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Enterocin AS-48RJ: a variant of enterocin AS-48 chromosomally encoded by *Enterococcus faecium* RJ16 isolated from food

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Abstract

The bacteriocinogenic strain RJ16 isolated from goat cheese has been identified as *Enterococcus faecium* by species-specific PCR, DNA–rRNA hybridization and rDNA sequencing. Purified bacteriocin from strain RJ16 is a carboxypeptidase A-resistant peptide with a molecular mass (7125 Da) very close to the cyclic peptide enterocin AS-48. Bacteriocin from strain RJ16 and AS-48 show identical antibacterial spectra, although the former is slightly less active on strains of *Listeria monocytogenes* and *Bacillus cereus*. Producer strains show cross-immunity. PCR amplification of total DNA from strain RJ16 with primers for the AS-48 structural gene and sequencing of the amplified fragment revealed an almost identical sequence (99.5%), except for a single mutation that predicts the change of Glu residue at position 20 of AS-48 to Val. Therefore, bacteriocin produced by *E. faecium* RJ16 should be considered a variant of AS-48, which we call AS-48RJ. PCR amplification revealed that strain RJ16 contains the complete *as-48* gene cluster. Hybridization with probes for *as-48* gene cluster revealed a chromosomal location of *as-48* genes in strain RJ16, being the first example of a chromosomal location of this bacteriocin trait. Strain RJ16 produced enzymes of interest in food processing (esterase, esterase lipase and phytase activities), and did not decarboxylate amino acids precursors for biogenic amines. Strain RJ16 did not exhibit haemolytic or gelatinase activities, and PCR amplification revealed the lack of genes encoding for known virulence determinants (aggregation substance, collagen adhesin, enterococcal surface protein, endocarditis antigens, as well as haemolysin and gelatinase production). Strain RJ16 was resistant to ciprofloxacin (MIC $> 2\,mg\,l^{-1}$) and levofloxacin (MIC $> 4\,mg\,l^{-1}$) and showed intermediate resistance to nitrofurantoin and erythromycin, but was sensitive to ampicillin, penicillin, streptomycin, gentamicin, rifampicin, chloramphenicol, tetracycline, quinupristin/dalfopristin, vancomycin and teicoplanin. Altogether, results from this study suggest that this broad-spectrum bacteriocin-producing strain may have a potential use in food preservation.

Keywords: Bacteriocin; *Enterococcus faecium*

Abbreviations: ABTS, 2-2′ azino-bis-(3 ethylbenzthiazoline-6-sulphonic acid; BSH, bile salt hydrolase

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Introduction

Enterococci are lactic acid bacteria widely distributed in the environment. The main habitat is the intestine of humans and warm-blooded animals [63]. They can be found in foods of animal origin (milk, cheese, fermented sausages), vegetables and plant materials because of their ability to survive heat treatments and adverse environmental conditions [35]. They also play an important technological role in manufacture of meat and dairy products [29,38,44] like development of aroma and ripening of different cheeses. However, they have been involved in food spoilage [27], in food intoxication [34,39], in nosocomial infections [47] and in the spreading of antibiotic resistance through the food chain [37,63], rising concerns about the safety of strains found in foods.

Enterococci fight competing bacteria by secreting a variety of anti-microbial proteins or peptides (bacteriocins). Enterococcal bacteriocins may be good candidates for food preservation [36], especially those with a broad-spectrum of inhibition like the cyclic peptide AS-48 (reviewed by Maqueda et al. [56]). In accordance with consumers demands for healthy, safe and fresh food, the interest in natural preservatives, including bacteriocins, is increasing and the use of bacteriocinogenic lactic acid bacterial as starters or as protective cultures in food fermentation processes offers promising perspectives [12,39].

The purpose of this study was to isolate strains of lactic acid bacteria producing broad-spectrum bacteriocins for future food applications. Strain RJ16 isolated from goat cheese was selected because it produced a broad-spectrum antimicrobial substance. This strain was further identified as *Enterococcus faecium*. Characterization at biochemical and genetic level of the bacteriocin produced by this strain indicates that it is a variant of the broad-spectrum cyclic peptide AS-48 produced by *E. faecalis* S-48 [32]. The chromosomal location of the genetic determinants of this bacteriocin and the absence of known virulence traits of the producer strain are also discussed in this work.

Materials and methods

Bacterial strains and growth conditions

The enterococcal strains used in this study and their sources are listed in Table 1. Strain RJ16 isolated from home-made goat cheese was selected as bacteriocin producer for its broad antimicrobial activity. *E. faecalis* A-48-32 [58] was used to produce bacteriocin AS-48, and *E. faecalis* S-47 [31] was used as test strain to determine bacteriocin activity. Other bacterial strains used to establish an inhibitory spectrum are listed in Table 2. All strains were grown in Brain–heart infusion broth (BHI) (Sharlab, Barcelona, Spain) or MRS (Sharlab) at 37 °C for 18 h. Solid media were prepared by adding 1.7% agar (BHI-agar or MRS-agar). Enterococci were routinely stored at 4 °C and maintained as frozen stocks at −80 °C in 40% glycerol.

Table 1. Bacterial strains and primers used in this study

	Strain or description	Reference or source
Bacteria		
E. faecalis	A-48-32	[58]
	S-47	[30]
	EJ97	[33]
	CECT 481	CECT
E. faecium	RJ16	This study
	CECT 410	CECT
E. durans	CECT 411T	CECT
E. hirae	CECT 214	CECT
E. mundtii	CECT 972	CECT
Primers		
Est AS-48F	5′-TATATCTAGAAATGAAATGCATTTCAA-3′	This study
Est AS-48R Res	5′-TATAGCATGCTATCATATTGTTAAATTA-3′	This study
AS-48F	5′-ATTTATACAAGCATAGGATCT-3′	This study
Res AS-48R	5′-CTTCTGACGCAGGCATTACA-3′	This study
ResBF	5′-ACTAACCTTAATAAAATCAG-3′	This study
$ResD_1R$	5′-ATCTTTCCTAACATAGAAT-3′	This study
cadf	5′-ATTGCATTATTTAGTTTAGT-3′	This study
cadr	5′-TGTTGCACCAGTTACCAC-3′	This study

CECT: Colección Española de Cultivos Tipo.