

PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF *LACTOBACILLUS SAKEI* ISOLATED FROM PORTUGUESE FERMENTED MEAT PRODUCTS FOR TETRACYCLINE RESISTANCE

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Abstract –The resistance profile presented by 31 different *Lactobacillus sakei* starter strains was investigated. These strains ability to function as resistant gene pools has granted an important focus, not only but also, regarding tetracycline resistance. Antibiotic susceptibility testing was performed by disc diffusion of the putative resistance responsible main genes, tet(K), tet(L) and tet(M) detection was done by multiplex-PCR. We found that between 87% and 97% of strains were resistant to vancomycin and trimethoprim–sulfamethoxazole. Also most strains were susceptible to protein synthesis inhibitors such as erythromycin, dalfopristin-quinupristin both with 19% resistance and chloramphenicol, and tetracycline with 29% resistant strains. For the phenotypically identified tetracycline resistant strains only one was detected as carrier for the tet(M) gene while 7 of the strains carried the tet(K). No strains were found to carry tet(L). Also, genetic resistance determinants for macrolides, as major antibiotic therapeutic alternatives, should also be evaluated for these same strains. Still, from our results, *L. sakei* can be selected as safe starter strains to be used on Portuguese traditional meat fermented products.

Key Words – Antibiotic resistance, LAB, Tet(M)

• INTRODUCTION

The genus *Lactobacillus* belongs to the lactic acid group of bacteria (LAB), these are fastidious, gram positive bacteria found in different environments from plant material, through manure, till meat and dairy products [1]. Regarding *Lactobacillus* spp. in food the status of generally regarded as safe (GRAS) has been attributed to these microorganisms mainly due to their frequent used on a large-scale as starter cultures in food industries (e.g. in the production of fermented milk products or sausages) without imposing a health risk for the consumers or the environment [1, 2]. Although most species proved to be susceptible to the majority of tested antibiotics resistance to tetracycline and erythromycin has been occasionally detected [4]. Lactobacilli potential to pose as antibiotic resistance gene transfer vectors, capable of supplying antibiotic resistance genes to food-borne or enteric pathogens, associated with their broad distribution in various environments translates into a potentially major concern for public health safety [5, 7].

Tetracyclines are a group of broad-spectrum antibiotics whose general usefulness has been reduced with the onset of bacterial resistance. Tetracycline resistance (Tc^R) can be mainly due to two different mechanisms. Associated with the activity of ribosomal protection proteins, RPPs, linked to the expression of various tet genes (e.g. tet(M)) and also with efflux proteins as those encoded by tet(K) and tet(L) [5, 7, 8]. Both efflux and RPP encoding genes are commonly associated with mobile genetic elements and is mostly acquired and passed by horizontal gene transfer to other, either commensal or pathogenic, bacteria [5, 7, 8].

• MATERIALS AND METHODS

The *Lactobacillus sakei* tested strains used in this study belonged to the collection of the *Laboratório de Tecnologia e Segurança dos Alimentos at the Faculty of Veterinary Medicine of Lisbon (UTL)*. *L. sakei* strains (n=31) were isolated from different Portuguese fermented/dry/smoked meat products (Linguiça, Paio, Chouriço de vinho, Chouriço de carne, Chouriço and Salsichão grosso) originating from different industries on Alentejo region (South Portugal) and collected during 2011/12. Strains were grown on MRS agar for 48h at 30°C under adequate anaerobic conditions.

Antibiotic susceptibility testing by disc diffusion was performed as described in CLSI,[6] for 7 different antibiotics: vancomycin, 30µg; trimethoprim–sulfamethoxazole, 25µg; erythromycin, 15µg; tetracycline, 30µg; gentamicin, 10µg; chloramphenicol, 30µg; and dalfopristin–quinupristin, 15µg. *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 were used as quality control strains. Strains were kept for 48h at 30°C under adequate anaerobic conditions for posterior halo size readings.

Detection of Tc^R genes was performed by multiplex-PCR according to Gevers *et al.*[3] for 3 tetracycline resistance associated genes *tet(K)*; *tet(M)* and *tet(L)*. Control strains were kindly provided by Prof. Constança Pomba and included *Staphylococcus aureus* PR7/08, gene *tet(M)* and *tet(K)* carrying strain and *Staphylococcus aureus* 124.1, gene *tet(M)* and *tet(L)* carrying strain.

• RESULTS AND DISCUSSION

Phenotypic strains characterization regarding antibiotic susceptibility results are shown in Figure 1. As previously described heterofermentative Lactobacilli such as *L. sakei* are intrinsically resistant to vancomycin, as well as trimethoprim/sulfamethoxazole [7, 8]. Our results revealed that, 87% (n=27) and 97% (n=30) resistant strains, for both mentioned antibiotics respectively. Lactobacilli are typically susceptible to protein synthesis inhibitors. However was reported resistance for erythromycin, 19% (n=6); chloramphenicol, 29% (n=9); dalfopristin–quinupristin, 19% (n=6) and tetracycline, 29%, (n=9). Aminoglycosides presented an exception, which was also corroborated in the present study with 61% of resistance (n=19).

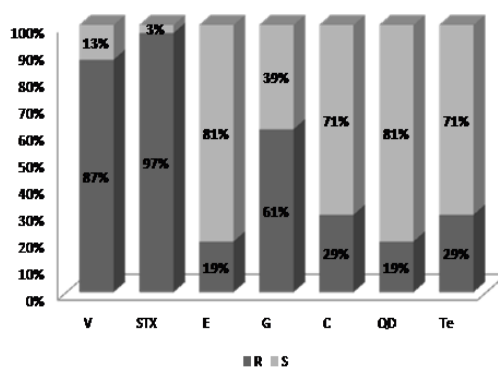


Figure 1. Disc susceptibility testing results for all tested *L. sakei* strains. V, vancomycin; STX, Trimethoprim/sulfamethoxazole; E, erythromycin; G, gentamicin; C, chloramphenicol; QD, dalfopristin–quinupristin and Te, tetracycline. R indicates resistance and S indicates susceptibility

Resistance to tetracycline has been mainly attributed to the existence of, not only, ribosomal protection proteins, encoded by one or more of *tet(M)*, *tet(O)* and *tet(W)*. But also, to *tet* efflux genes of which *tet(K)* and *tet(L)* have been primarily found in gram positive bacteria such as

LAB [5, 8]. Both mentioned resistance mechanisms have been linked to mobile transferable genetic elements [5, 8]. In our study was found that none of the tested strains carried the *tet(L)* gene and although some strains, 7, carried the *tet(K)* gene; no correlation was assured between the occurrence of this efflux gene and the development of resistance due to the fact that the gene was only present in 2 of the 9 resistant strains and also present in phenotypically susceptible strains (n=5). On the other hand, *tet(M)* was described as the main gene attributed to tetracycline resistance, although from our preliminary results we were only able to detect one resistant strain carrying this gene further PCR protocol optimization it will be required before inferring an alternative resistance mechanism [5, 7, 8]. As previously described both *tet(L)* and *tet(M)* are, in different ways, mainly responsible for tetracycline resistance patterns. Of the tested 31 *L. sakei* strains only one carried both genes *tet* genes and expressed such resistance [5, 7, 8].

Lactic acid bacteria such as the fermented meat product starter *L. sakei* may act as relevant genetic reservoir for antibiotic resistance genes that are horizontally transferable to either commensal or pathogenic bacteria. Still, from our results, *L. sakei* can be selected as safe starter strains to be used on Portuguese traditional meat fermented products.

• CONCLUSIONS

Tetracycline resistance was found for 29% of tested strains. None of the strains had the *tet(L)*, 7 strains carried the *tet(K)* although 2 were phenotypically resistant. It was only possible to confirm the existence of *tet(M)* for one strain. This same strain was *tet(K)* positive and expressed Tc^R. Still, from our results, *L. sakei* can be selected as safe starter strains from all susceptible to antibiotics, and be used on Portuguese traditional meat fermented products.

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