EXPRESSION, PURIFICATION AND ANTIMICROBIAL ACTIVITY OF RECOMBINANT PEDIOCIN PA-1 M31L, A PA-1 DERIVATIVE WITH ENHANCED STABILITY

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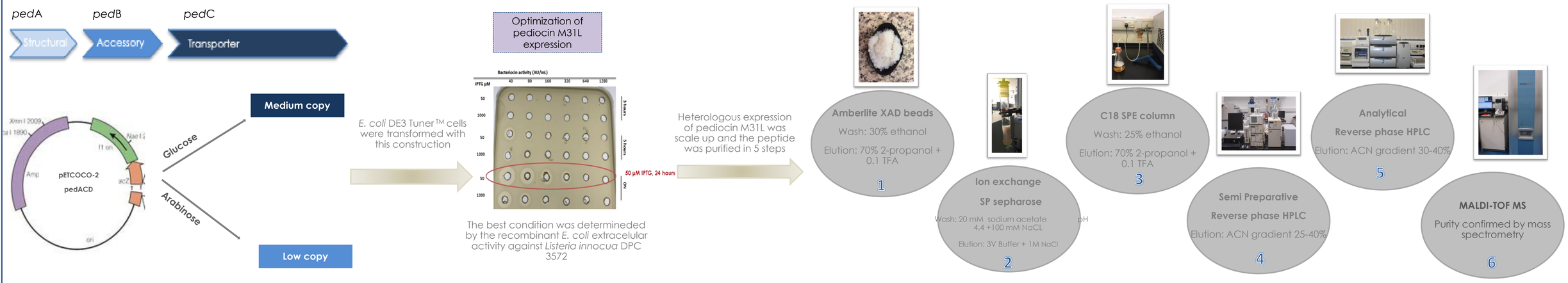
Introduction

Pediocin, the prototypical class IIa bacteriocin, is an efficient antilisterial molecule. Loss of pediocin PA-1 activity is attributed to methionine oxidation at position 31 and this can be overcome by substituting methionine for leucine (pediocin M31L). The aim of this study was to produce pediocin M31L with enhanced stability by recombinant expression in *E. coli* cells.

Methods



Results

Cloning of pediocin genes into the expression vector

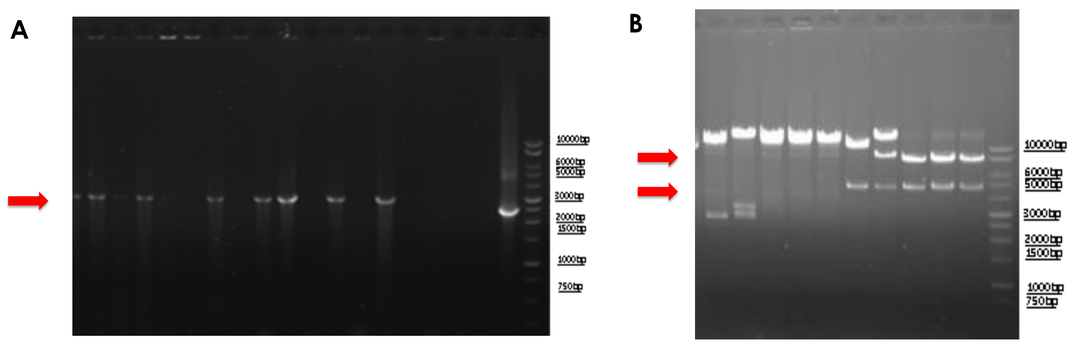


Figure 1. Confirmation of pediocin PA-1 genes in the expression vector petCOCO-2 by PCR (A) and restriction analysis (B). Red arrows indicade the expected fragment size in bp

The modified (M31L) structural, accessory and transport genes from the pediocin PA-1 operon were redesigned using codon bias for *E. coli and* after digestion with *SphI* and *AvrII* enzymes, the product was cloned into a modified pETCOCO-2 vector.

Heterologous expression of Pediocin M31L in *E. coli* DE3 Tuner ™ cells

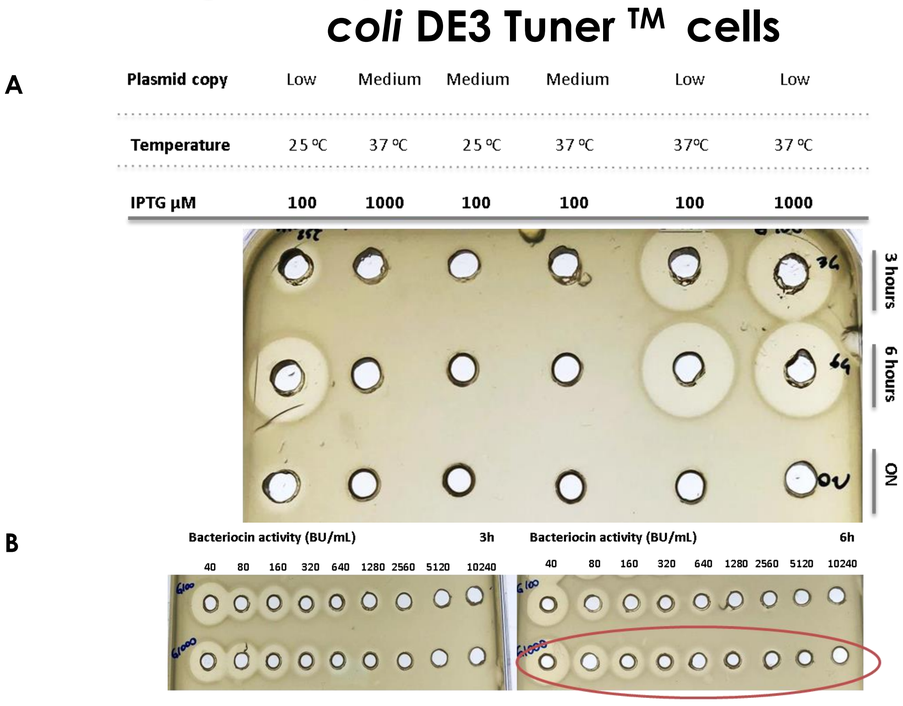


Figure 2. Antimicrobial activity of cell free supernant produced by recombinat *E. coli* against *L. Innocua* DPC 3572. Optimization of heterologous expression was assessed under different conditions including plasmid copy number (low vs medium), temperature (25 ºC vs 37 ºC) and IPTG concentration (0.1 mM vs 1 mM) (A). Bacteriocin activity (BU/mL) was quantified as follows: a^b /c where 'a' represents the dilution factor, 'b' the last dilution that produces an inhibition zone and 'c' the volume added in mL

Purification of pediocin PA-1 and pediocin M31L

Recombinant pediocin PA-1 M31L and natural pediocin PA-1 produced by *Pediococcus acidilactici* LMG 2351 were purified as described above. Pediocin PA-1 M31L with a mass of 4606 Da (theoretical mass of 4606 Da) was obtained at > 95% purity with a yield of 0.7 mgL^{-1} of culture.

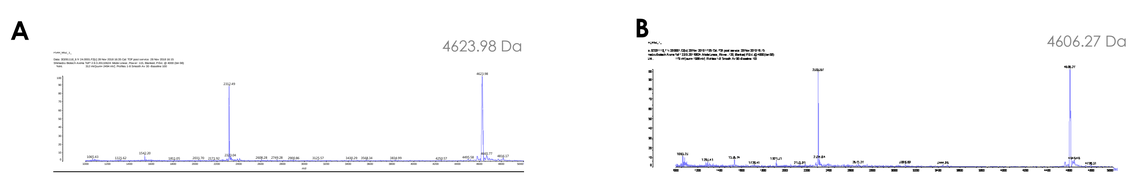


Figure 3. MALDI-TOF MS data. The mass of 4623.98 Da was detected for pediocin PA-1 produced by *P. acidilactici* LMG 2351 (A) and 4606.27 Da for the recombinant pediocin PA-1 M31L (B).

Comparison of antimicrobial spectrum and temperature stability of natural pediocin PA-1 and recombinant pedioicin M31L

	Pediocin PA-1	Pediocin M31L
Listeria Innocua DPC3572	++++	++++
L. monocytogenes DPC6894	+++	+++
L. monocytogenes DPC6893	+++	+++
L.monocytogenes DPC3362	++++	++++
Faecalibacteririum prausnitzii A2-165	–	–
Eubacterium rectale A1-86	–	–
Roseburia inulinivorans A2-194	–	–
Akkermansia muciniphila MucT	–	–
Ruminococcus bromii VPI 6883	–	–

Table 1. Spectrum of antimicrobial activity of pure pediocin PA-1 produced by *P. acidilactici* LMG2351 and recombinant pediocin M31L expressed in *E. coli* cells. Inhibition zones mesuared in millimeters: (+++++) > 20 mm; (++++) 15 -19.9 mm; (+++) 10 -14.9mm; (++) 5-9.9 mm; (+) < 5mm; (-) no inhibition zone.

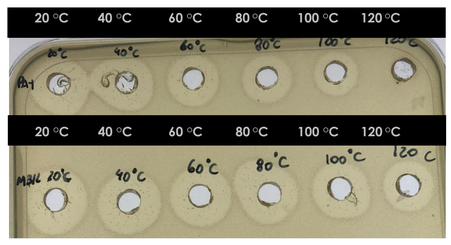


Figure 4. Temperature stability of natural pediocin PA-1 and recombinant pediocin M31L at 20, 40, 60, 80, 100 and 120ºC for 30 minutes. Antimicrobial activity was assessed against *L. Innocua* DPC3572.

Conclusions

Pediocin PA-1 M31L was successfully expressed and purified from recombinant *E. coli* cells. The pediocin M31L derivative displayed similar heat stability and antimicrobial activity to its natural form.

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