PURIFICATION AND MOLECULAR CHARACTERIZATION OF A TWO COMPONENT LACTOCOCCAL BACTERIOCIN

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The broad-spectrum bacteriocin produced by *L. lactis* IFPL105, isolated from raw goats´milk (Casla *et al.*, 1996), was purified to homogeneity by ionic and hydrophobic exchange and reverse phase chromatography. **Its activity consisted of two distinct peptides (α and β)** with average molecular masses of 3320 and 2848 Da (Fig. 1)

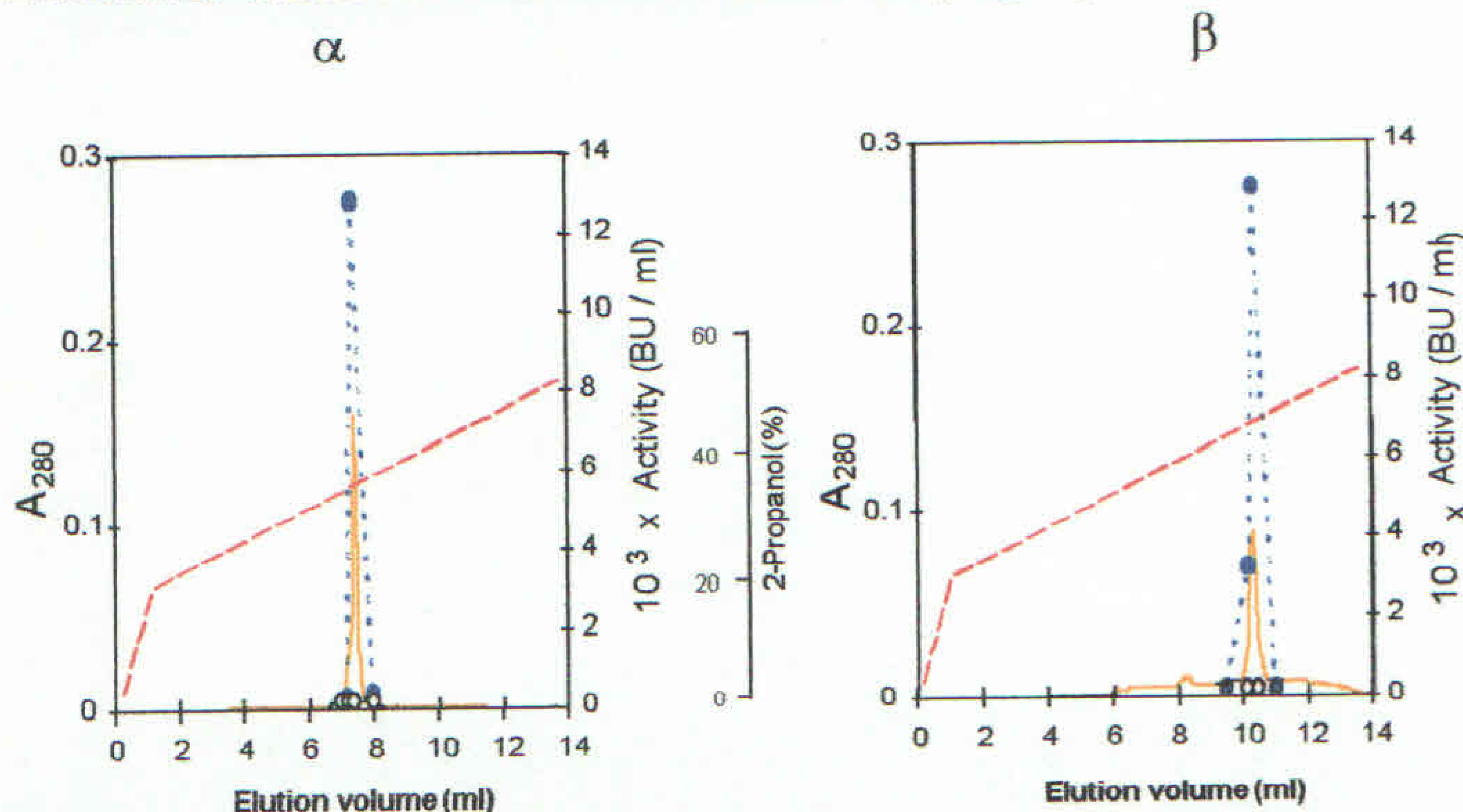

Fig. 1. Reverse -phase chromatography of the α and β peptides of the bacteriocin specified by plasmid pBAC105. Bacteriocin activity is indicated without (open circles) and with complementation (closed circles) of one peptide with the other. —— A_{280}; ------ 2-propanol.

The amino acid sequence of an internal fragment of the β peptide was found to be identical to an internal sequence of the peptide LtnB of lacticin 3147, one of the two components of the bacteriocin encoded in plasmid pMRCO1 of *L. lactis* DPC3147 (Dougherty *et al.*, 1998). Using PCR, **the genes encoding the α and β peptides** were cloned and sequenced and **were found to be identical to *ltnAB* of pMRCO1.**

Hybridization experiments showed that **the organization of the gene cluster in pBAC105** responsible for the production of the bacteriocin in *L. lactis* IFPL105 **is similar to that in pMRCO1**, and includes genes encoding enzymes homologous to those for lantibiotic biosynthesis and export (Fig. 2).

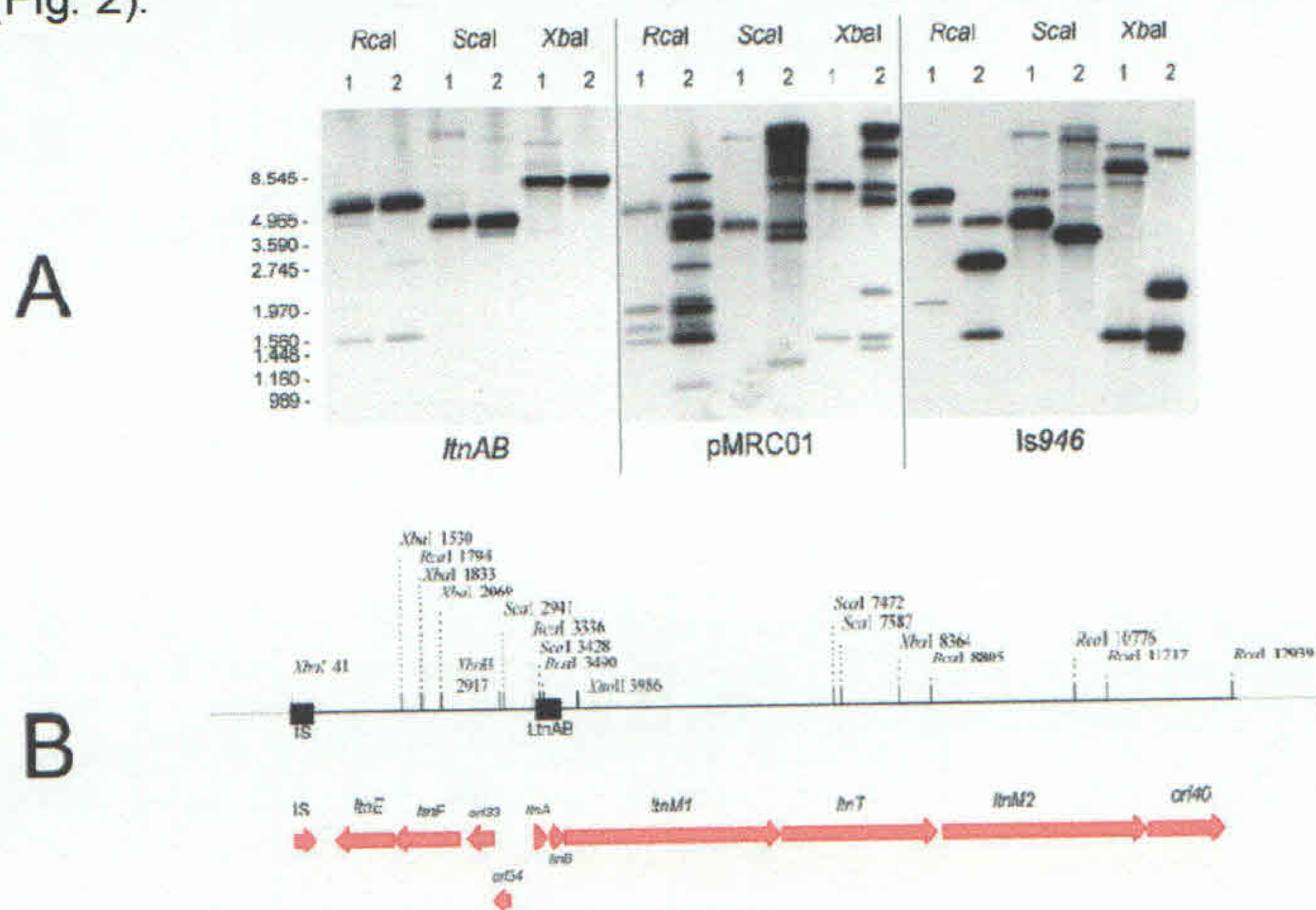

Fig. 2. (A) Southern hybridization analysis of *RcaI*, *ScaI*, and *XbaI* digests of the plasmids pBAC105 (1) and pMRC01 (2). The probes used were a PCR fragment of *ltnAB*, a *Sau3A* digest of plasmid pMRC01, and a PCR fragment of *IS946*. (B) Map of the region of plasmid pMRC01 responsible for bacteriocin production (Dougherty *et al.*, 1998). The black boxes, indicated LtnAB and IS represent the PCR fragments used as probes in A.

Comparison of the determined molecular masses and composition of the purified α and β peptides with those predicted for mature LtnA and LtnB **suggests post-tanslational modification of the peptides** (Table 1).

Amino acids	Peptide α		Peptide β	
	α[a]	LtnA[b]	β[a]	LtnB[b]
Trp[c]	ND	3	ND	0
Asp + Asn	4	4	1	1
Glu	1-2	1	0	0
Ser	0	3	0	3
Gly	2-3	2	0	0
His	1	1	0	0
Arg	0	0	1	1
Thr	0	4	3	8
Ala	3-4	2	7	4
Pro	0	0	3-4	3
Tyr	1	1	1	1
Val	0	0	0	0
Met	1	1	0	0
Cys	3	4	3	3
Ile	0	0	3	3
Leu	2	2	1-2	1
Phe	1	1	0	0
Lys	1	1	1	1
Total	20-23	30	24-26	29
Molecular mass[d]	3322	3428.4	2848	2985.5

Table 1. [a] The number of amino acid residues was calculated from the molar ratio relative to His (α) and Arg (β) after hydrolysis in 6 M HCl of the purified peptides. Unidentified residues are not shown.
[b] Amino acid residues predicted from the gene sequence after processing of LtnA and LtnB at the putative Gly-X cleavage site.
[c] Not determined.
[d]Molecular masses determined for the purified peptides (Mass Spectrometry) and predicted for mature LtnA and LtnB.

Lysis of logarithmic phase lactococcal cells is observed after the addition of the bacteriocin, but only **when an active autolysin (AcmA) is present** (Fig. 3).

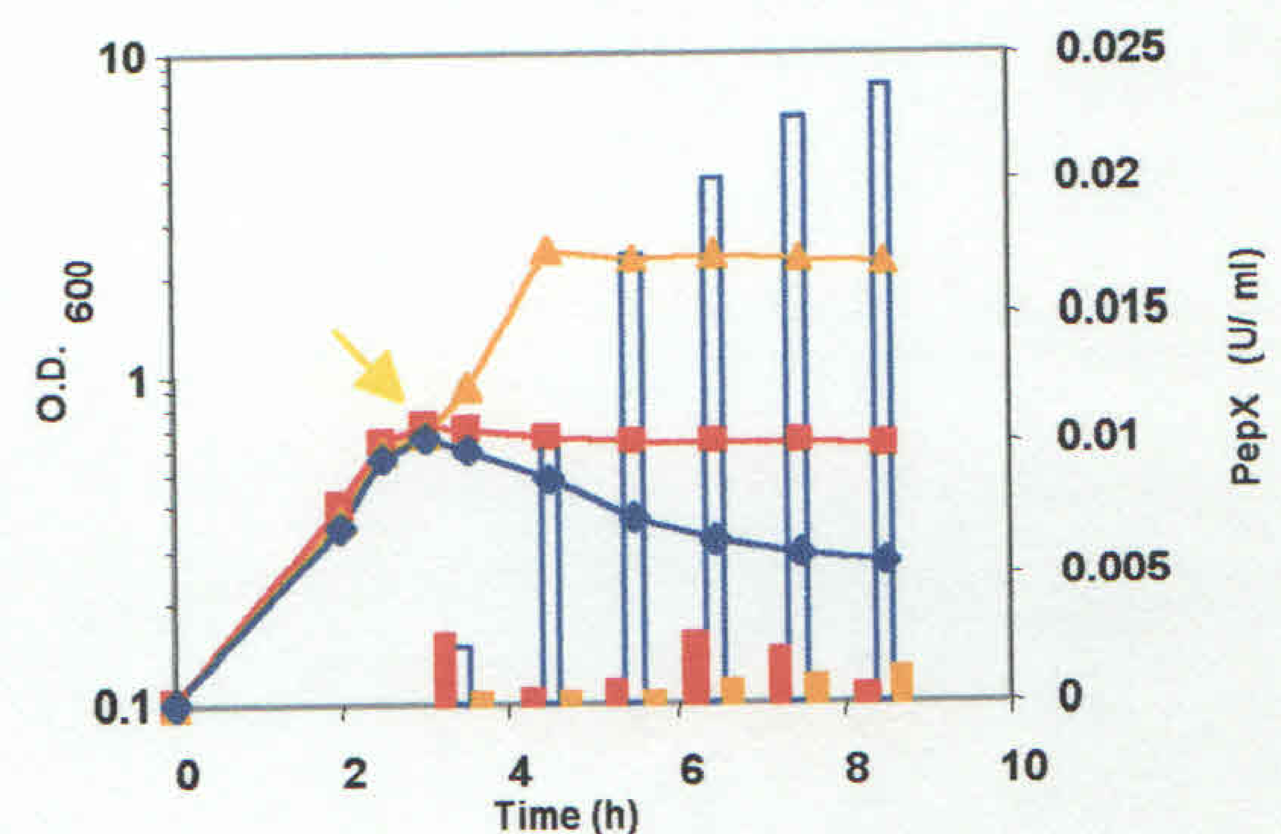

Fig. 3. Evolution of OD_{600} (lines) and release of PepX (bars) during growth at 30 °C of *L. lactis* MG1363 in M-17 broth (▲; orange bars) and during incubation with 300 AU/ml of bacteriocin of *L. lactis* MG1363 (◆; open bars) and *L. lactis* MG1363*acmAΔ1* (■; red bars). Arrow indicates point of bacteriocin addition.

Cell wall fragments of *L. lactis* MG1363, or of *L. lactis* MG1363*acmAΔ1* to which extracellular AcmA was added, were incubated with 300 UA/ml of bacteriocin. As this had no effect on lysis of these cell walls, this indicates that **the bacteriocin does not activate AcmA.**

The bacteriolytic effect was shown to be the result of loss of viability due to the action of the bacteriocin followed by the uncontrolled action of AcmA.

References

- Casla, D., Requena, T. and Gómez, R. (1996) *J. Appl. Bacteriol.* **81**, 35-41
- Dougherty, B. A., Hill, C., Weldman, J.F., Richardson, D.R., Venter, J.C and Ross, R.P. (1998) *Mol. Microbiol.* **29**, 1029-1038.