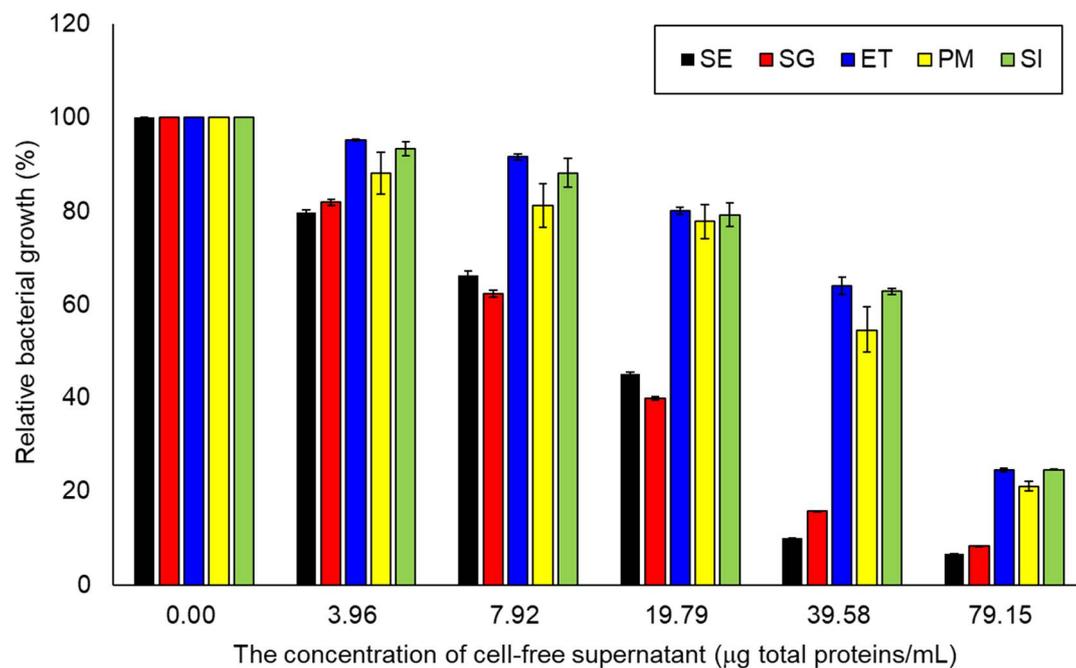


(A)



(B)

Figure 1. Antibacterial activity of cell-free supernatant from *L. plantarum* NIBR97. Antibacterial activities of the cell-free supernatant from the *L. plantarum* strains NIBR97, KCTC33131, KCTC21004 and KCTC13093 were examined against *S. Enteritidis* (A). The antimicrobial activity of cell-free supernatant from the NIBR97 were further examined against various susceptible bacteria, *Salmonella Gallinarum* (SG), *Edwardsiella tarda* (ET), *Pasteurella multocida* (PM) and *Streptococcus iniae* (SI), as well as *S. Enteritidis* (SE) (B). The Y axis in the graphs represents the relative growth of pathogenic bacteria (SE, SG, ET, PM or SI).

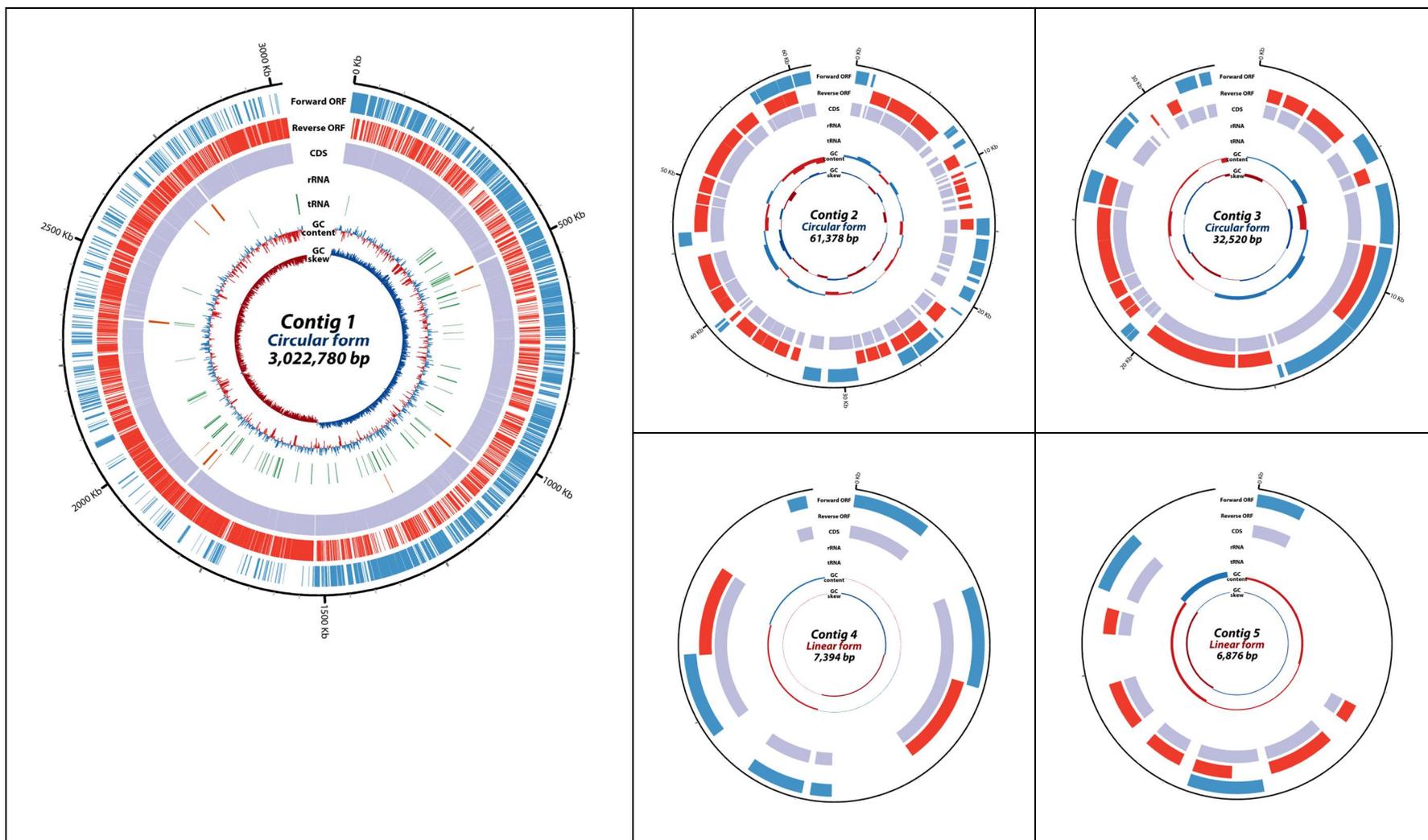


Figure 2. Overall features of the *L. plantarum* NIBR97 genome (Contig 1) and plasmids (Contig 2 to 5). The outer scale indicates the coordinates in base pairs. The open reading frames (ORF) is shown on the first two rings; first ring (blue) is the forward ORF and second ring (red) is the reverse ORF. The third and fourth circle shows the ORF which colored by gene annotation; third ring is forward ORF and fourth ring is reverse ORF. The fifth and sixth circle shows rRNA (green) and tRNA genes (orange). The next circle shows the GC content values. Purple and deep yellow colors indicate positive and negative sign, respectively. The inner-most circle shows GC skew, light green indicating negative values whereas deep orange for positive values.

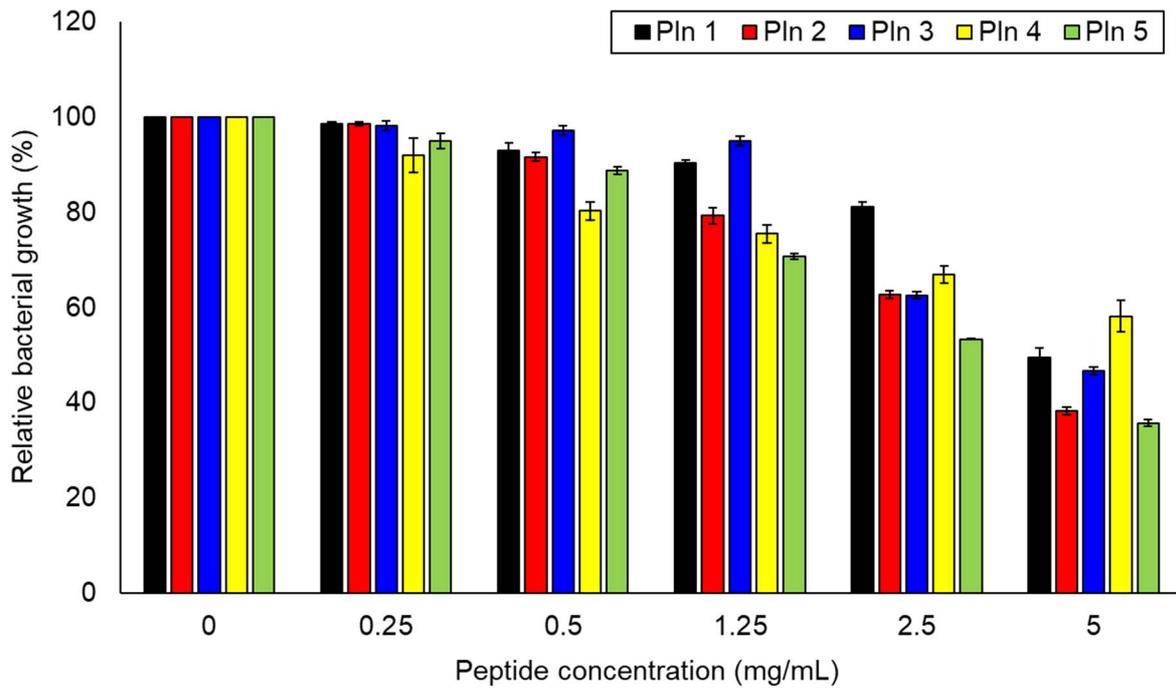


Figure 3. Antibacterial activity of synthetic Plantaricins identified from the *L. plantarum* NIBR97 genome. Pln 1, 2, 3, 4 and 5 were synthesized according to the amino acid sequences in Figure 3, and further examined for their antibacterial activity against a *Salmonella enterica* serotype, *S. Typhimurium*, whose MIC50s were determined as 5.48, 4.84, 5.17, 5.65 and 4.45 mg/mL, respectively. The Y axis in the graphs represents the relative growth of *S. Typhimurium*.

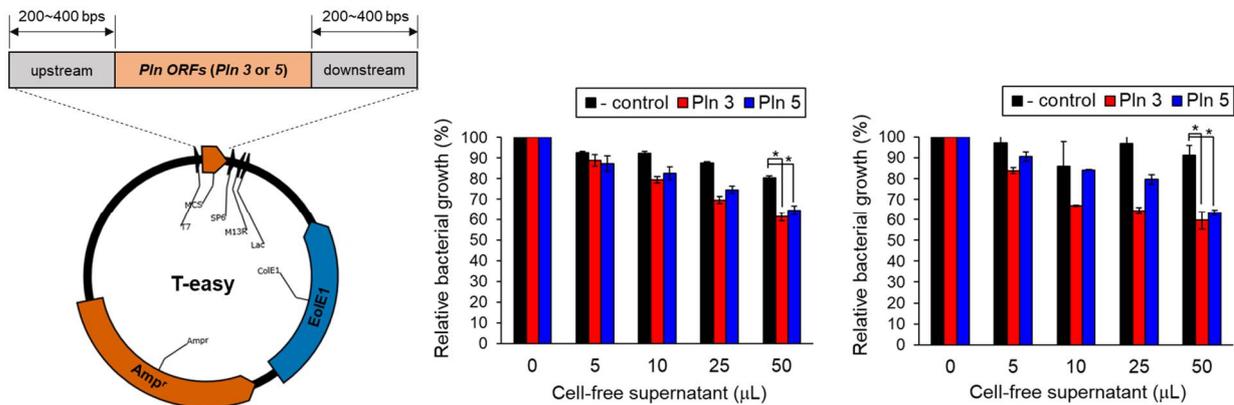


Figure 4. Antibacterial activity of the cell-free supernatant from *E. coli*. Top10 strain, harboring each *Pln* gene. The *E. coli*. Top10 strain (Invitrogen, Carlsbad, CA, USA), harboring each *Pln* gene, including its own intact promoter (upstream) and transcriptional terminator (downstream), was cloned into the pGEM®-T Easy Vector (Promega, Madison, WI, USA) (A) and its cell-free supernatant was used to examine antibacterial activities against a Gram-negative bacterium (pathogenic *E. coli*) (B) and a Gram-positive bacterium (*B. cereus*) (C). The Y axis and the * in the graphs represent the relative growth (%) of pathogenic bacteria and significant differences ($p < 0.05$) between - control and *Plns*, respectively.

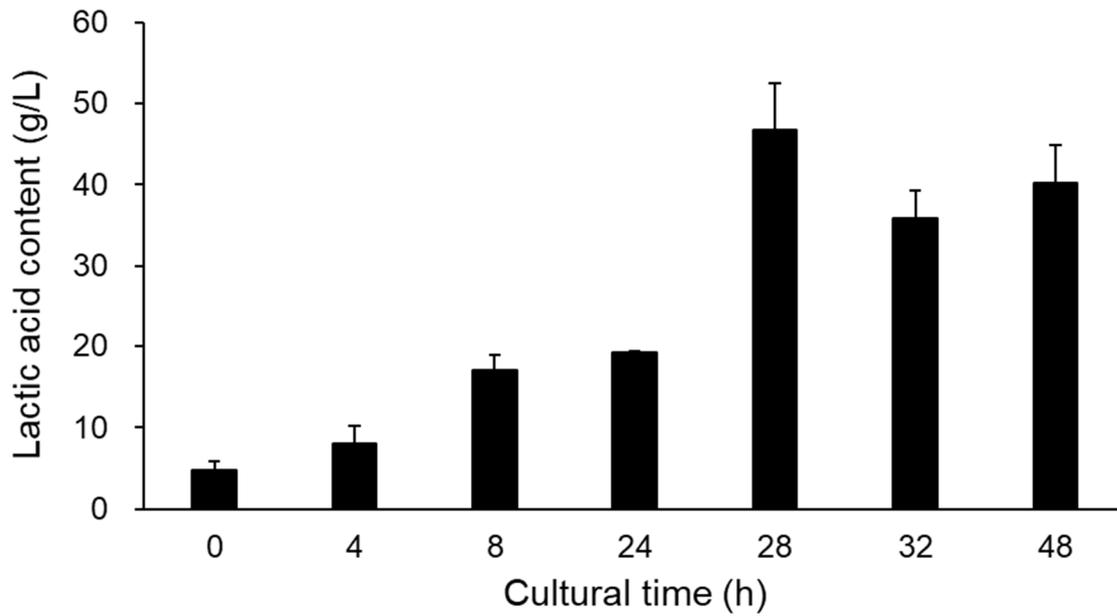


Figure 5. The content of lactic acid in the cell-free supernatant from *L. plantarum* NIBR97. *L. plantarum* NIBR97 was inoculated in MRS medium and cultured at 37 °C for the indicated time (X axis) in the graph to obtain the cell-free supernatant. Finally, the content of lactic acid in the cell-free supernatant was determined by thin layer chromatography (TLC) using the TLC silica gel 60 F254 Merck, Darmstadt, Germany) with nitromethane: 1-propanol: water (2:5:1.5, v/v/v) as a developing solvent and lactic acid (Sigma-Aldrich, St Louis, MO, USA) as a standard chemical. The Y axis in the graph represents the content of lactic acid (g/L) in the cell-free supernatant.

Table 1. Identification of ORFs predicted as AMPs from the genome assembly data of *L. plantarum* NIBR97. The AMPs were identified by NCBI blast (<https://blast.ncbi.nlm.nih.gov/>).

| No | ORF name | Peptide prediction | A.A. No | Identity | No | ORF name | Peptide prediction | A.A. No. | Identity |
|--|----------|---|---------|---|--|----------|--|----------|---|
| 1 | ORF00467 | MGTIGQLVLA YIAVLGFLIINIPHQAL NVAGWIGLTLWGGYLIVQAFDGGVV LGSLIGSVGIGVLSLAARYKKMPAIF NIPSLVSFVPGSQAYQMVRNFALGNY REAVSFTLQVIMITGAIALGFLLAELL NRLIAFCIRQWRLHRLS | 152 | > CAMPSQ1094 Grammistin Pp3 Length=25 Score = 22.3 bits (46), Expect = 5.8 Identities = 12/22 (55%), Positives = 15/22 (68%), Gaps = 1/22 (5%) > Renamed as plantaricin 1 | 6 | ORF02155 | MKSLDKIAGLGIEMAEKDLTTVEG GKNYSKTTWWYKSLTLGKVAEGTS SAWHGLG | 55 | > Bagel_II_172 179.2 Plantaricin_N Length=55 Score = 116 bits (291), Expect = 1e-036 Identities = 55/55 (100%), Positives = 55/55 (100%), Gaps = 0/55 (0%) |
| 2 | ORF01336 | MISLLEKHPNKITPHPIEKPKKQLPQR FPVKTPPENPQINPEKIYPEKQPEEPVR RD | 58 | > DBAASP_4483 4833 Indolicidin Length=13 Score = 25.4 bits (54), Expect = 0.076 Identities = 10/13 (77%), Positives = 11/13 (85%), Gaps = 0/13 (0%) > Renamed as plantaricin 2 | 7 | ORF02163 | MKKFLVLRDRELNAISGGVFHAYS ARGVRRNNYKSAVGPADWVISA VRG FIHG | 52 | > Bagel_II_169 176.2 Plantaricin_F Length=52 Score = 108 bits (270), Expect = 2e-033 Identities = 52/52 (100%), Positives = 52/52 (100%), Gaps = 0/52 (0%) |
| 3 | ORF01363 | MLKNIKKAFKHNRISSDKKLAKVMGG KKSRRQQVYNNGMPTGMYTSC | 46 | > APD_1473 AP02323 Bactofencin A Length=22 Score = 39.7 bits (91), Expect = 4e-007 Identities = 15/21 (71%), Positives = 17/21 (81%), Gaps = 0/21 (0%) > Renamed as plantaricin 3 | 8 | ORF02164 | MLQFEKLQYRSLPQKLLAKISGGFN RGGYNFGKSVRHVDAIGSVAGIR GILKSIR | 56 | > Bagel_II_168 175.2 Plantaricin_E Length=56 Score = 112 bits (281), Expect = 5e-035 Identities = 56/56 (100%), Positives = 56/56 (100%), Gaps = 0/56 (0%) |
| 4 | ORF01599 | LKTTWLASLLVTFWGA VLGVLVVTYL GGAMVEAL TATPIVREPFKAMAVGII AVMSGLLVTTHH | 65 | > CAMPSQ3668 Hymenochirin-5B Length=28 Score = 20.4 bits (41), Expect = 7.4 Identities = 10/25 (40%), Positives = 17/25 (68%), Gaps = 1/25 (4%) > Renamed as plantaricin 4 | 9 | ORF00645 | MTKRQHYRPVYAKTRWARWRYRL GWLLVLLVIIGSVVWGLAWLRWRS DAVVSFGDVRGVAVSQNDGYLDFA ALQNDGLKFVYLHATQGSYIDDN FASNRYRIVGTSLVGVVIHTFSFST AAAQAAAYFEKTVGDSIGNLPIAIV QYYGDYTDQTIAVRKSRAKLKALV TTLTQDYNRSCVWVSTPAVAKQIV KPALKDIDLWLDATAKTHQQGRRV MFMHYSRAVYRQNGTRQEFAGIL FNGSVTAYNKVVAQGLN | 258 | > Lysozyme M1 Compositional matrix adjust. 257/258(99%) 258/258(100%) 0/258(0%) |
| 5 | ORF01790 | MPESTEEIKKMEALIAKLDEQQQLK AKKRFLRNRLSQQARKARTKRLIEKA LY | 54 | > DBAASP_862 970 M-zodatoxin-Lt2a Latarcin-2a Ltc-2a Length=26 Score = 21.9 bits (45), Expect = 1.2 Identities = 10/21 (48%), Positives = 13/21 (62%), Gaps = 0/21 (0%) > Renamed as plantaricin 5 | 10 | ORF02421 | MKKISFKNADGSLNGKLIAGHSLLI VLIQQIFAMFGIKFTGDWSAIVAVIN TVLTILGMLGVITDVQIVTVPTVKS DEESQVEATANKVADEAQTPTSTV AAVNSSASSNTETTSESASQSGEKV V | 127 | > Bacteriophage holin Compositional matrix adjust. 127/127(100%) 127/127(100%) 0/127(0%) |
| <div style="background-color: yellow; border: 1px solid black; padding: 2px;">Uncharacterized proteins</div> | | | | | <div style="border: 1px solid black; padding: 2px;">Known proteins</div> | | | | |

Table 2. Transcriptomic analysis results of AMPs from *L. plantarum* NIBR97. The AMP transcripts were examined by RNA-sequencing. Lplan-ON and Lplan-OD05 indicate total RNA samples extracted from *L. plantarum* NIBR97 grown during stationary and exponential phases, respectively.

| Test_id | SampleA | SampleB | logFC | Absolute Fold Change | logCPM | p Value | FDR | Start | End | Strand | Description | Renamed |
|----------|----------|------------|-----------|----------------------|-----------|-----------|-----|--------|--------|--------|-----------------------|---------------|
| ORF00467 | Lplan-ON | Lplan-ODO5 | -0.086799 | 1.0620114 | 2.5828759 | 0.9523457 | 1 | 17279 | 17737 | + | hypothetical protein | Plantaricin 1 |
| ORF01336 | Lplan-ON | Lplan-ODO5 | -0.270876 | 1.2065403 | 4.3647043 | 0.84403 | 1 | 30744 | 30920 | + | hypothetical protein | Plantaricin 2 |
| ORF01363 | Lplan-ON | Lplan-ODO5 | -0.285383 | 1.2187342 | 2.2191936 | 0.8435393 | 1 | 2926 | 3066 | - | hypothetical protein | Plantaricin 3 |
| ORF01599 | Lplan-ON | Lplan-ODO5 | 0.1082445 | 1.0779158 | 2.5404791 | 0.9455275 | 1 | 105927 | 106124 | - | hypothetical protein | Plantaricin 4 |
| ORF01790 | Lplan-ON | Lplan-ODO5 | 0.0224743 | 1.0156999 | 2.5730455 | 0.9946698 | 1 | 1288 | 1452 | + | hypothetical protein | Plantaricin 5 |
| ORF02155 | Lplan-ON | Lplan-ODO5 | 0.9820619 | 1.9752865 | 1.2564042 | 0.4931495 | 1 | 96779 | 96946 | + | Plantaricin_N | |
| ORF02163 | | | | | | | | | | | Plantaricin_F | |
| ORF02164 | Lplan-ON | Lplan-ODO5 | -0.322824 | 1.2507762 | 1.0346486 | 0.8287364 | 1 | 102587 | 102745 | - | Plantaricin_E | |
| ORF00645 | Lplan-ON | Lplan-ODO5 | -0.560874 | 1.4751622 | 5.7206241 | 0.6808994 | 1 | 16330 | 17106 | - | Lysozyme M1 precursor | |
| ORF02421 | Lplan-ON | Lplan-ODO5 | -1.349274 | 2.5478396 | 3.0879399 | 0.3336474 | 1 | 148606 | 148989 | - | Bacteriophage holin | |