCTION

Lactobacillus species are considered as part of the normal microflora of the gastrointestinal and female genital tract [6], and also as major components of microflora involved in food fermentation [38].

Probiotic products that contain lactobacilli have long histories of safe use [10].

Bacteriosis caused by lactobacilli is considered to be rare but risk factors related to *Lactobacillus* species include impaired host defences and severe underlying diseases, as well as prior surgery and prolonged antibiotic therapy ineffective for lactobacilli [36].

However, widespread use of antibiotics could be responsible for significant changes in the composition of human microflora and for the rise of new pathogenic bacterial strains.

The most frequent clinical side effect of antibiotics is diarrhoea, with greater risks when they are prescribed for general purposes and taken orally [9].

Amongst the groups of patients most susceptible to these side effects are the elderly [26], the undernourished, and those who have either undergone surgery or who have been hospitalised for long periods.

Antibiotic susceptibility of probiotic micro-organisms is a fundamental requisite due to the possibility that an antibiotic-resistant strain may not be easily eliminated in the case of negative influence on the host [1, 18] and that antibiotic resistance could be subsequently transmitted to pathogenic or potentially pathogenic micro-organisms.

The data on drug resistance of the industrially important lactobacilli are rare but some species of lactic acid bacteria that are commonly used in the food industry, or naturally found in food raw material, are well known as intrinsically resistant to some antibiotics, e.g., vancomycin [42]. However, these bacteria are susceptible to many other antibiotics and they have not been reported to easily acquire antibiotic resistance determinants like the enterococci [11].

Within the context of a large-scale research project aimed at isolating and characterising lactic acid bacteria in food, this study examined the antibiotic resistance of *Lactobacillus rhamnosus* strains of potentially probiotic interest isolated from Parmigiano Reggiano cheese, a hard cooked Italian cheese produced with partly skimmed raw cow milk to which natural whey starter is added and which is then ripened for 12–24 months [15, 16, 32].

Parmigiano Reggiano cheese plays the leading role in the Italian dairy industry and could represent an optimal source of potentially probiotic bacteria thanks to the presence of high amounts of viable micro-organisms at the end of the ripening (up to 24 months), i.e., at the moment of consumption.

This aspect is not negligible, considering that recent studies showed low viability of probiotics in the market preparations [25].

The reason for focusing on *Lactobacillus rhamnosus* strains is tied to many studies, in which the positive effects of the attachment of this particular micro-organism to human intestinal mucosa are reported [3, 19, 30, 31, 35, 37].

2. MATERIALS AND METHODS

2.1. Bacterial strains

A total of 63 strains of *Lactobacillus rhamnosus*, isolated from different samples of Parmigiano Reggiano cheese (from 4 different dairy plants located in various areas of production) at the end of the ripening (24 months) [15, 16, 32], were used in the present study (Tab. I).

The strains had been phenotypically identified according to Hammes and Vogel [21] and by API 50 CH (Biomerieux, Marcy l'Étoile, France) according to Nigatu [34] (data not shown).

L. rhamnosus GG was isolated from a pharmaceutical preparation (Valio LTD, Helsinki, Finland) and revitalised in MRS broth (Oxoid, Milano, Italy) at 37 °C.

Table I. Provenience of *L. rhamnosus* strains isolated from Parmigiano Reggiano cheese at the end of the ripening.

	Dairy plants			
	A	B	E	G
Number of cheese samples	3	3	3	3
Number of isolates at 45 °C	21	10	11	21
Identified as *L. rhamnosus*	21	10	11	21
Last isolation dilution	–4	–3	–4	–4

L. rhamnosus type strain DSM 20021 was provided by the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany) and revitalised in MRS broth (Oxoid, Milano, Italy) at 37 °C according to the prescription of DSMZ.

RAPD-PCR analysis [4] was performed with the aim of differentiating the strains of *Lactobacillus rhamnosus* assayed (63 strains from Parmigiano Reggiano cheese plus *L.* GG) using as a reference *L. rhamnosus* DSM 20021^T.

PCR reaction was performed in a Mastercycler gradient (Eppendorf, Hamburg, Germany), using the primers M13 [24] and D8635 [2].

Amplification products were separated by electrophoresis on 1.5% (w/v) agarose gel in 0.5 × TBE buffer. RAPD-PCR profiles were obtained directly using the digital camera ImageMaster VDS (Amersham Pharmacia Biotech, Milano, Italy) and analysed with the pattern analysis software package, Gel Compar Version 4.1 (Applied Maths, Kortrijk, Belgium).

Calculation of similarities in the profiles of bands was based on the Pearson product-moment correlation coefficient. A dendrogram was obtained by means of the Unweighted Pair Group Method using the Arithmetic Average (UPGMA) clustering algorithm [44].

2.2. *Lactobacillus rhamnosus* species-specific PCR

The specific PCR was performed on 17 strains previously identified by RAPD-PCR as *L. rhamnosus* and belonging to different clusters and on 8 strains of *L. paracasei* subsp. *paracasei* from the DISTAAM collection (University of Molise), isolated from different cheeses. The conditions for specificity testing were the same as those reported for RAPD-PCR.

For comparative purposes the following type strains were used: *L. casei* DSM 20011^T, *L. paracasei* subsp. *paracasei* DSM 5622^T, and *L. rhamnosus* DSM 20021^T.

PCR reaction was performed in a Mastercycler gradient (Eppendorf, Hamburg, Germany), using the following primers:

Y2: 5' CCCACTGCTGCCTCCCGTAGGAGT 3' [45];

rhamn: 5' TGCATCTTGATTTAATTTTG 3' [45].

The amplification profile was an initial step of 94 °C for 3 min, and then 30 cycles of: 94 °C for 45 sec, 55 °C for 45 sec, and 72 °C for 1 min.

Amplification products were separated by electrophoresis on 1.5% (w/v) agarose gel in 0.5 × TBE buffer.

2.3. Antimicrobials

The susceptibility of *L. rhamnosus* strains was determined against 41 antibiotics. Antimicrobial susceptibility discs were obtained from Oxoid (Milano, Italy). The discs were stored in sealed containers with a desiccant at 4 °C. The antibiotics used for this study were cell wall synthesis, protein synthesis or nucleic acid synthesis inhibitors. Antibiotics were selected according to the experiment of Charteris et al. [14], and also by considering antibiotic treatments in standard use in hospitals.

2.4. Antimicrobial susceptibility test

Antibiotic susceptibility was semi-quantitatively determined by disc diffusion on

MRS agar using a modification of the National Committee for Clinical Laboratory Standards [33] as described by Charteris et al. [14]. Inhibition zone diameter was carefully measured after anaerobic incubation at 37 °C for 24 h (Anaerogen, Oxoid) using sliding callipers and the results (the mean of four determinations) were expressed in terms of resistance (R), moderate susceptibility (MS) or susceptibility (S) [7].

The statistical program Systat Version 11 (Systat Software, Inc., Richmond, USA) was utilised to calculate the similarity in the antibiotic-susceptibility profiles of the 65 strains. A dendrogram was obtained using the same parameters utilised in the RAPD-PCR analysis (average linkage and Pearson correlation) for comparative purposes.

3. RESULTS

3.1. Susceptibility to inhibitors of cell wall synthesis

Among penicillins (Tab. II), ampicillin, cloxacillin, mezlocillin, piperacillin and ticarcillin showed a strong inhibitory activity on all the strains tested; penicillin G inhibited 95% of the strains, while the remaining 5% were moderately susceptible to this antibiotic. *L. rhamnosus* DSM 20021^T and *L.* GG were sensible to the previous antibiotics.

Resistance was shown by 71% and 68% of strains isolated from Parmigiano Reggiano cheese to amoxicillin and oxacillin, respectively; *L. rhamnosus* DSM 20021^T and *L.* GG showed a moderate sensibility or resistance to these antibiotics, respectively.

Among cephalosporins, it was possible to note a strain-specific response to this class of antibiotics. In particular, a high percentage of *L. rhamnosus* strains showed resistance to cefadroxil, cephalexin and ceftriaxone, while a high percentage showed susceptibility to cefazolin, cefoperazone and cefuroxime. All the strains tested were resistant to cefixime, while 24%, 43% and 33% of them were, respectively, sensible, moderately sensible or resistant to ceftazidime. *L.* GG was sensible to cefazolin, moderately sensible to cefoperazone and resistant to all the other cephalosporins. DSM 20021^T showed sensibility to cefazolin, cefoperazone, ceftazidime and cefuroxime, moderate sensibility to cefadroxil and ceftriaxone and resistance to cephalexin and cefixime.

Assays for beta-lactamase inhibitors revealed susceptibility of all the strains to amoxicillin-clavulanic acid, ampicillin-sulbactam and piperacillin-tazobactam, while 76%, 10% and 14% of the assayed strains were sensible, moderately sensible or resistant to ticarcillin-clavulanic acid, respectively. DSM 20021^T showed sensibility to all β-lactamase inhibitors tested, while *L.* GG was resistant to ticarcillin-clavulanic acid.

Among the other single inhibitors of cell wall synthesis, all strains were susceptible to imipenem and resistant to vancomycin but a high resistance to bacitracin and teicoplanin was also evidenced by 73% and 95% of assayed strains, respectively. *L. rhamnosus* DSM 20021^T was susceptible to imipenem, moderately sensible to teicoplanin and resistant to bacitracin and vancomicyn, while *L.* GG showed sensibility to imipenem only.

3.2. Susceptibility to protein synthesis inhibitors

All strains showed susceptibility to tetracyclines (tetracycline, minocycline and doxicycline), to chloramphenicol and, among macrolides, to erythromycin, josamycin and spiramycin, while 93%, 5% and 2% were sensible, moderately sensible or resistant to clarithromycin, respectively.

Among lincosamides, clindamycin showed inhibitory effects against all the tested strains, while 14% and 86% of the strains were susceptible or resistant to lincomycin, respectively.

Almost all the strains were resistant to aminoglycosides (gentamicin and neomicyn). In fact, only 2% showed susceptibility to gentamicin. *L. rhamnosus* DSM 20021^T

Table II. Susceptibility to 41 antibiotics of *L. rhamnosus* (63 strains) isolated from Parmigiano Reggiano cheese, of *L. rhamnosus* DSM 20021^T and *L.* GG (% of susceptible, moderately susceptible or resistant strains).

Group	Name	Potency	Antimicrobial susceptibility [a]				
						DSM	
Inhibitors of cell wall synthesis			S	MS	R	20021^T	*L.* GG
Penicillins	Amoxicyllin	10 µg	2	27	71	MS	R
	Ampicillin	10 µg	100	0	0	S	S
	Cloxacillin	5 µg	100	0	0	S	S
	Mezlocillin	75 µg	100	0	0	S	S
	Oxacillin	1 µg	5	27	68	MS	R
	Penicillin G	10 I.U.*	95	5	0	S	S
	Piperacillin	100 µg	100	0	0	S	S
	Ticarcillin	75 µg	100	0	0	S	S
Cephalosporins	Cefadroxil	30 µg	8	19	73	MS	R
	Cephalexin	30 µg	5	0	95	R	R
	Cefazolin	30 µg	67	24	9	S	S
	Cefixime	5 µg	0	0	100	R	R
	Cefoperazone	75 µg	86	11	3	S	MS
	Ceftazidime	30 µg	24	43	33	S	R
	Ceftriaxone	30 µg	2	46	52	MS	R
	Cefuroxime	30 µg	71	5	24	S	R
β-lactamase inhibitors	Amoxicillin-clavulanic acid	30 µg	100	0	0	S	S
	Ampicillin-sulbactam	20 µg	100	0	0	S	S
	Piperacillin-tazobactam	110 µg	100	0	0	S	S
	Ticarcillin-clavulanic acid	85 µg	76	10	14	S	R
Single antibiotics	Imipenem	10 µg	100	0	0	S	S
	Bacitracin	10 I.U.*	24	3	73	R	R
	Teicoplanin	30 µg	2	3	95	MS	R
	Vancomycin	30 µg	0	0	100	R	R
Inhibitors of protein synthesis							
Tetracyclines	Doxicycline	30 µg	100	0	0	S	S
	Minocyclin	30 µg	100	0	0	S	S
	Tetracyclin	30 µg	100	0	0	S	S
Aminoglycosides	Gentamicin	10 µg	2	0	98	S	R
	Neomycin	30 µg	0	0	100	R	R
Macrolides	Clarithromycin	15 µg	93	5	2	S	MS
	Erythromycin	15 µg	100	0	0	S	S
	Josamycin	30 µg	100	0	0	S	S
	Spiramycin	100 µg	100	0	0	S	S
Lincosamides	Clindamycin	2 µg	100	0	0	S	S
	Lincomycin	2 µg	14	0	86	R	R
Single antibiotics	Chloramphenicol	30 µg	100	0	0	S	S
Inhibitors of nucleic acid synthesis							
Rifamicins	Rifampicin	5 µg	100	0	0	S	S
New quinolones	Enoxacin	10 µg	0	0	100	R	R
	Ofloxacin	5 µg	0	68	32	MS	MS
	Pefloxacin	5 µg	0	0	100	R	R
Sulphonamides + trimethoprim	Sulphamethoxazole + trimethoprim	25 µg	0	0	100	R	R

[a] Susceptibility expressed as S (Susceptible), MS (Moderately Susceptible) or R (Resistant) [14].
* International Units.

was susceptible to all the inhibitors of protein synthesis tested with the exception of neomycin and lincomycin; *L.* GG was moderately susceptible to clarithromycin, resistant to gentamicin, neomycin and lincomycin and susceptible to all the other aminoglycosides tested.

3.3. Susceptibility to inhibitors of nucleic acid synthesis

All strains were sensitive to rifampicin and resistant to enoxacin, pefloxacin and sulphamethoxazole-trimethoprim; 68% and 32% of the strains were moderately susceptible or resistant to ofloxacin, respectively.

L. rhamnosus DSM 20021^T and *L.* GG were susceptible to rifampicin, moderately sensible to ofloxacin and resistant to enoxacin, pefloxacin and sulphamethoxazole-trimethoprim.

3.4. Biodiversity of the isolates

The results of RAPD-PCR and antibiotic-susceptibility analyses of the 65 assayed strains are reported in the form of dendrograms in Figure 1.

The strains tested were ascribable to *L. rhamnosus* subsp. at a 64% similarity level at least (Fig. 1a). The effective identity of the isolates as *L. rhamnosus* was confirmed by species-specific PCR (Fig. 2). The low level of similarity with the type strain *L. rhamnosus* DSM 20021 highlighted a strong intra-specific biodiversity between the strains assayed.

Only in some cases it was possible to point out a high similarity of strains belonging to the same dairy plant, while in other cases a high similarity was ascertained between strains deriving from different dairy plants. This datum was also evidenced in Figure 1b.

Comparing similarity levels obtained from the dendrograms illustrated in Figure 1a and 1b, it was possible to observe the presence in the same cluster in both dendrograms only of certain strains.

4. DISCUSSION

The study of antibiotic resistance in strains of *L. rhamnosus* isolated from Parmigiano Reggiano gave different results.

The lactobacilli tested were susceptible to 18 antibiotics and manifested resistance to six types of antibiotics, while different percentages of strains were resistant to seventeen other antibiotics.

Among the inhibitors of cell wall synthesis, the *L. rhamnosus* strains assayed in the present work were susceptible to most penicillins, β-lactamase inhibitors and, among single antibiotics, to imipenem.

In particular, ampicillin, cloxacillin, mezlocillin, piperacillin and ticarcillin were strong inhibitors, while a low inhibition was ascertained with the use of amoxicillin and oxacillin.

Charteris et al. [14] tested the susceptibility of different *Lactobacillus* species to 44 antibiotics and, among the inhibitors of cell wall synthesis, the assayed strains exhibited susceptibility to almost all penicillins, cephalosporins and β-lactamase inhibitors.

In the study of Temmerman et al. [40], *L. rhamnosus* strains isolated from probiotic products were resistant to penicillin G.

Holley and Blaszyk [22] reported that penicillin G is more active than either amoxicillin or ampicillin against lactobacilli cultures, but our study showed a greater inhibitory action of ampicillin on *L. rhamnosus* strains from Parmigiano Reggiano cheese.

Beta-lactamase inhibitors, which are considered to be active on Gram-positive and Gram-negative bacteria, evidenced a great susceptibility of the strains isolated from Parmigiano Reggiano cheese, while a different response to cephalosporins was observed. In fact, among this group of antibiotics, cefazolin, cefoperazone and cefuroxime showed inhibitory effects, while a high resistance was observed to cefadroxil, cephalexin, cefixime and ceftriaxone. The response to ceftazidime was strain-specific: 43% were moderately sensible, 24% were

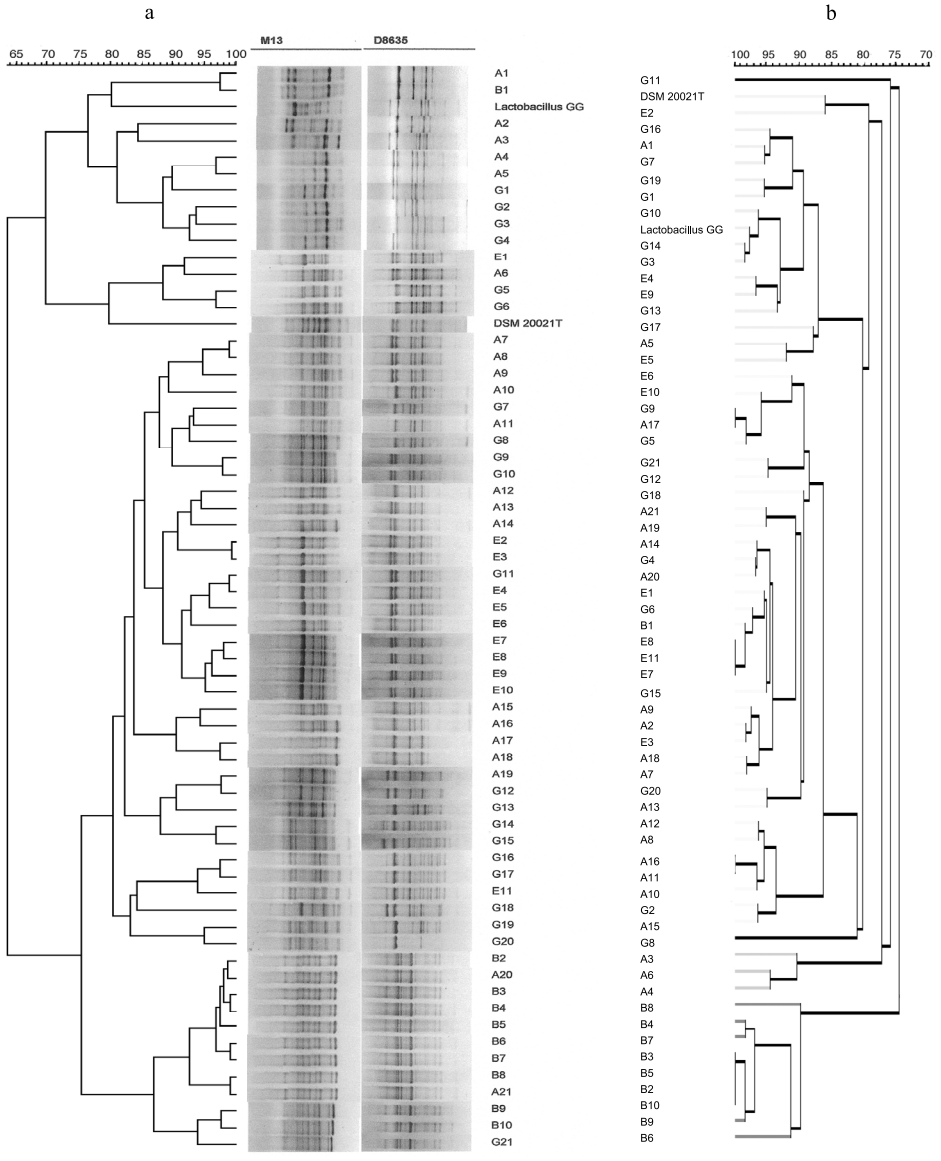

Figure 1. Cluster analysis obtained by RAPD-PCR performed with primers M13 and D8635 (a) and by antibiotic-resistance profiles (b) of 63 *Lactobacillus rhamnosus* strains isolated from Parmigiano Reggiano cheese, of the commercial strain *L.* GG and of the type strain *L. rhamnosus* DSM 20021.

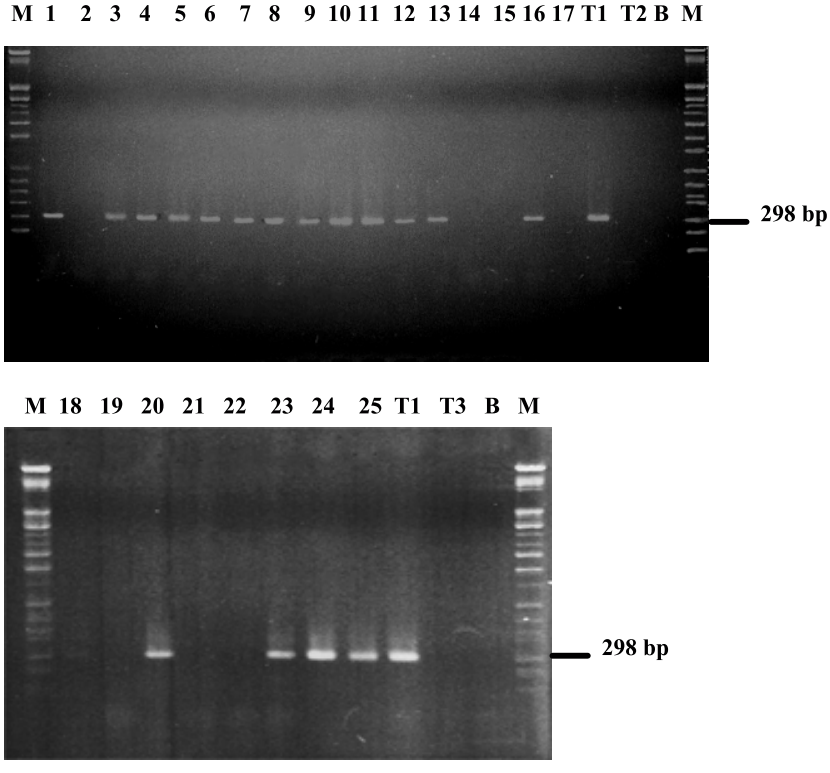

Figure 2. *Lactobacillus rhamnosus* species-specific PCR performed with primers *rhamn* and Y2 [45] on 17 lactobacilli isolated from Parmigiano Reggiano cheese. Lane M: molecular weight marker; B: blank; T1: *Lactobacillus rhamnosus* type strain DSM 20021; T2: *Lactobacillus paracasei* subsp. *paracasei* type strain DSM 5622; T3: *Lactobacillus casei* type strain DSM 20011; lanes 1, 3–13, 16, 20, 23–25: lactobacilli isolated from cheese, identified as *Lactobacillus rhamnosus* with RAPD-PCR analysis and belonging to different clusters; lanes 2, 14, 15, 17–19, 21, 22: *Lactobacillus paracasei* subsp. *paracasei* from the DISTAAM (University of Molise) collection and isolated from food.

sensible and 33% were resistant. A similar variability in the susceptibility of lactobacilli to cephalosporins was also evidenced by other authors [12, 14].

As previously described among single inhibitors of cell wall synthesis, imipenem revealed inhibitory effects on all the strains. This datum is in contrast to that shown in the study performed on *Lactobacillus* species from the Chr. Hansen Culture Collection by Danielsen and Wind [17].

Among other single antibiotic inhibitors of cell wall synthesis, vancomycin revealed a resistance of all the assayed strains, while bacitracin and teicoplanin revealed a high percentage of resistance. The inhibitory effects of these antibiotics on *Lactobacillus* species were previously reported by other authors [8, 12, 14].

Vancomycin and teicoplanin are both active against most Gram-positive bacteria. A number of *Lactobacillus* species, however, are intrinsically resistant to glycopeptides [18, 23, 39]. Differential susceptibility to vancomycin may be helpful in speciation of lactobacilli. The finding that all the strains

tested in the present study were resistant to this antibiotic is in accordance with the results of Hamilton-Miller and Shah [20] and with previous studies, which have shown that the vancomycin resistance in lactobacilli is intrinsic and chromosomally encoded [11].

Among the inhibitors of protein synthesis, tetracyclines (doxicycline, minocyclin and tetracycline) showed a great inhibitory action on the assayed lactobacilli. These results are in agreement with those reported by Charteris et al. [14] and by Baumgartner et al. [8]. However, in some cases a resistance to tetracycline was observed in *L. plantarum* strains [12].

Among aminoglycosides, gentamicin is especially indicated for the treatment of infections by Gram-negatives, and its inhibition potency showed an insignificant inhibition on the tested strains. The same result was evidenced with the use of neomycin, which, although very toxic, is regarded as an antibiotic for general purposes. For this reason, it is exclusively used for topical purposes or combined with bacitracin to fight against dysentery. Charteris et al. [14] reported the resistance of lactobacilli to aminoglycosides (amikacin, gentamicin, kanamicin, netilmicin and streptomycin) with variability in susceptibility only to netilmicin, whereas Cebeci and Gürakan [12] reported a high variability in susceptibility to gentamicin in *L. plantarum* strains. On the other hand, Baumgartner et al. [8] showed a high susceptibility of *L. rhamnosus* strains to gentamycin and neomycin.

Macrolides showed a strong inhibitory action on *L. rhamnosus* strains isolated from Parmigiano Reggiano cheese, while Baumgartner et al. [8] evidenced the susceptibility of both *L. plantarum* and *L. rhamnosus* strains to erythromicin.

Among lincosamides, clindamycin inhibited all the assayed strains, while the majority of them were resistant to lincomycin (86%). Lincomycin and clindamycin are especially active against Gram-negative bacteria. Moreover, the inhibitory action of clindamycin on lactobacilli has been widely shown by many authors [8, 12, 22], even if Charteris et al. [14] evidenced a resistance of some *L. casei* or *L. plantarum* strains to this antibiotic.

Chloramphenicol exerted a strong inhibitory action on all the assayed strains. Sensibility of *Lactobacillus* species to chloramphenicol was ascertained by many authors [5, 8, 14, 27], but in some cases a resistance to this antibiotic was observed [13, 29, 40].

Among the inhibitors of nucleic acid synthesis, a strong resistance of *L. rhamnosus* strains was observed against sulphonamides plus trimethoprim. The resistance to sulphamethoxazole found is not surprising since this antibiotic acts positively toward Gram-positive and -negative cocci and Gram-negative bacilli. This was in agreement with that shown by other authors [14, 27].

Among new quinolones, enoxacin and pefloxacin inhibited all the assayed strains; no susceptibility was ascertained to ofloxacin, but 68% of the strains were moderately susceptible to this antibiotic. Charteris et al. [14] reported the resistance of potentially probiotic lactobacilli to all quinolones, except ciprofloxacin.

The susceptibility of the assayed strains to rifampicin was previously described by Charteris et al. [14], but other authors have described a strong resistance to this antibiotic [8, 12].

The results obtained in the present study with regard to the susceptibility of *L. rhamnosus* strains isolated from Parmigiano Reggiano cheese to different antibiotics partially agree with those obtained by other authors, as previously evidenced. In some cases, the response to the different classes of antibiotics seems to depend on the species but, inside the species, it was possible to observe a strain-specific response for the antibiotic resistance.

RAPD-PCR and antibiotic-susceptibility analyses highlighted a strong intra-specific biodiversity between the strains tested.

The findings of strains isolated from the same sample of Parmigiano Reggiano cheese with very dissimilar profiles of RAPD-PCR bands testify the importance of the use of natural whey starter in this type of cheese as a source of a strong biodiversity [16]. On the other hand, the similarity in the profiles of RAPD-PCR bands for strains isolated from different samples of Parmigiano Reggiano cheese evidenced the influence of the raw milk, obtained from pastures of the same, defined geographic area, used in the manufacture of this kind of cheese.

The observation of RAPD-PCR (Fig. 1a) and antibiotic susceptibility profiles (Fig. 1b) gave way to some considerations.

In fact, only some strains, e.g., B2, B4, B5, B6, B7, B8, B9 and B10, belonged to the same cluster in both dendrograms. This result highlights the biodiversity among the strains isolated and allows the supposition that only in a few cases the presence of sister colonies could occur.

In fact, strains with a great similarity resulting from RAPD-PCR analysis (see strains B2 and A20 in Fig. 1a) generally showed very different antibiotic-susceptibility profiles. This could be imputable to the fact that RAPD-PCR technique is based on the random amplification of genomic DNA regions by specific primers, and the antibiotic-susceptibility/resistance factor is chromosomally coded [41]. However, it can be assumed that when lactic acid bacteria live in association with other microbes, the resulting contact with other bacteria is a precondition for horizontal gene transfer with the aid of conjugative transposons and plasmids [41].

Finally, Çataloluk and Gogebakan [11] pointed out that the presence of the resistance genes in the majority of the lactobacilli of intestinal origin suggests that transfer of such genes from an unknown origin during the passage from the intestinal tract is more likely.

This possibility worried microbiologists for a long time and today there is a strong tendency to avoid the distribution of bacteria with mobilisable antibiotic resistances.

The fact that *L. rhamnosus* strains isolated from Parmigiano Reggiano cheese gave different responses to the assayed antibiotics demonstrates the importance of individually testing the strains for their use as probiotics.

Moreover, the finding of resistance of *L. rhamnosus* GG to eighteen antibiotics (amoxicillin, oxacillin, cefadroxil, cephalexin, cefixime, ceftazidime, ceftriaxone, cefuroxime, ticarcillin-clavulanic acid, bacitracin, teicoplanin, vancomycin, gentamicin, neomycin, lincomycin, enoxacin, pefloxacin and sulphamethoxazole) suggests that it has become more and more imperative to regularly test the behaviour of all strains to be proposed as probiotics.

This is in agreement with the results obtained by Vanderhoof et al. [43] and Klein et al. [28] and allows the conclusion that the antibiotic resistance ascertained in *L. rhamnosus* GG is natural.

On the other hand, antibiotic resistance can be easily transferred via plasmids which, as stated by Tynkkynen et al. [42], are absent in *L.* GG.

This evidence, however, does not consent to conclude that all strains are plasmid-free and further investigations could point out the presence of plasmids in other strains ascribable to *L. rhamnosus*.

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