Cloning of pediocin PA-1 and its immunity genes from *Pediococcus acidilactici* K7 using pAMJ shuttle vector into *Lactococcus lactis* MG1363

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Received 17 October 2007; revised 25 March 2008; accepted 20 June 2008

The matured pediocin PA-1 encoding gene, *pedA* and its immunity counterpart, *pedB* from *Pediococcus acidilactici* K7 were cloned by PCR technique and ligated into the *E. coli*-lactic shuttle, protein expression vector pAMJ2008. The recombinant pAMJAB was constructed and sequenced to confirm the intactness of reading frame of the deduced pediocin PA-1 fusion protein. *Lactococcus lactis* MG1363 was electroporated with the plasmids and the transformation efficiency of ~ 2.3 × 10^3 μg^−1^ was observed. This was further confirmed by plasmid DNA isolation and analysis.

**Keywords**: *Pediococcus acidilactici*, pediocin PA-1, electroporation, P170 expression system, *Lactococcus lactis*

Heterologous expression of proteins in a food-grade organism such as lactic acid bacteria (LAB) is found to have immense commercial applications. Food-grade systems not only offer high quantity but also an economic production of the target protein. LAB, due to their GRAS status are being employed, of late, for the heterologous expression of various enzymes, hormones, bioactive compounds, antigens and membrane proteins. The large number of host-vector systems that have been developed are found to be valuable tools due to their food-grade controlled expression of foreign genes. Among them, the P170^6^, nisin inducible^5^ and the sugar inducible controlled expression systems are known to be commercially available^5^.

P170 expression system constitutes an important module of gene expression in *Lactococcus lactis* due to several reasons. Host bacterium *L. lactis* requires simple fermentation technology wherein the amount of native protein produced in the culture filtrate (CF) is low. Besides, P170 offers other advantages such as limited proteolytic activity, N-terminal signal peptide for secretion and a promoter regulated by pH and growth phase and elimination of the need for addition of an exogenous inducer^1^.

Pediocin PA-1 is a 4.6 kDa cationic membrane acting protein produced by *Pediococcus acidilactici*, that is being used for biopreservation and pharmaceutical applications^7^.

A laboratory isolate of *P. acidilactici* K7 producing pediocin PA-1 was used as a source for the pediocin PA-1 biosynthesis genes. The culture was grown in de Man, Rogosa and Sharp (MRS) broth (Himedia, Mumbai) at 37°C in a static condition. The total DNA of *P. acidilactici* K7 was isolated by the method of Mora et al^12^ and used as a template for PCR. The oligonucleotide primers (Sigma Aldrich) used for PCR cloning of *pedAB* genes were LlpF: 5′ CCAATATCATTGGAGATCTATACTACG 3′ as forward and BexR: 5′ CAAATATCCTTGAGATCTATACG 3′ as reverse primers. The restriction sites for *BglII* and *PstI*, marked in bold were introduced in the forward and reverse primers, respectively. PCR was carried out in a Perkin-Elmer (USA) thermocycler using Deep Vent DNA polymerase (MBI, Fermentas, Luthiana), following standard procedure described by Sambrook and Russell^13^.

The PCR product obtained (~528 bp) was double digested with *BglII* and *PstI*, purified by using Qiagen column and ligated to the double digested vector pAMJ2008 using T4 DNA ligase (MBI) to obtain the recombinant pAMJAB. Transformants were

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obtained using *E. coli* DH5α and analyzed by plasmid DNA isolation (alkaline-lysis method) and restriction digestion. Partial sequencing of the recombinant plasmid was carried out using the primer P170 fwd 5’CTGCTCCTCCTCTCCCTA GTGC 3’ at the University of Delhi, South Campus, New Delhi. For the construction of recombinant molecule and fusion protein translation, Clone Manager Ver. 4.0 was used.

The vector pAMJ2008 and the recombinant pAMJAB were electroporated into the competent cells of *Lactococcus lactis* MG1363 as per the protocol described in the P170 expression system manual (Biotechnologie Institute, Denmark) using the electroporation equipment of Biorad (Gene pulser X cell™). The conditions set for electroporation pulse were capacitance, 25 µF; voltage, 2 KV and resistance 200 Ω using a 0.2 cm cuvette. The transformed colonies of MG1363 were selected based on their growth on M17 agar medium (Hi Media) containing 0.5% glucose and erythromycin (Hi Media), 1 µg/mL. Plasmids from *L. lactis* MG1363 were isolated by the method of O’Sullivan and Klaenhammer followed by gel analysis.

In order to express matured pediocin PA-1 (without signal peptide) and its immunity counterpart, *pedAB* genes were cloned by PCR and ligated to the shuttle vector pAMJ2008 into its *BglII/PstI* sites. The deduced translated fusion protein was obtained upon DNA sequencing (Fig. 1). The result shows the intactness of the reading frame with the DAQA amino acids at the N-terminal of the target protein that acts as a recognition site for cleavage by signal peptidase. The translated fusion protein has the following features: The expected size of PedA fusion protein should be 8.5 kDa and the matured pediocin PA-1 of 5 kDa is expected to be secreted into the culture filtrate.

The partial DNA sequence of *pedB* gene was also obtained that had been truncated at 3’end due to incomplete sequencing. Sequencing results in combination with BLAST analysis further confirm cent per cent homology of *pedAB* genes with that reported in literature, Acc nos. M83924 and our previously reported sequences. In this study, *pedB* gene was cloned to co-express in combination with *pedA* gene since *L. lactis* MG1363 is sensitive to the

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**Fig. 1**—Construction of recombinant plasmid pAMJAB: a-Restriction digestion and analysis of the pAMJAB plasmid. Recombinant pAMJAB digested with *Hind* III (lane 1) and double digested with *BglII/PstI* enzymes (lane 2). M is 100 bp ladder (MBI Fermentas). Arrow indicates the insert release of expected size; b-molecular map of recombinant and diagrammatic representation of pediocin fusion protein. Arrow indicates position of proteolytic cleavage site; and, C-deduced amino acid sequence and fusion protein construct of SP310mut-pedAB obtained upon nucleotide sequencing.
pediocin PA-1 (data not shown). In heterologous expression of pediocin in LAB, co-expression of immunity protein is required since majority of the lactic hosts are sensitive to the pediocin PA-1.

The *E. coli* shuttle vector pAMJ2008 and the recombinant were electroporated into the host *L. lactis* MG1363 and the efficiency of transformation was calculated based on colonies obtained in M17 agar plate containing erythromycin as a selection marker. The results indicated high efficiency transformation. The transformation efficiency was $2 \times 10^3$ recombinant $\mu$g$^{-1}$ of DNA when compared to the vector ($3 \times 10^3$ transformants $\mu$g$^{-1}$). The field strength and time constant for electroporation was also within the range of expected results (data not shown). Transformation results were further confirmed by plasmid DNA isolation and analysis (Fig. 2). The two independent clones were subjected to gel analysis and compared with the plasmid isolated from *E. coli*.

Results described in this study show the successful construction of the recombinant plasmid pAMJAB molecule and upon its restriction analysis and sequence determination, electroporation into *L. lactis* MG1363. Since *L. lactis* is one of the GRAS status bacteria, approaches towards the production of pharmaceutical compounds and bioactive peptides are attractive. Further, P170 expression system is an environmentally regulated gene expression system being used recently for the production of several antigens and bioactive peptides. In this study, we made an attempt to clone pediocin PA-1 gene for its hyper-expression. For bacteriocins that are being produced at very low amount by the native microorganisms and require complex cultivation medium for the growth, recombinant DNA approach is being suggested. Since pediocin PA-1 is an antilisterial low molecular weight peptide to be used for food conservation and pharmaceutical purposes, heterologous production using P170 system is an attractive concept. Further studies on the optimization of conditions for expression of biologically active peptide is underway.

**Acknowledgement**

Authors are thankful to the Biotechnologie Institute, Denmark for providing the P170 expression system. We express our gratitude to Dr V Prakash, Director, CFTRI Mysore for the facilities.

**References**


![Fig. 2 — Agarose gel electrophoretic analysis of the plasmid isolated from *L. lactis* MG1363. R1 and R2, two independent pAMJAB recombinants; V, vector; H, DNA isolated from host MG1363, as a control and Er, pAMJAB isolated from *E. coli* DH5α by alkaline lysis. M is a λ *Bst*II digest (MBI Fermentas). Arrow indicates the expected size of the plasmid observed.](image-url)


