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Heterologous Expression and Partial Purification of Plantaricin Produced by *Lactiplantibacillus plantarum* COY2906

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Abstract

Background and Objective: *Lactiplantibacillus plantarum* COY2906 was isolated from virgin coconut oil, a strain known for its production of plantaricin which acts as a bio-preservative. The aim of this study was to investigate specific plantaricin genes of *pln*A, *pln*EF, *pln*N, *pln*J *and pln*K, precipitate the plantaricin with ammonium sulfate and assess antimicrobial activity of the crude plantaricin.

Material and Methods: Growth analysis of strain COY2906 was monitored using spectrophotometer. Amplification and detection of gene targets were carried out using real-time polymerase chain reaction (Real-Time PCR). Crude plantaricin was assessed using 40 and 70% (w/v) ammonium sulphate. Antimicrobial activity was assessed using well-diffusion assay and the molecular mass of partially purified protein was assessed using matrix-assisted laser desorption ionization mass spectrometry (MALDI-TOF-MS).

Results and Conclusion: The *plantarum* strain COY2906 was cultured in MRS broth at 37 °C under anaerobic conditions and harvested after 19 h or in the middle of the stationary phase to maximize production of plantaricin. Relative expression level of *pln*A, *pln*EF, *pln*N and *pln*J were over-expressed, while that of *pln*K was not. To achieve plantaricin, cell-free supernatant was precipitated with 40 and 70% ammonium sulphate, resulting in crude protein concentrations of 41.33 and 148 µg.ml^{-1}, respectively. Crude protein had no antimicrobial activities, cell-free supernatant of the strain COY2906 showed a comparable antimicrobial efficacy to that of sodium ampicillin at 100 µg.ml^{-1}. Matrix-assisted laser desorption ionization mass spectrometry spectrum did not show the presence of plantaricin A, plantaricin EF, plantaricin N and plantaricin J after precipitation with 70% ammonium sulphate. However, plantaricin K was detected in the spectrum. Regarding the results, further analysis on the detection of plantaricin is recommended using matrix-assisted laser desorption ionization mass spectrometry. This may involve modifying the solvent or increasing concentration of ammonium sulphate to assess its activities and characteristics.

Conflict of interest: The authors declare no conflict of interest.

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1. Introduction

Lactiplantibacillus (*L.*) *plantarum* belongs to the group of lactic acid bacteria (LAB). It is an aerotolerant, Gram-positive, catalase-negative homofermentative bacterium that is commonly identified in various environments, including healthy intestinal mucosa [1], skins [2], dairy products [3] and nutrient-rich environments [4]. *L. plantarum* produces organic acids, hydrogen peroxide, diacetyl and bacteriocins [4]. Bacteriocins are ribosomally-synthesized peptides, typically ranging 20-60 amino acids. These biochemicals can inhibit Gram-negative and Gram-positive bacteria [5]. Bacteriocins are toxic to similar and dissimilar bacterial strains and include ability to eliminate or inhibit pathogens. However, they do not include threats to their cells because these bacteria typically produce immunity proteins, which

shield them from being affected by their bacteriocins [6]. Bacteriocins produced by *L. plantarum* are called plantaricins and classified under class II. Plantaricins are small heat-stable peptides (< 10 kDa) that are cationic and hydrophobic. They possess an amphiphilic α-helical structure and act as membrane-permeabilizing peptides. Furthermore, their isoelectric points typically range 8.3-10.0 [7]. Plantaricins were reported as heterologous and widely used as antimicrobials [8], decreasing agents for human colorectal cancers [9], biopreservatives [10], antioxidants [11] and biofilms [12] as well as other functions. Plantaricins are massively used as broad-spectrum antimicrobial peptides to inhibit food pathogen bacteria. They achieve this by inserting into the cytoplasmic membranes, forming pores and disrupting nucleic acid and protein syntheses [5,7]. Heterologous plantaricins, which differ in their amino acid compositions, show distinct characteristics. Genes responsible for encoding plantaricin are arranged in an operon cluster located in the genomes, plasmids and other genetic elements [5]. Location of the coding genes affects variations observed in plantaricins. So, diversity of gene expression can be identified and measured based on the protein activity. Genes that encode proteins can be reported as "turn on" when their activities can be assessed.

Plantaricins are commonly precipitated using ammonium sulfate, polyethyleneimine, ethanol, acetone and polyethylene glycol (nonionic polymer) as well as isoelectric precipitation [13]. However, ammonium sulfate precipitation is the most frequently used method. Previous studies reported that *L. plantarum* strain COY2906 was isolated from virgin coconut oil and encoded plantaricin gene in plasmids and chromosomes. The genome consists of several encoding genes such as *pln*W, *pln*V, *pln*U, *pln*H, *pln*G, *pln*E, *pln*F, *pln*D, *pln*C, *pln*B, *pln*A, *pln*P, *pln*O, *pln*N, *pln*M, *pln*J, *pln*K and *pln*L [14]. The whole genome of strain COY2906 includes *plnK* gene; however, *pln*Q and *pln*I were not detected in the genome, compared to *L. plantarum* strain WCFS1. This study focused on *pln*A, *pln*E, *pln*F, *pln*N, *pln*J and *pln*K due to their well-documented roles as natural antimicrobial peptides. Two peptide bacteriocins (plantaricin E/F and plantaricin J/K) were produced in response to the peptide pheromone plantaricin A [15]. However, activities and characteristics of plantaricin A, plantaricin E, plantaricin F, plantaricin N, plantaricin J and plantaricin K of the strain COY2906 are currently unknown. Therefore, the aims of the current study were to identify heterologous expression of plantaricin genes and assess activity of the crude plantaricin in *L. plantarum* strain COY2906.

2. Materials and Methods

2.1 Sample Collection and Culture Media

The *L. plantarum* strain COY2906 was formerly isolated from virgin coconut oil [14,16] and cultivated in Man, Rogosa and Sharpe (MRS) broth (Becton, Dickinson and Company, Franklin Lakes NJ, USA) at 37 °C under anaerobic conditions. Genome was deposited at GenBank (accession no. NZ_BOVM00000000).

2.2 Growth Analysis of L. plantarum

The *L. plantarum* strain COY2906 was cultivated in MRS broth at 37 °C under anaerobic conditions. Growth was monitored using spectrophotometer (Molecular Device SpectraMax M5, San Jose, USA) at 600 nm every 1 h [17,18].

2.3 RNA Extraction

The RNA extraction was carried out based on a modified ethanol precipitation protocol. Strain COY2906 was cultivated in MRS broth at 37 °C and harvested at the middle-stationary phase with a cell concentration of nearly10^8 CFU.ml^{-1}. Culture was centrifuged and the supernatant was carefully removed. Briefly, 300 µl of a solution containing 50 mM sodium acetate, 10 mM ethylenediamine tetraacetic acid (pH 5.0) (HiMedia Laboratories, Maharashtra, India), 25 µl of 10% sodium dodecyl sulfate (Merck, Darmstadt, Germany) and 300 µL of phenol (Wako Pure, Osaka, Japan) were added to the pellet. The resulting mixture was incubated at 65 °C for 5 min using water bath, followed by centrifugation at 14,000 g for 5 min. The upper solution was transferred to a new tube, mixed with 300 µl of phenol: chloroform solution (1:1) and centrifuged at 14,000 g for 5 min. It was then transferred into a new tube and mixed with 300 µl of chloroform (Wako Pure, Osaka, Japan). The supernatant was centrifuged and the upper solution was transferred into a new tube. Then, 10 µl of 3M sodium acetate (pH 5.3) and 257 µl of absolute ethanol (Wako Pure, Osaka, Japan) were mixed with the solution. The mixture was set for one night and then centrifuged at 14,000 g for 30 min. The supernatant was carefully removed, mixed with 500 µl of 70% ethanol, centrifuged at 14,000 g for 1 min at 4 °C and dried at room temperature for 2 h. Pellet was suspended in 20 µl of nuclease free deionized water. Concentration and quality of the extracted RNA were assessed by measuring the absorbance at 260 nm using NanoDrop device (Thermo Fisher Scientific, Maryland, USA).

2.4 Gene Expression of Plantaricin

Briefly, cDNA of the strain COY2906 was synthesized using TaqMan reverse-transcription reagents (Applied Biosystems, Foster City, USA). Amplification and detection of the gene targets (*pln*A, *pln*EF. *pln*N, *pln*E and *pln*J) were carried out using real-time polymerase chain reaction (real-time PCR) (ABI Step One Plus, Thermo Fisher Scientific, USA) and Power SYBR Green PCR master mix (Thermo Fisher Scientific, Maryland, USA). Based on the available literature, 16S rRNA was selected as a housekeeping gene and specific primers were used as shown in Table 1. Results were analyzed using Livak method of the comparative critical threshold (ΔCT) [19]. Amplification of each gene was carried out in triplicate.

2.5 Partial Purification of Plantaricin

Cell-free supernatant (CFS) of the strain COY2906 was filtered through 0.20-µm pore size filters (Advantec Toyo Roshi Kaisha, Tokyo, Japan). Crude plantaricin was assessed using 40% and 70% ($w.v^{-1}$) ammonium sulphate (Merck, Darmstadt, Germany) with continuous stirring at 4°C overnight. The resulting precipitate was collected by centrifuging the mixture at 14,000 g for 30 min at 4 °C and resuspended in 10 mM phosphate buffer. This was subjected to dialysis through a membrane to remove salts [22].

2.6 Protein Concentration Assay

The total protein assessment was carried out using Takara Bradford protein assay kit protocol (Takara Bio, Shiga, Japan) and calibration curve equation of y=0.0003x + 0.2276 with a correlation coefficient of R=0.9442. This was assessed twice for each experiment.

2.7 Antimicrobial Activity of the Crude Protein

The antimicrobial activity was assessed using well-diffusion assay. Bacteria strains such as *Bacillus subtilis*, *Escherichia coli* K12 JM109 and *Staphylococcus aureus* JCM 20624 were cultivated in Luria Bertani (LB) broth (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) until reaching the logarithmic phase. To prepare the plates, Mueller-Hinton agar (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) was used as 50 µl (10^5 $CFU.ml^{-1}$) and wells with a diameter of approximately 6.5 mm were created. Then, 100 µl of the crude protein were added to each well and incubated aerobically at 30 °C. Sodium ampicillin (Avantor, USA) at 100 $\mu g.ml^{-1}$ was used as a positive control. The diameter zone was measured using cross method by excluding the well size [23].

2.8 Matrix-assisted Laser Desorption Ionization Mass Spectrometry Analysis of the Partially Purified Protein

Molecular mass of partially purified protein was assessed using matrix-assisted laser desorption ionization mass spectrometry (MALDI-TOF-MS) based on the standard procedures using Shimadzu AXIMA-Resonance spectrum-eter (Shimadzu, Kyoto, Japan). For the analysis, 5 µl of the partially purified protein crystal were carefully mixed with the analytic solution (α-cyano-4 hydroxyc-innamic acid in 50% acetonitrile aqueous solution/0.1% trifluoroacetic acid aqueous solution) (Merck, Darmstadt, Germany) [24]. Then, 1 µl of the prepared solution was spotted onto a plate and set to dry at room temperature for 10 min before analysis. Sample was analyzed using MALDI-TOF MS equipment and Axima Resonance software.

2.9 Statistical Analysis

Data included mean values with standard deviation (SD) and significant differences were analyzed using one-way of variance (ANOVA), unpaired T-test and GraphPad Prism 10 software (GraphPad, Boston, Massachusetts, USA) for MacOS Ventura v.13.1. Generally, $p \leq 0.05$ was considered as significant difference.

3. Results and Discussion

3.1 Growth Analysis of *Lactiplantibacillus plantarum*

The growth curve was plotted at 600 nm with intervals of 1 h, which was indicative of the cell number. This was carried out to identify the middle of the stationary phase of cell growth. Bacteriocin is a secondary metabolite and a ribosomal synthesized antimicrobial peptide. Furthermore, it is produced during the exponential phase and reached its maximum at the stationary phase of the strain growth curve [25]. Data in Figure 1 showed that the lag, exponential, stationary and death phases of the strain COY2906 occurred at 0-2, 3-16, 17-22 and 23-28 h, respectively.

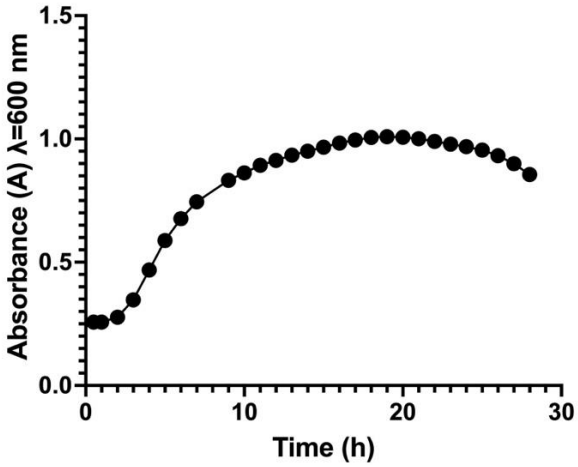

Figure 1. Growth curve of the *Lactiplantibacillus plantarum* Strain COY2906

De Giani et al. reported that *L. plantarum* Strain PBS067 produced a bacteriocin-like compound during the stationary phase of the growth after 16 h of incubation at 37 °C, which successfully inhibited *E. coli* ATCC 25922 and *S. aureus* ATCC 6538 [26]. Furthermore, *L. plantarum* MXG-68 produced bacteriocin during the late lag phase (4 h) and reached its maximum value in the middle of the stationary phase (24 h) [27]. For further analysis, cells of the COY2906 strain were harvested at 19 h, corresponding to the midpoint of the stationary phase.

3.2 Relative Expression of the Plantaricin Genes

The aim of this study was to assess the process; by which, information encoded in plantaricin genes of *L. plantarum* Strain COY2906 was used to synthesize a functional bacteriocin. Plantaricin genes investigated in this study were *pln*A, *pln*EF, *pln*N, *pln*J and *pln*K. To maximize production of plantaricin, cells were harvested at 19 h, which corresponded to the midpoint of the stationary phase of *L.*

plantarum Strain COY2906. Hurtado et al. (2011) reported that the relative expression (RE) level was over-expressed when RE was >1. A value of 1 meant a 100% gene expression rate in the test condition as in the control. Meanwhile, a value below 1 was indicative of gene down-regulation, compared to the control [21]. In the middle of the stationary phase of growth, the RE of *pln*A, *pln*EF, *pln*N and *pln*J were overexpressed while that of *pln*K were not (Figure 2).

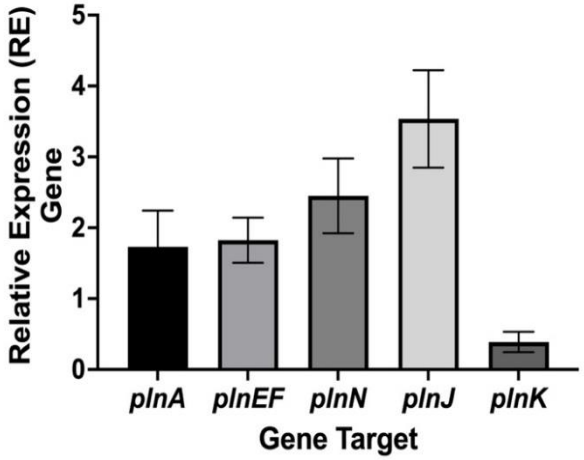

Figure 2. Relative expression (RE) level of plantaricin genes; *pln*A, *pln*EF, *pln*N, *pln*J and *pln*K of *Lactiplantibacillus plantarum* Strain COY2906 cultivated in De Man, Rogosa and Sharpe broth for 19 h. Data include mean values with standard deviation (n = 2)

3.3 Crude Protein Concentration

The CFS containing plantaricin, produced by *L. plantarum* Strain COY2906, was harvested at 19 h and precipitated using 40 and 70% ammonium sulphate. Based on Figure 3, concentrations of the crude precipitated protein were 41.33 and 148 µg.ml^{-1}, respectively.

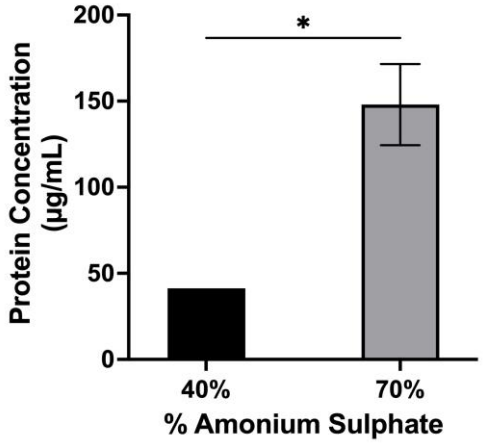

Figure 3. Crude protein concentrations were precipitated with 40 and 70% ammonium sulphate. Data included mean values with standard deviation (n = 2). *Significant differences between the protein concentration and concentr-ation of ammonium sulfate using unpaired T-test ($p \leq 0.05$)

These concentrations were used for the analysis in the study. Furthermore, they increased directly proportional to the concentration of ammonium sulphate [22,28]. Ammonium sulphate was widely used for the precipitation of proteins with unknown sequences due to its high solubility, high stability to protein structure, compatibility with high ionic strength solution, low cost and availability of pure materials [29]. Plantaricin containing positively and negatively charged regions often aggregate even under low salinity conditions [7]. However, presence of salts, anions and cations helps neutralize charges on the protein surface and prevent aggregation. As the salt concentration further increases, the surface of plantaricin becomes highly charged, causing the molecules to reaggregate [13,29]. Therefore, it was necessary to increase and decrease solubility by increasing concentration of ammonium sulphate.

3.4 Crude Bacteriocin Activity

Antimicrobial activities of the crude bacteriocin were assessed to assess its effectiveness. The crude protein was assessed using 40 and 70% ammonium sulphate; thereby, producing concentrations of 41.33 and 148 µg.ml^{-1}, respectively. Positive control included sodium ampicillin at 100 µg.ml^{-1}. The CFS of Strain COY2906 showed antimicrobial activities against the indicator bacteria, including *B. subtillis*, *E. coli* K12 JM109 and *S. aureus* JCM 20624. However, a crude protein with concentrations of 41.33 and 148 µg.ml^{-1} did not show any antimicrobial activities (Table 2). Considering that *pln*A, *pln*EF, *pln*N, *pln*J *and pln*K genes were present in COY2906 strain, they might not detect in the crude bacteriocin; thus, resulting in lack of bioactivities.

Lei et al. (2019) reported that *L. plantarum* zrx03 isolated from infants' faces did not demonstrate antimicrobial activity when precipitated with 40% ammonium sulphate. Additionally, it showed less than 8-mm inhibition zones against *S. aureus*, *B. subtillis*, *B. anthracis*, *E. coli* and *Salmonella* when precipitated with 70% ammonium sulfate [22]. Muhammad et al. stated that crude proteins of *L. plantarum* strain KLDS 1.0344 isolated from Mongolian conventional fermented cheese and extracted with 60% ammonium sulphate did not demonstrate any antimicrobial activity at pH 7 and 8 against *Listeria monocytogenes*, *S. aureus*, *S. typhimurium* and *E. coli* O_{157}:H_7 [30]. Activities of the crude bacteriocin were affected by its precipitation and the mode of action of plantaricin. Precipitation is a pre-purification process for the extraction of plantaricin from *L. plantarum* Strain COY2906 and is commonly achieved using ammonium sulfates, polyethylene glycol (PEG) and organic solvents such as alcohol, acetone and ethyl acetate [31]. There were two possible reasons why the crude protein did not exhibit antimicrobial activity. First, plantaricin was a class II bacteriocin, small peptide (<10 kDa) heat-stable molecule [7].

Table 1. Primers used in this study

Genes	Forward	Reverse	Product Size	References
16S rRNA	GATGCATAGCCGACCTGAGA	CTCCGTCAGACTTTCGTCCA	114	[20]
plnA	AAAATTCAAATTAAAGGTATGAAGCAA	CCCCATCTGCAAAGAATACG	108	
plnEF	GTTTTAATCGGGGCGGTTAT	ATACCACGAATGCCTGCAAC	85	
plnN	GCCGGGTTAGGTATCGAAAT	TCCCAGCAATGTAAGGCTCT	102	[21]
plnJ	TAAGTTGAACGGGGTTGTTG	TAACGACGGATTGCTCTGC	102	
plnK	TTCTGGTAACCGTCGGAGTC	ATCCCTTGAACCACCAAGC	97	

Table 2. Antimicrobial activity of the crude protein precipitated with 40 and 70% ammonium sulphate

	Zone of Inhibition (mm)			
	E. coli K12 JM109^T	*B. subtilis*	*S. aureus* JCM 20624^T	Reference
CFS of *L. plantarum* COY 2906	12.25 ± 0.75^a	10 ± 0.75^{ab}	13.5 ± 0.25^b	[14]
Positive Control	14.25 ± 0.3****	11 ± 0.3****	12.25 ± 0.3****	on study
Crude Protein (µg/mL)				
41.33	-	-	-	on study
148.0	-	-	-	on study

Table 3. Molecular weights of the plantaricins based on GenBank information

Protein Target	Molecular Weight	Amino Acid	Accession Number
Plantaricin A	~ 5,41 KDa	48	WP_003641979
Plantaricin EF	~ 3.59 KDa	31	AYE60565
Plantaricin N	~ 5,96	55	AFJ79562
Plantaricin J	~ 5,87 KDa – 6.07 KDa	53-55	AFJ79560 WP_003641973
Plantaricin K	~ 6,29 KDa	57	AFJ79559

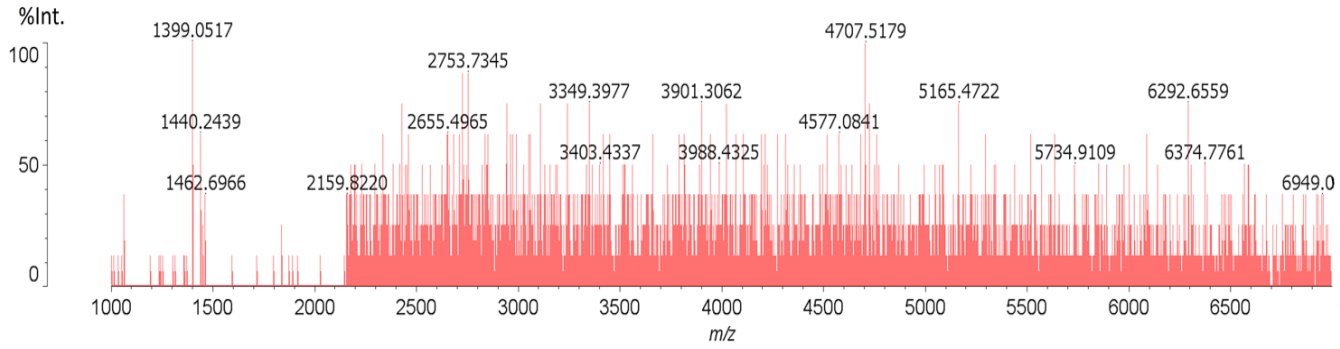

Figure 4. The mass spectrum of plantaricin precipitated with 70% ammonium sulphate using matrix-assisted laser desorption ionization mass spectrometry

Typically, small peptide bacteriocin was precipitated using high ammonium sulphate of 75-80% [31]. Second, ammonium sulphate could not efficiently precipitate plantaricin in this study, suggesting that use of PEG and organic solvent might be more appropriate. According to Lei et al. (2019), a crude extract prepared by ethyl acetate and n-butanol included significantly a higher antimicrobial activity than that the ammonium sulphate did [22]. Plantaricin provided a broad inhibitory activity against Gram-positive and Gram-negative bacteria by inducing formation of pores, which led to the efflux of intracellular K^+ ions, inorganic phosphate, ATP and UV-absorbing substances [31]. Additionally, it could disrupt integrity of the cell wall or inhibit protein and nucleic acid syntheses [7]; thereby, ensuring efficient killing of the target bacteria. There is a possibility that the produced plantaricin was not bactericidal and bacteriostatic. However, further studies are needed to verify this hypothesis.

3.5 Antimicrobial Spectrum of the Bacteriocin

In this study, MALDI-TOF-MS was one of the tools used for the plantaricin characterization to assess molecular weight and structure of macromolecules with high sensitivity over a wide range of detectable masses. However, bacteriocin spectrum was compromised due to contamination in the crude protein sample. Table 3 presents information on molecular weight and amino acids of the biochemical. However, the spectrum in Figure 4 did not indicate presence of plantaricin A, plantaricin EF, plantaricin N and plantaricin J precipitated with 70% ammonium sulphate. In contrast,

plantaricin K was detected with a molecular weight of 6,292 KDa, which was 2 Da greater than the value achieved from the GenBank, possibly due to the presence of a disulfide bond [32].

Song et al. reported that plantaricin ZJ5 was successfully isolated and sequenced, showing a molecular weight of 57.2 Da higher than that of the value achieved. This difference in molecular weight was attributed to a leader peptide characterized by two conserved glycine residues that formed a Gly-Gly site and facilitateed transport of the mature polypeptide across the plasma membrane [33]. Considering these results, it is recommended to purify and sequence plantaricin K for further analysis.

4. Conclusion

This study showed heterologous plantaricin genes (*pln*A, *pln*EF, *pln*N, *pln*J and *pln*K) expressed in *L. plantarum* Strain COY2906. To carry out the precipitation of plantaricins with ammonium sulphate, a high concentration is necessary to achieve effective results in killing or inhibiting pathogenic bacteria. Moreover, appropriate precipitation with ammonium sulfate yields clear MALDI-TOF-MS spectra and can depict characteristics of the plantaricins. It is suggested to study expression of all the genes to increase concentration of ammonium sulphate or change precipitated protein solvents.

5. Acknowledgements

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6. Conflict of Interest

The authors report no conflict of interest.

7. Authors Contributions

Conceptualization, Y.S.; methodology, Y.S; software, Y.S and J.L..; validation, N.R, N.R. and R.S; formal analysis, Y.S..; writing-original draft preparation, Y.S.; writing-review and editing, Y.S. and J.L.; supervision, Y.S., R.S. and A.P.W.; funding acquisition, Y.S.

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بیان ژن‌های دگرساخت[1] و خالص‌سازی نسبی پلانتاریسین تولید شده توسط *لاکتی‌پلانتی‌باسیلوس پلانتاروم* COY2906

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چکیده

سابقه و هدف: *لاکتی‌پلانتی‌باسیلوس (ل.) پلانتاروم* COY2906 از روغن نارگیل بکر جدا شد، سویه‌ای که به‌دلیل تولید پلانتاریسین، به‌عنوان نگهدارنده زیستی، شناخته شده است. هدف از این مطالعه بررسی ژن‌های اختصاصی پلانتاریسین *plnA*، *plnEF*، *plnN*، *plnJ* و *plnK*، رسوب‌دهی پلانتاریسین با آمونیوم سولفات و ارزیابی فعالیت ضدمیکروبی پلانتاریسین خام بود.

مواد وروش‌ها: تجزیه و تحلیل رشد سویه COY2906 با استفاده از طیف سنج پایش شد. تکثیر و شناسایی اهداف ژنی با استفاده از واکنش زنجیره‌ای پلیمراز بلادرنگ (Real-Time PCR) انجام شد. پلانتاریسین خام با استفاده از آمونیوم سولفات ۴۰ و ۷۰ درصد وزنی حجمی ارزیابی شد. فعالیت ضدمیکروبی با استفاده از روش انتشار-چاهک و جرم مولکولی پروتئین نسبتا خالص شده با استفاده از طیف سنجی جرمی یونیزاسیون دفعی لیزر به کمک ماتریس (MALDI-TOF-MS) ارزیابی شد.

یافته‌ها و نتیجه‌گیری: *ل. پلانتاروم* سویه COY2906 در آبگوشت MRS و دمای ۳۷ درجه سلسیوس کشت بی‌هوازی داده شد. پس از ۱۹ ساعت یا در وسط فاز سکون برداشت شد تا تولید پلانتاریسین به حداکثر برسد. سطح نسبی بیان ژن *plnA*، *plnEF*، *plnN* و *plnJ* بیش از حد بیان شد، در حالی‌که *plnK* اینطور نبود. برای دستیابی به پلانتاریسین، مایع رویی بدون سلول با ۴۰ و ۷۰ درصد آمونیوم سولفات رسوب داده شد که در نتیجه غلظت پروتئین خام به ترتیب به ۴۱/۳۳ و ۱۴۸ $\mu g.ml^{-1}$ رسید. نتایج نشان داد که پروتئین خام فاقد فعالیت ضدمیکروبی است، مایع رویی بدون سلول سویه COY2906 اثر ضدمیکروبی مشابه سدیم آمپی سیلین در $\mu g.ml^{-1}$ ۱۰۰ نشان داد. پس از رسوب با سولفات آمونیوم ۷۰ درصد، طیف سنجی جرمی یونیزاسیون دفعی لیزر به کمک ماتریس، اثری از حضور پلانتاریسین A، پلانتاریسین EF، پلانتاریسین N و پلانتاریسین J را نشان نداد. با این حال، پلانتاریسین K در طیف تشخیص داده شد. با توجه به نتایج، تجزیه و تحلیل بیشتر در مورد تشخیص پلانتاریسین با استفاده از طیف سنجی جرمی یونیزاسیون دفعی لیزر به کمک ماتریس توصیه می‌شود. برای ارزیابی فعالیت‌ها و ویژگی‌های آن می‌توان اصلاح حلال یا افزایش غلظت آمونیوم سولفات را توصیه کرد.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

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واژگان کلیدی
- فعالیت ضدمیکروبی
- پروتئین خام
- بیان ژن
- *لاکتیپلانتیباسیلوس پلانتاروم*
- *پلانتاریسین*

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[1] Heterologou Genes- ژن‌هایی که از نظر کارکرد و منشا متفاوت هستند.