Molecular characterisation of new organisation of \textit{plnEF} and \textit{plw} loci of bacteriocin genes harbour concomitantly in \textit{Lactobacillus plantarum} I-UL4

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Abstract

\textbf{Background:} Bacteriocin-producing Lactic acid bacteria (LAB) have vast applications in human and animal health, as well as in food industry. The structural, immunity, regulatory, export and modification genes are required for effective bacteriocin biosynthesis. Variations in gene sequence, composition and organisation will affect the antimicrobial spectrum of bacteriocin greatly. \textit{Lactobacillus plantarum} I-UL4 is a novel multiple bacteriocin producer that harbours both \textit{plw} and \textit{plnEF} structural genes simultaneous which has not been reported elsewhere. Therefore, molecular characterisation of bacteriocin genes that harboured in \textit{L. plantarum} I-UL4 was conducted in this study.

\textbf{Results and discussion:} Under optimised conditions, 8 genes (\textit{brnQ1}, \textit{napA1}, \textit{plnL}, \textit{plnD}, \textit{plnEF}, \textit{plnI}, \textit{plnG} and \textit{plnH}) of \textit{plnEF} locus and 2 genes (\textit{plw} and \textit{plwG}) of \textit{plw} locus were amplified successfully from genomic DNA extracted from \textit{L. plantarum} I-UL4 using specific primers designed from 24 \textit{pln} genes selected randomly from reported \textit{plw}, \textit{plS}, \textit{pln423} and \textit{plnF} loci. DNA sequence analysis of the flanking region of the amplified genes revealed the presence of two \textit{pln} loci, UL4-\textit{plw} and UL4-\textit{plnEF} loci, which were chromosomally encoded as shown by Southern hybridisation. UL4-\textit{plw} locus that contained three ORFs were arranged in one operon and possessed remarkable amino acid sequence of LMG2379-\textit{plw} locus, suggesting it was highly conserved. Interestingly, the UL4-\textit{plnEF} locus appeared to be a composite \textit{pln} locus of JDM1-\textit{plnEF} and J51-\textit{plnEF} locus in terms of genetic composition and organisation, whereby twenty complete and one partial open reading frames (ORFs) were aligned and organised successfully into five operons. Furthermore, a mutation was detected in \textit{plnF} structural gene which has contributed to a longer bacteriocin peptide.

\textbf{Conclusions:} Plantaricin EF and plantaricin W encoded by \textit{plnEF} and \textit{plnW} loci are classified as class I bacteriocin and class II bacteriocin molecules respectively. The concurrent presence of two \textit{pln} loci encoding bacteriocins from two different classes has contributed greatly to the broad inhibitory spectrum of \textit{L. plantarum} I-UL4. The new genetic composition and organisation of \textit{plnEF} locus and concurrent presence of \textit{plnEF} and \textit{plnW} loci indicated that \textit{L. plantarum} I-UL4 is a novel multiple bacteriocin producer that possesses vast potentials in various industries.

\textbf{Keywords:} Molecular characterisation, Genetic organisation, Genetic location, \textit{pln} genes, \textit{plnEF} locus, \textit{plw} locus, Bacteriocin gene, \textit{Lactobacillus plantarum} I-UL4

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Background

Lactic acid bacteria (LAB) is a group of bacteria frequently isolated from food. LAB genera that have important role in food and animal industries are Lactococcus, Leuconostoc, Pediococcus, Lactobacillus, and Streptococcus [1]. Extensive reports have shown LAB have capability to produce various compounds, such as acetic acid, hydrogen peroxide, ethanol, diacetyl and bacteriocins that contribute to the inhibitory effects to pathogenic microorganisms [2, 3]. Bacteriocins are ribosomal synthesised peptides or proteins that release extracellularly to inhibit bacteria closely related to the producing strains [4]. The inhibitory activities are mainly mediated through pore formation on cytoplasmic membrane or by inhibiting cell wall synthesis of sensitive bacteria [5–7]. Bacteriocins and bacteriocin-producing LAB have received special attention due to their potential applications in human and animal health, as well as in food industry [8–11]. The structural, immunity, regulatory, export and modification genes of bacteriocin that commonly arrange into one or more operon structures are required for effective bacteriocin biosynthesis [12, 13].

Despite a number of bacteriocins produced by Lactobacillus plantarum that generally known as plantaricin have been described [14–18], only a few plantaricin (pln; with italic formatted is used to describe gene) loci have been characterised genetically. The structure and organisation of pln loci may be simple or complex. The relatively simple pln loci are found in one operon, such as plw locus that encodes Class I two-peptide plantaricin W [19], pls locus that encodes Class IIb plantaricin S [20] and pln423 locus that encodes Class IIa plantaricin 423 [21]. The relatively complex pln locus is plnEF locus that distributes widely among L. plantarum isolated from various ecological niches. The well characterised plnEF locus has been reported for L. plantarum C11 [22], WCFS1 [23], JDM1 [24], J23 [25], J51 [26], NC8 [27] and V90 [28]. The reported plnEF loci have been designated as plnEF locus for L. plantarum JDM1, C11, WCFS1, V90, J51, NC8 and J23 respectively. The size of the reported plnEF loci are between 18 and 19 kb with 22 to 26 genes and they are organised in five to six operons in mosaic like structure encoding four types of class IIb plantaricins and three regulatory networks [28].

Probiotic effects of bacteriocin-containing postbiotic produced by L. plantarum have been reported for rats and livestock animals [29–34]. The bacteriocin-containing postbiotic of L. plantarum 1-UL4 isolated from tapai ubi (fermented tapioca, a Malaysian traditional fermented food) has been shown to has broad inhibitory spectrum against various Gram-positive (Bacillus cereus, Staphylococcus aureus, Streptococcus pneumoniae, Enterococcus faecalis, Enterococcus faecium and Pediococcus acidilactici) and Gram-negative bacteria (Escherichia coli and Salmonella typhimurium) [35, 36]. According to Moghadam et al. [37], L. plantarum 1-UL4 is a multiple bacteriocin producer that harbours both plw and plnEF structural genes. The simultaneous detection of both plw and plnEF that encode for plantaricin W and plantaricin EF respectively has not been reported elsewhere [37]. Furthermore, the genetic loci of plnEF are in high plasticity and possess many variable regions with respect to their mosaic genetic composition and regulatory network [28]. Hence, the characterisation of pln loci is important as variations in gene sequence, gene composition and organisation will affect the antimicrobial spectrum of bacteriocin that release in extracellular environment. In addition, new open reading frame (ORF) can be discovered in close proximity to the known bacteriocin genes. Therefore, molecular characterisation of plnEF and plw loci of bacteriocin genes that harbour concomitantly in Lactobacillus plantarum 1-UL4 were conducted in this study.

Results and discussion

pln genes of L. plantarum 1-UL4

The pln genes of L. plantarum 1-UL4 were detected by PCR using gene-specific primers designed from 24 pln genes selected randomly from reported plw [19], pls [20], pln423 [21] and plnEF [22, 27] loci. Under optimised conditions, 8 genes (brnQ1, napA1, plnL, plnD, plnC, plnM, plnN, plnO and plnK) of plnEF locus and 2 genes (plw and plwG) of plw locus were amplified successfully. The identities of amplified pln genes were further confirmed by DNA sequence analyses, whereby high DNA sequence identity (ranging from 96 to 100%) that correspond to respective pln gene was obtained for all amplified DNA fragments (Table 1). In contrast, 11 pln genes (plnA, plnB, plnC, plnM, plnN, plnO, plnP, plnJ, plnK, plnC8, plnC8HK) of plnEF locus and all the selected genes from pls and pln423 loci were absent in the studied strain as confirmed further by gradient PCR analysis, inferring that L. plantarum 1-UL4 harbours plw and plnEF loci simultaneously as reported by Moghadam et al. [37]. Although several studies reported the presence of plnEF gene in bacteriocinogenic L. plantarum isolated from fermented foods by PCR screening, none of the reported isolates harboured plw structural gene [38–40] simultaneously. In addition, only plnEF structural gene was found in the complete genome sequence of L. plantarum WCFS1 [23] and L. plantarum JDM1 [24]. Therefore, L. plantarum 1-UL4 is the first L. plantarum strain that has been reported to harbour both plw and plnEF structural genes concomitantly, which have contributed greatly to the broad inhibitory spectrum of bacteriocin-containing postbiotic produced by L. plantarum 1-UL4 against
Table 1 Nucleotide sequence characteristics of PCR-amplified pln genes harboured in Lactobacillus plantarum I-UL4 in comparison to the pln genes reported for Lactobacillus plantarum JDM1, C11, WCFS1, V90, JS1, NC8, J23 and LMG2379

| pln genes | Length (bp) | Function of gene | Nucleotide sequence identity (%) |
|-----------|-------------|------------------|----------------------------------|
|            |             |                  | JDM1   | C11  | WCFS1 | V90   | JS1   | NC8   | J23   | LMG2379 |
| bmQ1       | 1,088       | Amino acid transport protein | 98     | ND    | 98    | ND    | 98    | ND    | ND    | ND     |
| napA1      | 738         | Na+/H+ antiporter      | 98     | ND    | 99    | ND    | 99    | 98    | 98    | ND     |
| plwG       | 279         | Prebacteriocin         | ND     | ND    | ND    | ND    | ND    | ND    | ND    | ND     |
| plwH       | 558         | Immunity              | 98     | 98    | 98    | 98    | 98    | 98    | 98    | 99    |
| plnH       | 382         | Putative immunity protein | 98    | 96    | 96    | 96    | 96    | 96    | 96    | ND     |
| plnD       | 365         | Response regulator     | 96     | 96    | 96    | 95    | 96    | 100   | 96    | ND     |
| plnI       | 394         | ABC transporter        | 99     | 99    | 99    | 98    | 99    | ND    | ND    | ND     |
| plnF       | 926         | Accessory protein      | 99     | 98    | 98    | 98    | 98    | ND    | ND    | ND     |
| plnG       | 279         | Prebacteriocin         | 99     | 99    | 99    | 98    | 99    | 99    | 99    | 99    |
| plw        | 797         | ABC transporter        | ND     | ND    | ND    | ND    | ND    | ND    | ND    | ND     |

ND not detected; the respective gene was not detected in the reference strain.

Various Gram-positive (Bacillus cereus, Staphylococcus aureus, Streptococcus pneumoniae, Enterococcus faecalis, Enterococcus faecium and Pedicoccus acidilactici) and Gram-negative bacteria (Escherichia coli and Salmonella typhimurium) as reported by Lim [35] and Thanh et al. [36]. Moreover, the pln genes in plnEF locus of L. plantarum I-UL4 [UL4-plnEF locus; for simplicity, the ORF, peptide or locus of a strain was abbreviated as (name of the strain)-(ORF, peptide or locus)] were different from the reported plnEF loci.

Characterisation of UL4-plw locus

The upstream and downstream DNA sequences of plw and plwG were amplified and analysed from genomic DNA of L. plantarum I-UL4 (plw loci of L. plantarum I-UL4 were deposited at GenBank/EMBL/DDBJ with accession number of GU322921). A contig of 2.77 kb termed UL4-plw locus was successfully assembled and DNA sequence analysis of UL4-plw locus revealed the presence of three ORFs (plwβ, plwα and plwG) that arranged in one operon with same orientation. Both plwβ and plwα were 100% identical to LMG2379-plwβ and LMG2379-plwα respectively [19]. plwα and plwβ are the structural genes that encode for Class I two-peptide lantibiotic, plantaricin W, whereby the mature peptides are modified to contain lanthionine, methyl Lanthionine and dehydrated residues [19]. The last ORF, plwG, that encoded for ABC-transporter was highly similar (more than 99.7% identities) to LMG2379-plwG [19].

Characterisation of UL4-plnEF locus

The upstream and downstream DNA sequences of bmQ1, napA1, plwL, plnD, plnEF, plnI, plnG and plnH in plnEF locus were successfully amplified and sequenced from genomic DNA of L. plantarum I-UL4 and a contig of 17.58 kb that designated as UL4-plnEF locus was obtained by careful alignment and assembly (plnEF loci of L. plantarum I-UL4 were deposited at GenBank/EMBL/DDBJ with accession number of GU138149). The amino acid sequence of deduced peptides encoded by UL4-plnEF locus and the reported plnEF loci are shown in Table 2. Figure 1 shows the putative promoters that were searched manually by sequence alignment and comparison to the promoter sequences reported for pln operons. The promoter sequences that identified in the UL4-plnEF locus were consisted of a pair of direct repeat which was located at the upstream of −35 region. Each pair of the repeats was separated by a spacer of 12 nucleotides that rich in adenine and thymine. The characteristic of promoters identified in UL4-plnEF locus were highly identical to the reported plnEF loci [28]. The direct repeat pair is important for the regulation of bacteriocin biosynthesis at transcriptional level as this consensus direct repeat serves as DNA binding sites for response regulator (RR) to initiate the transcription process [41, 42]. Changes in nucleotide sequence of the repeat such as point substitutions, deletion of repeat or alteration in the length of spacer region can abolish or reduce the binding of RR and subsequently suppress the gene expression [43]. The promoter motifs of pln operons were also found in other bacteriocin systems such as gene cluster of sakacin A [44, 45], sakacin P [46, 47], carnobacteriocin A [48], carnobacteriocin B2 [49] and enterocin A [50, 51], indicating similar regulatory mechanism was used for the production of various bacteriocins.

Biocomputational analyses of UL4-plnEF locus revealed the presence of 20 complete and one partial ORFs. The comparison of genetic organisation of UL4-plnEF locus and reported plnEF loci are illustrated in a genetic map as shown in Figure 2. Five putative operons...
that preceded by a putative promoter were deduced from the UL4-plnEF locus. The operons of orf345, plnLR, and plnEFI were predicted to encode for a two-peptide bacteriocin and immunity protein respectively. The UL4IF-UL4HK-plnD operon was predicted to regulate bacteriocin production at transcriptional level. The last predicted operon, plnGHTUVW, was responsible for maturation and secretion of bacteriocins and bacteriocin-like peptides as proposed by Diep et al. [22, 28].

Three ORFs of brnQ1, napA1 and DHelicase that amplified and sequenced from the genomic DNA of L. plantarum I-UL4 were also found in the reported operons. However, their functions have not been related to any bacteriocin production.

### Table 2 Characteristics of the predicted ORFs encoded by UL4-plnEF locus amplified from Lactobacillus plantarum I-UL4

| Predicted ORFs | Orientation (+ or −) | Nucleotide coordinates | Gene and peptide length (bp: aa) | 15 bp upstream of the start codon (5′−3′) | Homologous gene and function | Re-designated as |
|---------------|----------------------|------------------------|--------------------------------|------------------------------------------|-----------------------------|------------------|
| ef1           | +                    | 829–2,205              | 1,377: 458                     | GGAGGAGAGGACT                              | brnQ1: amino acid transporter | brnQ1            |
| ef2           | +                    | 2,238–3,434            | 1,197: 398                     | TAAAGACCTTTGATGG                            | napA1: Na+/H+ antiporter    | napA1           |
| ef3           | +                    | 3,809–3,982            | 174: 57                        | GAAAAGGTGATAAAA                            | orf3: putative bacteriocin  | orf3            |
| ef4           | +                    | 3,998–4,177            | 180: 59                        | AAAGAAATGGTAAAA                            | orf4: putative bacteriocin  | orf4            |
| ef5           | +                    | 4,283–4,525            | 243: 80                        | TTGGTTGCTTTTATTA                            | orf5: putative immunity protein | orf5            |
| ef6           | −                    | 4,970–5,083            | 114:37                         | GTAAGGCACACGTATA                            | plnR: unknown               | plnR            |
| ef7           | −                    | 5,108–5,776            | 669: 222                       | CTCGGGGGATTAAAA                            | plnL: putative immunity protein | plnL            |
| ef8           | +                    | 6,369–6,536            | 168: 55                        | GAGGGGTTATTATT                              | Putative induction factor | UL4IF           |
| ef9           | +                    | 6,554–7,894            | 1,341: 446                     | TAGGGTTGCTTTCA                              | HK: histidine Protein Kinase | UL4HK           |
| ef10          | +                    | 7,895–8,638            | 744: 247                       | TTGGAGGAAAAATGA                            | plnD: response regulator    | plnD            |
| ef11          | −                    | 8,932–9,705            | 774: 257                       | GGCGGATTTA                                  | plnI: immunity protein      | plnI            |
| ef12          | −                    | 9,784–9,963            | 180: 59                        | GGGGAGATACAAATT                             | plnF: plantaricin EF precursor | plnF            |
| ef13          | −                    | 9,988–10,158           | 171: 56                        | CAGGGGGATTATT                              | plnE: plantaricin EF precursor | plnE            |
| ef14          | +                    | 10,424–12,574          | 2,151: 716                     | GAGGGGAGTACAAGT                             | plnG: ABC transporter       | plnG            |
| ef15          | +                    | 12,591–13,967          | 1,377: 458                     | GGGGAAATCTGAATA                             | plnH: accessory protein     | plnH            |
| ef16          | +                    | 14,057–14,746          | 690: 229                       | CGAAAGAGGAAGTTA                            | plnT: unknown               | plnT            |
| ef17          | +                    | 14,814–15,482          | 669: 222                       | CTTGGGAGGCTTTGAT                              | plnU: unknown               | plnU            |
| ef18          | +                    | 15,569–16,249          | 681: 226                       | TGGATGGGAAAGAC                              | plnV: unknown               | plnV            |
| ef19          | +                    | 16,343–17,029          | 687: 228                       | GATGGAGTGATTGAAA                            | plnW: unknown               | plnW            |
| ef20          | +                    | 17,167–17,370          | 204: 67                        | AGGGAGTTGTTAAT                              | orfZ1: unknown               | UL4orfZ1        |
| ef21          | −                    | 17,465–17,588          | >124:40                        | ND                                          | DHelicase: DNA helicase      | DHelicase       |

Underlined nucleotides are putative RBS. No RBS could be detected for ef5 which was re-designated as plnR. ef8 did not show homology to any entries in database but the deduced peptide sequence contained GG motif. ef21 was partially sequenced and hence upstream sequence of ef21 is not available.

ND not detected.

(orf345, plnLR, UL4IF-UL4HK-plnD, plnEFI and plnGHTUVW) that preceded by a putative promoter were deduced from the UL4-plnEF locus. The operons of orf345, plnLR and plnEFI were predicted to encode for a two-peptide bacteriocin and immunity protein respectively. The UL4IF-UL4HK-plnD operon was predicted to regulate bacteriocin production at transcriptional level. The last predicted operon, plnGHTUVW, was responsible for maturation and secretion of bacteriocins and bacteriocin-like peptides as proposed by Diep et al. [22, 28]. Three ORFs of brnQ1, napA1 and DHelicase that amplified and sequenced from the genomic DNA of L. plantarum I-UL4 were also found in the reported operons. However, their functions have not been related to any bacteriocin production.

Three-component regulatory system of UL4IF-UL4HK-plnD was detected in the UL4-plnEF locus as compared to plnABCD or plnC8IF-plnCHK-plnD regulatory operon that reported for plnEF locus by Diep et al. [28]. UL4IF was found to encode a putative induction factor (IF) that usually activates transcription process of regulated genes. The leader peptide of UL4IF contained a double-glycine (GG) motif and the mature peptide consisted of 28 amino acids. The calculated pl and the MW of the mature peptide was 11.26 and 3321.98 Da, respectively. The IFs that identified in bacteriocin
systems are a small bacteriocin-like peptide having several physicochemical properties of bacteriocin. Both IF and bacteriocin are synthesised as a precursor peptide containing GG leader peptide and hence the same maturation and secretion system has been suggested for both IF and bacteriocin. In addition, the mature peptide of both IF and bacteriocin has high pI and low MW. Although IF and bacteriocin share several physicochemical properties, IF can be discriminated from bacteriocin in the way that IF possess little or no bacteriocin activity and the gene encoding IF is always located in the same transcription unit and preceded the gene encoding histidine protein kinase (HPK) and RR [47, 48, 50, 52, 53].

UL4HK that encoded for HPK and plnD regulatory operon was semi-conserved in which plnD was found in all regulatory operons regardless of the HPK type, suggesting that the interaction between IF and HPK is highly specific while the interaction between HPK and RR is less specific. The results obtained in this study were further supported by the notion of antimicrobial activity of L. plantarum J23 containing plnC8IF-plnC8HK-plnD regulatory operon that only could be detected when induced by plnC8IF and not plnA [25].

The plnEFI operon encoded for plantaricin EF and its cognate immunity protein was present in L. plantarum I-UL4. No variation of amino acid was detected in UL4-plnE as compared to the reported plnE. However, UL4-plnF was seven amino acids longer than the reported plnF peptide due to the insertion of two nucleotides at the stop codon resulting in additional translation of seven amino acids N′-YSSSHQV- C′ prior to TAA stop codon (Figure 4). Thus, the mature UL4-plnF contains 41 amino acids with the calculated MW of 4.492 kDa, as compared to 34 amino acids of the reported plnF. The pI of UL4-plnF is 9.99 which is 0.28 unit lower than the plnF of the reported plnEF loci. A similar case was demonstrated by Rojo-Bezares et al. [25] for J23-plnJ, whereby J23-plnJ was reported to be 28 amino acids longer than the reported plnJ (55 amino acids) and a great reduction in antimicrobial activity was observed in the J23-plnJ

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| Operon | Strain | L-repeat | R-repeat | −35 | −10 |
|--------|--------|----------|----------|-----|-----|
| crf454S | UL4 | TACTATAATTGACT | TACTATAATTGACT | AAAAAAT | CTTTTTTT |
| crf454S | JS1 | TACTATAATTGACT | TACTATAATTGACT | AAAAAAT | CTTTTTTT |
| LR | UL4 | TACCATATTGACAT | TACCATATTGACAT | AAAAAAT | CTTTTTTT |
| LR | JS1 | TACCATATTGACAT | TACCATATTGACAT | AAAAAAT | CTTTTTTT |
| JKR | C11 | TACCATATTGACAT | TACCATATTGACAT | AAAAAAT | CTTTTTTT |
| JKR | JS1 | TACCATATTGACAT | TACCATATTGACAT | AAAAAAT | CTTTTTTT |
| JKR | V90 | TACCATATTGACAT | TACCATATTGACAT | AAAAAAT | CTTTTTTT |
| JKR | N08 | TACCATATTGACAT | TACCATATTGACAT | AAAAAAT | CTTTTTTT |
| JLR | V28 | TACCATATTGACAT | TACCATATTGACAT | AAAAAAT | CTTTTTTT |

**Figure 1** Putative promoters of UL4-plnEF locus that searched by DNA sequence alignment and comparison to the promoter sequences reported for plnEF loci. The promoters that identified in UL4-plnEF locus were consisted of a pair of direct repeat which was located at the upstream of −35 region. Each pair of the repeats was separated by a spacer of 12 nucleotides that are highlighted in grey-boxes. Putative −35 and −10 sequences are indicated with boldface.
peptide. However, the antimicrobial activity of the UL4-plnF has yet to be determined.

Another bacteriocin-like operon orf345 that previously described in L. plantarum J51 [26] was detected in UL4-plnEF locus as well. Operon UL4-orf345 contained three ORFs of orf3, orf4 and orf5, which was highly identical to those described for L. plantarum J51. However, one amino acid mismatch was detected in both orf3 and orf4 [26] respectively and GG leader peptide was detected in both UL4-orf3 and UL4-orf4 [54]. The mature peptide of UL4-orf3 and UL4-orf4 has highly cationic property with calculated pI of 11.45 and 9.87 respectively. Hence, UL4-orf345 operon resembles a bacteriocin and immunity operon encodes for a two-peptide bacteriocin together with its cognate immunity protein.

A class II bacteriocin, plantaricin JK together with its dedicated immunity and a hypothetical protein with unknown function were encoded by plnJKLR operon [22]. The plnJKLR operon was found as a degenerated operon, plnLR, in the UL4-plnEF locus. In addition, similar degenerated form of orfZ123 operon (orfZ1 alone) was detected in L. plantarum I-UL4. According to Diep et al. [28], this operon was degenerated greatly, whereby either orfZ1 or orfZ3 was detected in the reported plnEF loci.

**Figure 2** Genetic map for the comparison of genetic organisation of UL4-plnEF locus and reported plnEF loci of L. plantarum strains. ORFs are represented by arrow-blocks. The promoter sequences are indicated by small block arrows. brnQ1 and napA1 signify the upper boundary while DNA helicase signify the lower boundary of plnEF loci. The DNA sequence of UL4-DHelicase, J51-plnW, NC8-plnG and J23-plnY was partially analysed. C11-, WCFS1- and V90-plnEF loci were identical. However, brnQ1, napA1, plnS, plnT and DHelicase were not described in C11; brnQ1, napA1 and DHelicase were not described in V90, plnS and plnT were "fused" in V90. The genetic map was generated using information retrieved from GenBank with accession number of CP001617 (JDM1), X94434 (C11), NC_004567 (WCFS1), FJ809773 (V90), DQ340868 (J51), AF522077 (NC8) and DQ323671 (J23) respectively.
Bacteriocins with GG leader peptides were processed and secreted by a dedicated ABC-transporter. A highly conserved secretion operon, either plnGHTUVW or plnGHSTUVW was found in those reported plnEF loci. The major difference between plnGHTUVW or plnGHSTUVW operon is that plnT of plnGHTUVW operon is a fusion gene of plnS and plnT of plnGHSTUVW operon [28]. The secretion operon that detected in UL4-plnEF locus was plnGHTUVW operon. UL4-plnH appeared to be a fusion gene of plnS and plnT found in C11, WCF51, J51, NC8 and J23. UL4-plnT shared 99.1 and 96.9% amino acid sequence identity to JDM1- and V90-plnT, respectively. The plnTUVW encoded putative proteins that belong to Abi family and they contained protease CAAX motif [55]. It was noted that some identified bacteriocin immunity proteins belong to Abi family and Kjos et al. [56] have shown the involvement of several Abi proteins in bacteriocin self-immunity [28, 57, 58]. However, the role of plnTUVW in bacteriocin system still remains unknown.

**Genetic location of plw and plnEF loci**

The genetic location of plw locus has not been reported elsewhere. However, plnEF locus of *L. plantarum* WCF51

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**Figure 3** DNA sequence alignment of UL4HK with HPK of reported plnEF loci. Low amino acid sequence identity at N-terminal receptor domain of HPKs was detected. Amino acids that identical to UL4HK are represented by dot.
and *L. plantarum* JDM1 [24] have been reported to be located on chromosomal DNA. *L. plantarum* I-UL4 that employed in this study harboured multiple plasmids as shown by agarose gel electrophoresis of the genomic DNA (Figure 5). Therefore, Southern hybridisation of genomic DNA with three DNA probes, namely 16S probe, EF probe and W probe, were carried out to determine the genetic location of UL4-*plnEF* and UL4-*plw* loci that harboured in *L. plantarum* I-UL4. The 16S probe generated in this study was 100% complementary to the 16S rDNA sequence of *L. plantarum* I-UL4, which was specific to chromosomal DNA rather than plasmid DNA. The hybridisation signals generated by 16S probe would differentiate and confirm the identification of chromosomal DNA band from plasmid DNA bands separated by agarose gel electrophoresis. The hybridisation signal of EF probe and W probe were detected at the same DNA band as the 16S probe (Figure 5), indicating that *plnEF* and *plw* loci were located on chromosomal DNA since the location of 16S rDNA is only found in chromosomal DNA of *L. plantarum* I-UL4.

**Conclusions**

*L. plantarum* I-UL4 was shown to be a multiple bacteriocin producer, harbouring *plw* and new mosaic *plnEF* loci that chromosomally encoded as shown by Southern hybridisation. This is the first report of a *L. plantarum* strain harbouring the combination of *plw* and *plnEF* loci concomitantly. The plantaricin W and plantaricin EF encoded by *plw* and *plnEF* loci respectively are two different classes of bacteriocin, in which plantaricin W is a class I bacteriocin molecule while plantaricin EF is a class II bacteriocin molecule. UL4-*plw* locus was highly conserved and contained remarkable amino acid sequence of LMG2379-*plw* locus. However, the UL4-*plnEF* locus appeared to be a composite *pln* locus of JDM1-*plnEF* and J51-*plnEF* locus in terms of genetic composition and organisation. The new genetic composition and organisation of *plnEF* locus and concurrent presence of *plnEF* and *plnW* loci indicated that *L. plantarum* I-UL4 is a novel multiple bacteriocin producer that possesses vast potentials in various industries.

**Methods**

**Bacterial strain and culture conditions**

*L. plantarum* I-UL4 isolated from fermented tapioca, “tapai ubi” was used in this study [35]. The strain was deposited at the Microbial Culture Collection Unit (UNICC) of Institute of Bioscience, Universiti Putra Malaysia with deposition number UPMC5. The studied strain was grown in de Man-Rogosa-Sharpe (MRS) media (Merck, Germany) at 30°C [59] under anaerobic condition.

**Genomic DNA extraction**

The genomic DNA of *L. plantarum* I-UL4 was extracted using the method described by de los Reyes-Gavilán et al. [60] with minor modifications. Briefly, a single
colony of L. plantarum 1-UL4 was inoculated into 10 ml of MRS broth and incubated at 30°C for overnight. Bacteria cells were then harvested from 1 ml of overnight culture by centrifugation at 16,000 × g for 10 min at 4°C, followed by incubating the cell pellet for 1 h at 37°C in 200 μl of TEG buffer (25 mM Tris–HCl, 10 mM EDTA and 50 mM glucose at pH 8.0) containing 15 mg ml⁻¹ lysozyme (Sigma, USA). Subsequently, 100 μl of 15% (w/v) SDS was added and mixed by gentle inversion to lyse the cells. Then, 300 μl of 3 M cold sodium acetate buffer (pH 5.2) was added and the mixture was inverted gently, followed by incubation on ice for 5 min. The mixture was then centrifuged at 16,000 × g for 10 min at 4°C to precipitate the proteins. The resulting supernatant was transferred into a clean microcentrifuge tube and mixed with an equal volume of phenol:chloroform:isoo mylalcohol solution (Amresco, USA). After centrifugation at 16,000 × g for 15 min at 4°C, the aqueous phase containing DNA was transferred to a new microcentrifuge tube. Two sample volumes of cold absolute ethanol was then added to the aqueous phase, followed by gently mixing and incubated overnight at −20°C to precipitate the DNA. The mixture was centrifuged at 16,000 × g for 15 min at 4°C to collect the DNA after overnight incubation. DNA pellet was then washed with 1 ml of 70% (v/v) cold ethanol and air-dried in a laminar air flow before re-suspended in 40 μl of 1× TE buffer (10 mM Tris–HCl and 1 mM EDTA at pH 7.0). RNA was removed by adding RNase A (Fermentas, Germany) to a final concentration of 0.4 mg ml⁻¹, followed by incubation at 37°C for 15 min.

**Detection of pln genes**

Gene-specific primers were designed specifically based on the published pln genes sequences selected randomly from plw [19], plS [20], pln423 [21] and plnEF [22, 27] loci using internet-based software, PRIMER3 [61]. The specificity of each primer is listed in Table 3. PCR amplification was carried out in 25 μl reaction mixture containing 1× Taq buffer, 0.2 μM of each dNTPs, 2 mM MgCl₂ (Fermentas, Germany), 0.08 μM of each forward and reverse primers, 1 unit of Taq DNA polymerase and 500 ng of genomic DNA extracted from the studied strain. PCR reaction was performed with MyCycler™ Thermal Cycler (BioRad, USA) using following program: (a) initial denaturation at 95°C for 5 min, (b) 30 cycles of denaturation at 95°C for 1 min, (c) annealing at 53°C for 1 min, (d) extension at 72°C for 1 min, and (d) final extension at 72°C for 7 min. PCR products were analysed using 1% (w/v) agarose gel electrophoresis. Gradient PCR with annealing temperature of 50–60°C was carried out for primers that produced negative results. Two positive controls (PLANT1 and LOWLAC primers that specific to partial 16S rDNA of L. plantarum [62]) and a negative control (without DNA template) were included in PCR amplification to monitor the functionality of DNA template, PCR components and contamination. The positive controls produced specific PCR fragment of 996 bp.
### Table 3  PCR primers that designed for the detection of *pln* genes haboured in *Lactobacillus plantarum* I-UL4

| Target gene | Function | Primer sequence (5′–3′) | Size (bp) | References |
|-------------|----------|--------------------------|-----------|------------|
| *brnQ1*     | Amino acid transport protein | F: ATGCTCTTTGGGATGTTTTT  R: ACGATGAATAGGCGTGAGG | 1,068 | [23] |
| *napA1*     | Na⁺/H⁺ antiporter | F: AAGTATTACGCCCGCATTA  R: TTTAACCACATGCGAAGAA | 798 | [23] |
| *plnI*      | Prebacteriocin | F: TAAACGACCTGTCTTG  R: AAATCAGGAAATACATCATGC | 475 | [22] |
| *plnK*      | Prebacteriocin | F: CTGTAGACATTGCTAACCATC  R: ACTGCTGGCCTGGAAGAG | 246 | [22] |
| *plnL*      | Putative immunity protein | F: TAGATCGCGCCTCGTAAGA  R: CGTACCTCGGCCAAGTGG | 442 | [22] |
| *plnM*      | Unknown function | F: TGGCTGAAAGAATTTACAGGATT  R: CAAACGCAACCATCAAAATA | 171 | [22] |
| *plnN*      | Prebacteriocin | F: ATGGCAGCGGTAGTATCG  R: CCTAACATCAGCATGCCAC | 146 | [22] |
| *plnO*      | Glycosyl transferase group 2 family | F: CGGAAGCCCCCTTTAATTTG  R: TCTTGGCACCCCTCTGATT | 580 | [22] |
| *plnP*      | Protease CAAX family | F: TCCGAAAGTATGGCAAAATGA  R: AAAGTTCCCCAAAGCACCC | 437 | [22] |
| *plnA*      | Induction factor | F: CAAAATTAAGGTAGGCAACTC  R: TCTTCTACGTGTTATTTGCCAG | 113 | [22] |
| *plnB*      | Histidine kinase | F: CTGCGGTGCGCGGATGATGG  R: GCATATTAGGCTGCTGCTT | 531 | [22] |
| *plnC*      | Response regulator | F: GCCGACAGGAGATTTACAAAGA  R: CCATTATTGTGTTTTCCAGTCAG | 437 | [22] |
| *plnD*      | Response regulator | F: TGAGGACAAAAAACAGACGAC  R: GCATTGCGGGTAATTTACG | 415 | [22] |
| *plnEF*     | Prebacteriocin | F: GCCATAGTTAAATTTCCCC  R: CAGTTGGCCGCAAAAAAG | 428 | [22] |
| *plnI*      | Immunity | F: CGTATTAGGCTGCTGCTT | 424 | [22] |
| *plnG*      | ABC transporter | F: TGGCGGTATGATGATGTA | 454 | [22] |
| *plnH*      | Accessory protein | F: AGTGTACCGGATCCGTTT | 986 | [22] |
| *plw*       | Prebacteriocin | F: AGTCTGAGTAAGAATGCTATTG  R: TCACAAGAAAATATTCCA | 389 | [19] |
| *plwG*      | ABC transporter | F: GGTTACTGGACTAGGATGGCGATTG  R: CGCTCTCCTGGCATATGCTTAC | 1,034 | [19] |
| *plnCB*     | Prebacteriocin | F: GTGTTGGATAGATAAGCAGC | 207 | [27] |
| *plNCBH*K   | Histidine kinase | F: AGCCGCCGAGATGGTAGGAC  R: AATCCCTTTATTTTTGGCAGTCAG | 790 | [27] |
| *plcC*      | ABC transporter | F: GGCGCTTTCTGCTTGT  R: ACCCGGTGGTGCTGATGTC | 301 | [21] |
| *plcD*      | Accessory protein | F: TGACATCAGATAATGCTATTG  R: GGAAACCATCAACAGCAGCAC | 950 | [21] |
| *plS*       | Prebacteriocin | F: GCCTTACCGGTAATGCCC  R: CTGGTGATGATCCTGAGTT | 320 | [20] |
| 16S rDNA    | Positive control | PLANT1: ATCATGATTTAGTATCG  LOWLAC: CGACGACCATGAAACCATG | 996 | [62] |

*F* forward primer, *R* reverse primer.
Amplification and characterisation of pln loci

Primers were designed to analyse the upstream and downstream DNA sequences of pln genes (Table 4) amplified from L. plantarum I-UL4 genomic DNA. The PCR reaction mixture and program were as described in the experiment of "Detection of pln genes"; but slightly longer time of 8 min was used for each extension cycle. The DNA Walking SpeedUp™ Kit II (Seegene, Germany) was used to amplify the upstream and downstream DNA sequences of pln genes according to the manufacturer’s recommendations when the reference DNA sequence was not available.

DNA sequence analysis of PCR amplified fragments

The PCR products were separated by 1% (w/v) agarose gel electrophoresis. The desired DNA fragments were excised from the agarose gel using clean scalpel and purified by using Wizard® SV Gel and PCR Clean-Up System (Promega, USA). The nucleotide sequence of the amplified fragments were analysed by ABI PRISM™ 3730 x l DNA Analyzer using BigDye® Terminator v3.0 Cycle Sequencing Kit performed by First Base Laboratories Sdn. Bhd. (Malaysia).

DNA alignment and deduced amino acid sequence analysis

The computer software, BioEdit version 7.0.5.2 [63] was used to process and assemble nucleotide sequences, to calculate the percentage identity of DNA and deduced amino acid sequences and to perform the alignment of multiple sequences. ORF-Finder program (http://www.ncbi.nlm.nih.gov/gorf/), GeneMark [64] and Glimmer [65] were then used to determine ORF. Similarity search of nucleotide sequence was performed using Basic Local Alignment Search Tool (BlastN) program (http://blast.ncbi.nlm.nih.gov/). DNA sequence located at the upstream of start codon of each ORF was searched for the putative ribosomal binding site (RBS) manually by comparing the reported DNA sequence of RBS (5′-AGGAGG-3, which is complementary to 3′ end of 16S rRNA 5′CCUCCU-3′) of L. plantarum [66]. Putative promoter was also searched manually by comparing amplified DNA sequence with promoter sequences reported for pln operons [28]. Isoelectric point (pI) and molecular mass (MW) of the deduced peptide were calculated using ExPASY Compute pI/MW program (http://expasy.org/tools/pi_tool.html) and conserved protein domains were identified using CD-search program (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) available at NCBI website.

Determination of genetic location of pln loci

Southern hybridisation was carried out to determine the genetic location of pln loci that harbour plnEF and plw structural genes were either chromosomally or plasmid encoded. Genomic DNA of L. plantarum I-UL4 was separated by 0.7% (w/v) agarose gel electrophoresis and visualized by UV transillumination. The separated genomic DNA bands were then depurinated, denatured and transferred onto the Immobilon-Ny + Transfer Membrane (Millipore, USA) according to the instructions of manufacturer. Three DNA probes of 16Sprobe EF probe and W probe that developed from PCR products generated from primers listed in Table 1 were labelled using the NEBlot® Phototope® Kit (New England Biolabs, USA) according to the manufacturer’s instruction. The probes were 100% specific to 16S rDNA [62], plnEF [22] structural gene and plw [19] structural gene of L. plantarum I-UL4, respectively. The Southern blot membrane containing separated genomic DNA bands of L. plantarum I-UL4 was prehybridised with DNA probes at 58°C for 40 min, followed by further hybridisation at 53°C for 18 h. The hybridised membrane was then processed and visualised further using Phototope®–Star Detection Kit

| Primer designation | Primer sequence (5′–3′) |
|--------------------|------------------------|
| bmQ1-napA1         | F: ATGCTCTTTGGAGATTTTTT<br>R: AGCATGAAATAGGGGTGAGG |
| napA1-L            | F: CGGGCTTGAGTGGTTTCTT<br>R: TACTTTACGGAGCGGCATCT |
| R-plnD            | F: AGCACGCCCATCATAATTC<br>R: AAACCTTGGCGTGCTATTT |
| plnD-I            | F: TGAGGACAACACACTGACG<br>R: AGCTGTCCTTTGAGCCCTAG |
| plnEF              | F: CGTTATGCTTGTAGTTT<br>R: AGCGGATATAGTTCAAGCCAT |
| plnEF-G           | F: CGGTACGCCCAAAACTAAGAAAT<br>R: TCAATCACCCTGTTGAGAAAA |
| plnG-H           | F: TTATTTGGCCGTGTTTGGT<br>R: CGGCCACCTTCAACTAAATA |
| plwG1            | F: CGAAGTGTTGACTTTG<br>R: CCAGATTGCACAACGCTAGTA |
| bmQ1-walk         | F: ATTTTGAGATGCCAGTCCTGAGT<br>R: RCAAACAGTGCCACAACGCTAGTA |
| plnU-helicase     | F: ATTGTGAGATGCCAGCTGAGT<br>R: TGGTCCACATGATCTGTCCTC |
| Plw               | F: CGCGTTGCAATGACAAATAT<br>R: CCACGAACTGGGAAATATTATCA |
| plwGTSP           | F: AGATGGGCGGACTACAGCAGT<br>F2: GGTTGAAATTGAGAAAGGACAG<br>F3: TGTAGCACATGCACTATCAACCA |

F forward primer, R reverse primer

Table 4 PCR primers that designed for the upstream and downstream DNA sequence amplification of PCR amplified pln genes harboured in Lactobacillus plantarum I-UL4
(New England Biolabs, USA) performed according to the manufacturer’s instruction.

End note
The DNA sequences for both plw and plnEF loci of \textit{L. plantarum} I-UL4 were deposited at [GenBank/EMBL/DDB] with accession numbers GU322921 and GU138149 respectively.

Abbreviations
- \textit{pln}: plantaricin; ORF: open reading frame; RBS: ribosomal binding site; IF: induction factor; HKP: histidine protein kinase; RR: response regulator; pl: isoelectric point; MW: molecular mass.

Authors’ contributions
HFT carried out the molecular characterisation study of \textit{pln} genes, participated in the sequence alignment and drafted manuscript. HLF participated in the design, conceived and coordination of this study; and helped to draft and revised the manuscript. RAR participated in the design of the study and helped to draft the manuscript. MPA participated in the design of the study and the sequence alignment. TCL participated in the design of the study and helped to draft the manuscript. KY participated in the molecular characterisation study of \textit{pln} genes. All authors read and approved the final manuscript.

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Acknowledgements
This work was supported by the Long-Term Research Grant Scheme (LRGS) granted by the Ministry of Education Malaysia.

Compliance with ethical guidelines
Competing interests
The authors declare that they have no competing interests.

Received: 1 May 2015 Accepted: 2 June 2015 Published online: 16 June 2015

References
1. Ray B, Bhunia A (2008) Fundamental food microbiology, 4th edn. CRC Press, New York
2. Piard JC, Desmazeud M (1991) Inhibition factors produced by lactic acid bacteria: Oxygen metabolites and catalasobin end products. Le Lait 71:525–541
3. Piard JC, Desmazeud M (1992) Inhibiting factors produced by lactic acid bacteria: bacteriocins and other antibacterial substances. Le Lait 72:113–142
4. Tagg JR, Dajani AS, Wannamaker LW (1976) Bacteriocins of Gram-positive bacteria. Bacteriol Rev 40:722–756
5. Salbon E, Contreras B, Vandamme E (2000) Antimicrobial peptides of lactic acid bacteria mode of action, genetics and biosynthesis. Adv Biochem Eng Biotechnol 68:21–60
6. Hechard Y, Sahl HG (2002) Mode of action of modified and unmodified bacteriocins from Gram-positive bacteria. Biochimie 84:545–557
7. Bauer R, Dicks LMT (2005) Mode of action of lipid II-targeting lactobacillics. Int J Food Microbiol 92:1–206
8. Hoover DG, Chen H (2005) Bacteriocins with potential for use in foods. In: Davidson PM, Sofos JN, Branen AL (eds) Antimicrobials in food, 3rd edn. CRC Press, New York, pp 389–428
9. Giller Q, Negro LM, Riley MA (2005) Genetically engineered bacteriocins and their potential as the next generation of antimicrobials. Curr Pharm Des 11:1065–1075
10. Giller Q, Etzion A, Riley MA (2008) The dual role of bacteriocins as anti- and probiotics. Appl Microbiol Biotechnol 81:591–606
11. Galvez A, Abriouel H, Lopez RL, Omar NB (2007) Bacteriocin-based strategies for food biopreservation. Int J Food Microbiol 120:51–70
12. Dimov S, Ivanova R, Hanzanova N (2005) Genetics of bacteriocins biosynthesis by lactic acid bacteria. Biotechnol Biotechnol Equip 19:4–10
13. Klaenhammer TR (1993) Genetics of bacteriocins produced by lactic acid bacteria. FEMS Microbiol Rev 12:39–86
14. Smouai S, Elleuch L, Bejar W, Karray-Rebai I, Ayadi I, Jaouadi F et al (2010) Inhibition of fungi and Gram-negative bacteria by bacteriocin BacN635 produced by \textit{Lactobacillus plantarum} sp. TN635. Appl Biochem Biotechnol 162:1132–1146
15. Todorov SD, Nyati H, Meincken M, Dicks LMT (2007) Partial characterisation of bacteriocin \textit{AMA-K} produced by \textit{Lactobacillus plantarum} \textit{AMA-K} isolated from naturally fermented milk from Zimbabwe. Food Control 18:656–664
16. Todorov SD, Dicks LMT (2005) \textit{Lactobacillus plantarum} isolated from molasses produces bacteriocins active against Gram-negative bacteria. Enzyme Microbial Technol 36:318–326
17. Todorov SD, Dicks LMT (2004) Influence of growth conditions on the production of a bacteriocin by \textit{Lactococcus lactis} subsp. \textit{lactic} S34BR, a strain isolated from barley beer. J Basic Microbiol 44:305–316
18. Messi P, Bondi M, Sabia C, Batini R, Manicardi G (2001) Detection and preliminary characterisation of a bacteriocin (plantaricin 356) produced by a \textit{Lactobacillus plantarum} strain. Int J Food Microbiol 64:193–198
19. Holo H, Jeknic Z, Daeschel M, Stevanovic S, Nes IF (2001) Plantaricin \textit{W} from \textit{Lactobacillus plantarum} belongs to a new family of two-peptide lactobacilitins. Microbiology 147:643–651
20. Stephens SK, Fioriano B, Cathcart DP, Bayley SA, Witt VF, Jiménez-Díaz R et al (1998) Molecular analysis of the locus responsible for production of plantaricin \textit{S}, a two-peptide bacteriocin produced by \textit{Lactobacillus plantarum}. Appl Environ Microbiol 64:1871–1877
21. van Reenen CA, Chikindas ML, Van Zyl WH, Dicks LMT (2003) Characterisation and heterologous expression of a class IIa bacteriocin, plantaricin 423 from \textit{Lactobacillus plantarum} 423, in \textit{Saccharomyces cerevisiae}. Int J Food Microbiol 81:29–40
22. Diep DB, Havarstein LS, Nes IF (1996) Characterisation of the locus responsible for the bacteriocin production in \textit{Lactobacillus plantarum} \textit{J23}. J Bacteriol 178:4472–4483
23. Kleerebezem M, Boekhorst J, van Kranenburg R, Molenaar D, Kuipers OP, van der Oost J et al (2008) Characterisation of a new organisation of the plantaricin \textit{S}, a two-peptide bacteriocin produced by \textit{Lactobacillus plantarum}. J Genet 191:5020–5021
24. Rojo-Bezares B, Sánchez Y, Navarro L, Jiménez-Díaz R, Zarazaga M, Ruiz-Larraea F et al (2008) Characterisation of a new organisation of the plantaricin locus in the inducible bacteriocin-producing \textit{Lactobacillus plantarum} 323 of grape must origin. Archiv Microbiol 189:491–499
25. Navarro L, Rojo-Bezares B, Saenz Y, Diez L, Zarazaga M, Ruiz-Larraea F et al (2008) Comparative study of the \textit{pln} locus of the quorum-sensing regulated bacteriocin-producing \textit{Lactobacillus plantarum} \textit{JDM1}. Plantaricin \textit{S}. Int J Food Microbiol 128:390–394
26. Maldonado A, Ruiz-Barba JL, Jimenez-Diaz R (2003) Purification and genetic characterisation of plantaricin \textit{NCO}, a novel nucleotide-inducible two-peptide bacteriocin from \textit{Lactobacillus plantarum} \textit{NC8}. Appl Environ Microbiol 69:383–389
27. Diep DB, Strauss DM, Kjos M, Torres C, Nes IF (2009) An overview of the mosaic bacteriocin \textit{pln} loci from \textit{Lactobacillus plantarum}. Peptides 30:1562–1574
28. Huy NT, Thanh NT, Foo HL, Bejo MH, Azhar BK (2010) Feeding of different levels of metabolite combinations produced by \textit{Lactobacillus plantarum} on growth performance, fecal lactic acid bacteria and Enterobacteriaeae count, volatile fatty acids and villi height in broilers. J Anim Sci 81:205–214
30. Loh TC, Cheong SW, Foo HL, Law FL (2009) Effects on growth performance, faecal microflora and plasma cholesterol after supplementation of spray-dried metabolite to postweaning rats. Czech J Anim Sci 54:10–16
31. Loh TC, Hanon HA, Foo HL, Law FL (2008) Effects of feeding spray-dried metabolites of Lactobacillus casei subsp. lactic–RW18 in post-weaning rats. Int J Probiotics Prebiotics 3:1–6
32. Loh TC, Lee TM, Foo HL, Law FL, Rajon MA (2008) Growth performance and faecal microflora of rats offered metabolites from lactic acid bacteria. J Appl Animal Res 34:61–64
33. Thanh NT, Loh TC, Foo HL, Bejo MH, Azhar BK (2009) Effects of feeding metabolite combinations produced by Lactobacillus plantarum on growth performance, faecal microbial population, small intestine villus height and faecal volatile fatty acids in broilers. Br Poult Sci 50:298–306
34. Foo HL, Loh TC, Law FL, Lim YS, Kufli CN, Rusul G (2003) Effect of feeding Lactobacillus plantarum LL-UL4 isolated from Malaysian Tempeh on growth performance, faecal flora and lactic acid bacteria and plasma cholesterol concentrations in postweaning rats. Food Sci Biotechnol 12:403–408
35. Lim YS (2003) Master thesis. Universiti Putra Malaysia, Malaysia
36. Thanh NT, Loh TC, Foo HL, Bejo MH, Kaisim AB (2010) Inhibitory activity of metabolites produced by strains of Lactobacillus plantarum isolated from Malaysian fermented food. Int J Probiotics Prebiotics 5(1):37–43
37. Moghadam MS, Foo HL, Leow TC, Raha AR, Kasim AB (2010) Inhibitory activity of bacteriocin-like peptides released by Lactobacillus plantarum strains isolated from poto poto, a Congolese fermented maize product, and genetic fingerprinting of their plantaricin operons. Int J Food Microbiol 148:478–483
38. Omar NB, Abriouel H, Lucas R, Martinez-Camano M, Guyot JP, Galvez A (2006) Isolation of Bacteriocinogenic Lactobacillus plantarum strains from ben saiglia, a traditional fermented гeurk from Burkina Faso. Int J Food Microbiol 112:44–50
39. Omar NB, Abriouel H, Keleke S, Sanchez Valenzuela A, Martinez-Camano M, Lucas Lopez R et al (2008) Bacteriocin-producing Lactobacillus strains isolated from poto poto, a Congolese fermented maize product, and genetic fingerprinting of their plantaricin operons. Int J Food Microbiol 130:18–29
40. Knoll C, Divoll B, du Toit M (2008) Genetic screening of lactic acid bacteria of oenological origin for bacteriocin encoding genes. Food Microbiol 25:983–991
41. Risoen PA, Havarstein LS, Diep DB, Nes IF (1998) Identification of the leader peptide of colicin V shares consensus sequences with leader peptides that are common among peptide bacteriocins produced by Gram-positive bacteria. Microbiology 144:2383–2389
42. O’Keeffe T, Hill C, Ross RP (1999) Characterisation and heterologous expression of the genes encoding enterocin A production, immunity, and regulation in Lactobacillus plantarum DPC1:146. Appl Environ Microbiol 65:1506–1515
43. Diep DB, Havarstein LS, Nes IF (1995) A bacteriocin-like peptide induces bacteriocin synthesis in Lactobacillus plantarum C11. Mol Microbiol 18:631–639
44. Axelsson L, Kupfers OP, de Vos WM, Stiles ME, Quadri LEN (2001) A two-component signal-transduction cascade in Camobacterium psicoli LV17B: two signaling peptides and one sensor-transmitter. Peptides 22:1597–1601
45. Havarstein LS, Holo, Nes IF (1994) The leader peptide of colicin V shares consensus sequences with leader peptides that are common among peptide bacteriocins produced by Gram-positive bacteria. Microbiology 140:2383–2389
46. Pei J, Grishin NV (2001) Type II CAAX prenyl endoproteidases belong to a novel superfamly of putative membrane-bound metalloproteases. Trends Biochem Sci 26:275–277
47. Kjos M, Snipen L, Salehian Z, Nes IF, Diep DB (2010) The Abi proteins and their involvement in bacteriocin self-immunity. J Bacteriol 192:2068–2076
48. Kostina V, Myskowski SM, Kvinn LA, Chem DN, Varki N, Kansal RG et al (2005) Mutational analysis of the group A streptococcal operon encoding streptolysin S and its virulence role in invasive infection. Mol Microbiol 56:681–695
49. Lux T, Nuhn M, Hakenbeck R, Reichmann P (2007) Diversity of bacteriocins and activity spectrum in Streptococcus pneumoniae. J Bacteriol 189:7741–7751
50. de Man JC, Rogosa M, Sharpe ME (1966) A medium for the cultivation of lactobacilli. J Appl Bacteriol 23:130–135
51. de los Reyes-Gavilán CG, Limosovit GK, Tailliez L, Accolas JP (1992) Lactobacillus helveticus-specific DNA probe detects restriction fragment length polymorphisms in this species. Appl Environ Microbiol 58:3429–3432
52. Rozen S, Skaletsky HJ (2000) Primer3 on the WWW for general users and for biologist programmers. Methods Mol Biol 132:365–386
53. Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98
54. Chagnaud P, Machinis K, Coutte LA, Marecat A, Mercenier A (2001) Rapid PCR-based procedure to identify lactic acid bacteria: application to six common Lactobacillus species. J Microbiol Methods 44:139–148
55. Pei J, Grishin NV (2001) Type II CAAX prenyl endoproteidases belong to a novel superfamly of putative membrane-bound metalloproteases. Trends Biochem Sci 26:275–277
56. Chagnaud P, Machinis K, Coutte LA, Marecat A, Mercenier A (2001) Rapid PCR-based procedure to identify lactic acid bacteria: application to six common Lactobacillus species. J Microbiol Methods 44:139–148
57. Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98
58. Lukashin A, Borodovsky M (1998) GeneMark.hmm: new solutions for gene finding. Nucleic Acids Res 26:1107–1115
59. Delcher AL, Bratke KA, Powers EC, Salzberg SL (2007) Identifying bacterial genes and endosymbiont DNA with Glimmer. Bioinformatics 23:673–679
60. Pouwels PH, Leer RJ (1993) Genetics of lactobacilli: plasmids and gene expression. Antonie van Leeuwenhoek 64:85–107

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