Nat. Volatiles & Essent. Oils, 2021; 8(5): 1861 - 1872

NVEO
Natural Volatiles & Essential Oils

Screening and Characterization of Bacteriocin Produced by Alcaligenes faecalis (Strain MW4) Isolated from Vegetable Waste

K.C.P. Rajamanikandan[1], S. Vairamuthu[1], G. Kaviyarasan[1], D. Arvind Prasanth[1*] And D.J. Mukesh Kumar[2]

[1 & 1] Department of Microbiology, School of Biosciences, Periyar University, Salem-636 011, Tamilnadu, India.*

[2] Postdoctoral Researcher, Biomaterial Lab, CAS in Botany, University of Madras, Chennai – 600 025, India.

ABSTRACT:

Bacteriocins are defined as proteinaceous compounds produced by almost all bacteria that specifically kill the closely interrelated species. Due to the emergence of antibiotic resistance, this bacteriocin have evolved as an alternative with several advantages such as target specificity and cost effectiveness. The objective of this present study is to screen and isolate the bacteriocin producing bacteria from vegetable waste. In this regard, Vegetable waste material was grinded with distilled water and was plated on to MRS (deMan Rogosa and Sharpe) and BHI (Brain Heart Infusion) agar plate. A total of 10 bacterial colonies were isolated from this sample and found to be bacteriocin producing organism by agar well diffusion method. Among these ten isolates, strain no. MW-4 showed good activity against clinical and cattle field environmental pathogens. The strain MW-4 was identified as *Alcaligenes faecalis* by 16S rRNA gene sequencing and the protein profiling was confirmed the presence of low molecular weight bacteriocin around 3-5 KDa. Spectrum analysis shows an evidence of peptides in its distinctive absorption bands equivalent to N-H stretching of proteins and peptide bonds. The results of this study suggest that the strain MW4 could be used as a potential bio-control agent in the cattle field. However further studies are required to clarify the type and nature of bacteriocin and predict the structure of peptide linkage so that it can find the application in bio-control agent in the treatment of environmental pathogens.

Keywords: Bacteriocin, *Alcaligenes faecalis*, antibacterial compound, vegetable waste, clinical pathogens, FTIR

Introduction

Bacteriocins are considered as the proteinaceous compounds produced by most of the bacteria including Lactic acid bacteria (LABs) which inhibits the growth of other bacterial strains (Parada *et al.,* 2007)**.** Bacteriocins are highly specific antibacterial proteins produced by active strains mainly against the some other strains of identical or related species (Djadouni & Kihal, 2012). Bacteriocins are potent, highly specific microbial peptides produced under stressful circumstances, causing quick elimination of neighboring cells and have attracted this extensive studies by many researchers. These small peptides (typically containing less than 60 amino acids) eliminate or prevent the growth of specific bacterial strains from closely interrelated species (Hassan *et al.*, 2020). These bacteriocins are of different types such as Class I, II, III and IV based on structural and functional characteristics (Karpinski and Szkaradkiewicz, 2013)**.** The uses of these microbial peptides are gaining more and more attention not only as an alternative therapeutic agent but also in the food industries as preservative agents to avoid the worsening and spoilage of food (Muhammad *et al.*, 2015)**.** Some bacteriocins are considered to be lantibiotics due to their altered amino acid Lanthionine structure as one of their portions. Fruits and Vegetable wastes gives a better source of isolating a microorganism possessing antagonistic properties (Ruiz Rodríguez *et al*., 2021)**.** However, work on bacteriocinogenic organisms from vegetable wastes has been limited. Hence, the present study is

carried out to isolate and identify the bacteriocins producing bacterial isolates from spoiled vegetables to treat against the clinical and cattle field environmental pathogens.

MATERIALS AND METHODS:

ISOLATION OF BACTERIOCIN PRODUCING ORGANISM:

The vegetable waste was collected in a sterile container from Suramangalam market, Salem, Tamilnadu and brought to the laboratory in sterile condition. This material was grinded with sterile distilled water in a mortar and pestle. 1 ml of grinded sample was serially diluted up to 10^{-6} dilution by using sterile distilled water. 0.1 ml of sample from 10^{-2} to 10^{-6} were plated on to MRS (deMan Rogosa and Sharpe) (Menconi *et al*., 2014) for the presence of lactic acid production and BHI (Brain Heart Infusion) agar medium for non-lactic acid production. The plates were incubated for 24 h at 37^0C. The bacterial isolates with different colony morphology were purified and sub cultured onto specific agar plates.

SCREENING OF ANTIBACTERIAL ACTIVITY:

a. Culture Preparation for bacteriocin:

The culture preparation method was followed by **Anacarso 2015**. The selected strain was grown in MRS (deMan Rogosa and Sharpe) & BHI broth separately at 37^0C for 24 h. Then bacterial cultures were subjected to centrifugation for separation of cells at 5000 rpm for 15 m to obtain cell-free supernatant (CFS) that were adjusted to pH 6 with sterilized 1M NaOH and used as crude bacteriocins for the detection of inhibitory activity.

b. Test culture preparation:

The preserved Clinical pathogenic bacterial culture of *Bacillus subtilis,* ATCC cultures such as *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853) were obtained from Department of Microbiology, Periyar university, Salem and cattle field environmental pathogens namely *Pseudomonas spp*., (PP-I K) and *E.coli* (PP-I U) were isolated in medical Microbiology laboratory are used as test organisms for evaluating the antibacterial activity (Gomashe *et al*., 2014)**.**

c. Detection of inhibitory activity of bacteriocin by Agar well diffusion assay:

The inhibitory activity of bacteriocin was achieved by agar well diffusion method (Gomashe et al., 2014)**.** Around 5mm of diameter wells were cut on solidified MRS & BHI agar media previously swabbed by clinical pathogen namely *Bacillus subtilis*, ATCC cultures: *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853) and cattle field environmental pathogens PP-I K, PP-I U separately. Then add 100µl of previously prepared crude bacteriocin supernatants were loaded into the each well separately. Incubate the plates at 37^0C for 24 h and observe the broad spectrum of inhibition for further analysis.

PARTIAL PURIFICATION & BACTERIOCIN ACTIVITY OF MW-4 STRAIN:

The partial purification of best potential bacteriocin producing strain (MW-4) was done by the following method of Sambrook and Russell, 2001**.** The overnight bacterial culture was centrifuged at 5000 rpm for 10 m and the cells were separated. Bacteriocin present in the cell-free

supernatant (CFS) was successively precipitated by 80% ammonium sulfate by stirring at 4^0C for 1 h, after which the precipitates were collected by centrifugation at 10,000 rpm for 20m and dissolved in distilled water. The precipitates were subjected to dialysis process by dissolving in 0.05M phosphate buffer solution (pH-7) with 0.22μm filter dialysis bag and agitated gently with magnetic stirrer at 4°C for 24 h. The resulting dialyzed supernatant was confirmed for bacteriocin activity by agar well diffusion assay.

DETERMINATION OF MOLECULAR SIZE:

The molecular weight of bacteriocin was estimated by SDS-PAGE using horizontal electrophoresis units (Medox Co). Following electrophoresis, gels were stained with Coomassie Brilliant Blue R-250 by constant revolution at 120 rpm for overnight and destained by methanol to remove the excess stains and observe the protein bands. A protein ladder (10kDa) was used for estimating the molecular weight of bacteriocin (Hassan *et al.,* 2020)**.**

HPLC ANALYSIS OF CFS:

The active fractions of partially purified sample MW-4 was further checked for homogeneity by subjecting the resulting protein to HPLC analysis (Thermo Finnigan Surveyor) using C18 column by Empower 2 software. The elution conditions employed for HPLC were as follows: solvent system 0.1% aqueous TFA (A solution) and acetonitrile containing 20% a solution; Injection volume - 10µl by auto sampler; temperature - 25^0C; Run time- 30 min; UV detection range - 220 nm and 280 nm. The protein was eluted with acetonitrile and 0.2 % formic acid in 50:50 ratio for first 5 m and then with a linear concentration gradient of B solution. Retention time of peptides were determined by HPLC and observed at 220nm.

PEPTIDE CONFIRMATION BY FTIR:

A Perkin-Elmer infrared spectrophotometer (Perkin Elmer, Uberlingen, Germany) was used for the investigation of the surface functional groups of the protein bacteriocin compound. First, the MW4 primary metabolites was extracted by using methanol solvent and metabolites were grinded by KBr pellets (spectroscopic grade) with MilliQ water and the samples were evenly applied onto a ZnSe optical plate. Then plate is placed under dried vacuum for approximately 15 m and then analyzed by FTIR spectroscopy. The samples were scanned between 400 and 4000 cm^{-1} in the spectral range.

STRAIN IDENTIFICATION AND 16s RIBOSOMAL RNA GENE SEQUENCING

The Bacteriocin producing potential strain MW4 was subjected to gram staining and 16S rRNA sequencing. The DNA Extraction was performed. The PCR reaction was carried out using the forward primers (5'-AGA GTT TGA TCC TGG CTC AG-3') and reverse primers (5' – TAC GGC TAC CTT GTT ACG ACT T -3'). The amplified PCR product was analyzed using 1% agarose gel electrophoresis and visualized under UV trans-illuminator. The amplified PCR product was purified using Gene elute column and the purified DNA was sequenced out using Beckman Coulter CEQ 8000 auto analyzer by Bioserve Technologies Pvt. Ltd. After determining the 16S rRNA gene sequence, each base sequence

was matched with the base sequences of related strains in GenBank database to define the phylogenetic location of the strain (Ilham *et al.*, 2013)**.**

RESULTS & DISCUSSION:

SERIAL DILUTION & SCREENING OF MICROORGANISM:

In this present study, a total of 10 bacterial strains were isolated from vegetable waste in Suramangalam, Salem district. Among the 10 strains, 4 strains namely MW-1 to MW-4 were selected based on cream colored colonies on the MRS agar plates indicating the presence of lactic acid production and 6 strains namely BW-1 to BW-6 were identified as non-lactic acid production from BHI agar plates. In the antibacterial activity, non-lactic acid bacterial isolates namely BW-1 to BW-6 will not produce any inhibition effect except BW-2. BW-2 isolate produce very lower inhibition effect when compare to the MW strains (Table - 1).

In agar well diffusion assay, the potential strain of bacteriocin producers were cultured and processed. Then the culture broth & cell free supernatant (CFS) obtained from bacteriocin producer strains were tested for inhibitory activity against the test organisms such as clinical pathogens, ATCC cultures and cattle field pathogens (Oliveira *et al.,* 2018). In this, only one bacterial strain MW4 from MRS agar plates showed the best inhibitory activity against both clinical pathogen *Bacillus Subtilis,* ATCC culture: *Staphylococcus aureus* (ATCC 25923) and cattle field environmental pathogens [*Pseudomonas spp.,* (PP-I K), *E.coli* (PP-I U)] [(Table - 2) (Graph – 1)] (Khochamit *et al.*, 2015)**.** From this result, both MW & BW strains will not produce any inhibitory effect against *Pseudomonas aeruginosa* ATCC strain no. 27853 and conclude that bacteriocin compound produced from MW-4 strain will not have inhibitory activity in this ATCC culture.

PARTIAL PURIFICATION OF BACTERIOCIN:

Bacteriocin produced MW-4 strain was partially purified by two-step procedure, including ammonium sulfate precipitation and dialysis method. Primarily, the cell-free supernatant (CFS) containing bacteriocin was precipitated by 40, 60, and 80% ammonium sulfate (Zhang *et al.,* 2018)**.** The resultant precipitate was filtered by 0.22μm filtered dialysis bag using0.05M phosphate buffer solution (pH-7). Partially purified dialyzed supernatant was tested and results showed that better antibacterial activity. The partially purified bacteriocin compound confirmed a strong antibacterial activity against the ATCC strain No: (ATCC 25923) *Staphylococcus aureus* followed by clinical pathogen *Bacillus subtilis* and Cattle field environmental pathogen such as PP-I K, PP-I U [(Table - 3) (Graph – 2) (Figure – 1)].

DETERMINATION OF MOLECULAR SIZE & HPLC ANALYSIS:

The protein profile was analyzed by SDS-PAGE & showed the presence of protein band approximately 3-5 KDa thereby confirming the presence of low molecular weight bacteriocin. Based on this result, this bacteriocin may be thermo-stable peptide which acts on membrane structure and confirmed that MW-4 strain disrupt the integrity of cell membrane, resulting in the leakage of proteins (Figure – 2) (Hassan *et al.,* 2020)**.**

Then, cell free supernatant of MW- 4 was subjected to HPLC column. The HPLC profile of the bacteriocin revealed the presence of two major peaks eluted earlier at 90% and 95% by elution

Buffer e3acetonitrile and formic acid, corresponding to the retention times of 3.069 and 3.102 min, respectively and of minor peak at retention time 25.691 (Figure – 3a). Purified fractionated samples were individually collected and re-chromatographed on the same condition. Two major active peaks and one minor peak were designated as fractions A and B, respectively and considered to be presence of bacteriocin compound (Figure – 3b) *(Kaur et al.*, 2013)**.**

FTIR ANALYSIS:

The FTIR spectral analysis, the intracellular metabolite extract of MW4 strain revealed the spectral range between 400 and 4000 cm^{-1}. From the spectrum analysis result, we analyzed the peak values with the help of spectrum chart available in NIST library, the peak signal recorded at 615.77 may be due to C-H (alkyne) and the sharp peak observed at 1061.93 cm^{-1}may be due to presence of C-N stretch aliphatic amine. The peak at 1286.07, 1405.76 &1467.82 cm^{-1} may be revealed due to presence of carbon, nitrogen and oxygen bonds (C–N stretch & N–O asymmetric stretch). The vibration stretch recorded at 1467.82 & 1515.74 cm^{-1} represents the presence of nitro compounds (N–O asymmetric stretch). Again, the vibration stretches upto 1628.97 & 2076.09 cm^{-1} due to the presence of primary amines and conjugated amines (N–H &–C ≡ C– stretch). Finally a broad band appears at 3449.48 cm^{-1} may be due to presence of hydroxyl and phenolic groups (O–H stretch). Analysis of the spectrum showed a distinctive absorption bands equivalent to N-H stretching of proteins and peptide bonds (Bizani *et al.*, 2005) (Al-Thubiani *et al.*, 2018), concrete evidence that the substance contained a peptide in its structure. (Figure – 4)

STRAIN IDENTIFICATION AND 16s RRNA GENE SEQUENCING:

The bacterial strain MW4 showed Gram negative, rod shaped, arranged singly and no pigments were produced. The analyzed base sequence of PCR amplified product was compared with the base sequences of related strains recorded in the GenBank database to observe the correlation between genes. Based on the result, the isolated strain had a 99% homology to that of *Alcaligenes faecalis*. In addition, to determine the molecular phylogenic relationship with the existing *Alcaligenes faecalis* based on the structure of the 16S rRNA gene, a phylogenetic tree was prepared, and the results are shown in figure – 5 (Ilham *et al.*, 2013). The sequence was deposited in genbank with the accession number KT023112. Therefore, the lactic acid Bacteria that was isolated from vegetable waste was named *Alcaligenes faecalis* MW-4.

CONCLUSION:

In this study, the isolation of bacteriocin producing strain from vegetable waste was collected from the Suramangalam market, Salem district. A total of 10 bacterial strains were isolated from the fermented vegetable waste and screened for bacteriocin production. The crude bacteriocin was prepared and tested against the clinical and cattle field environmental pathogens. 10 bacterial strains exhibited antimicrobial activity against test organisms. Then partially purified strain MW4 bacteriocin was tested against the clinical pathogens and cattle field environmental pathogens. In this result, Strain MW4 exhibited higher activity against *Staphylococcus aureus* (ATCC 25923); Cattle field environmental pathogens PP-I K, PP-I U and clinical pathogen *Bacillus Subtilis*. The protein profile of bacteriocin producer strain MW4 confirms the presence of low molecular weight protein bands approximately 3-5 KDa. The HPLC profile revealed the presence of two major active peaks and

one minor peak were observed and considered to be presence of bacteriocin compound. Spectrum analysis showed a distinctive absorption bands gave an evidence of peptide bonds in its structure. Hence, this result shows that the evidence of bacteriocin compound have antibacterial activity of peptides produced by *Alcaligenes faecalis* MW4 has a bactericidal effect in cattle field organisms by disrupting the membrane function of target organisms.

ACKNOWLEDGEMENT:

We are very thankful to Department of Microbiology, School of Biosciences, Periyar University, Salem for giving the opportunity to carry out this research work. We are grateful by the never-ending inspiration and support of all the colleagues and friends. We thank the DST- FIST for providing sophisticated laboratory facilities to carry out this research work with reference No. SR / FST / LSI – 640 / 2015 (C) dated 30/5/2016.

AUTHOR'S CONTRIBUTION:

RMK designed the whole experiment, sample collection, and complete draft of this study. SVM isolated the organism and performed the biochemical characterization. GKY gives the support of research design. MK interprets FTIR results and edited the paper. AP organized the whole paper and approved the final form of manuscript.

FUNDING:

There is no funding to this report.

ETHICAL APPROVAL:

This study does not involve the use of animals or human subjects.

Table – 1: Isolation & screening of antibacterial activity by crude bacteriocin

Isolated Vegetable waste	Activity	
	Clinical pathogen & ATCC cultures	Cattle field pathogens
MW-1	+	++
MW-2	+	-
MW-3	+	+
MW-4	++	++
BW-1	-	-
BW-2	-	+

BW-3	-	-
BW-4	-	-
BW-5	-	-
BW-6	-	-

Table-2: Inhibitory activity of the bacterial isolates against clinical & Cattle field pathogens

Bacterial Isolates	**Clinical pathogen**	**ATCC cultures**		**Zone of diameter (mm)**	**Cattle field pathogens**		**Zone of diameter (mm)**
	Bacillus subtilis	*Staphylococcus aureus* (ATCC 25923)	*Pseudomonas aeruginosa* (ATCC 27853)		PP-I K	PP-I U	
BW-2		-	-	-	-	+	12
MW-1	+	-	-	20	+	+	20,13
MW-2	+	-	-	35	-	-	-
MW-3	+	-	-	28	+	-	20
MW-4	**+**	**+**	**-**	**33, 28**	**+**	**+**	**15, 29**

Graph – 1 Inhibitory activity of the bacterial isolates against clinical and Cattle field pathogens

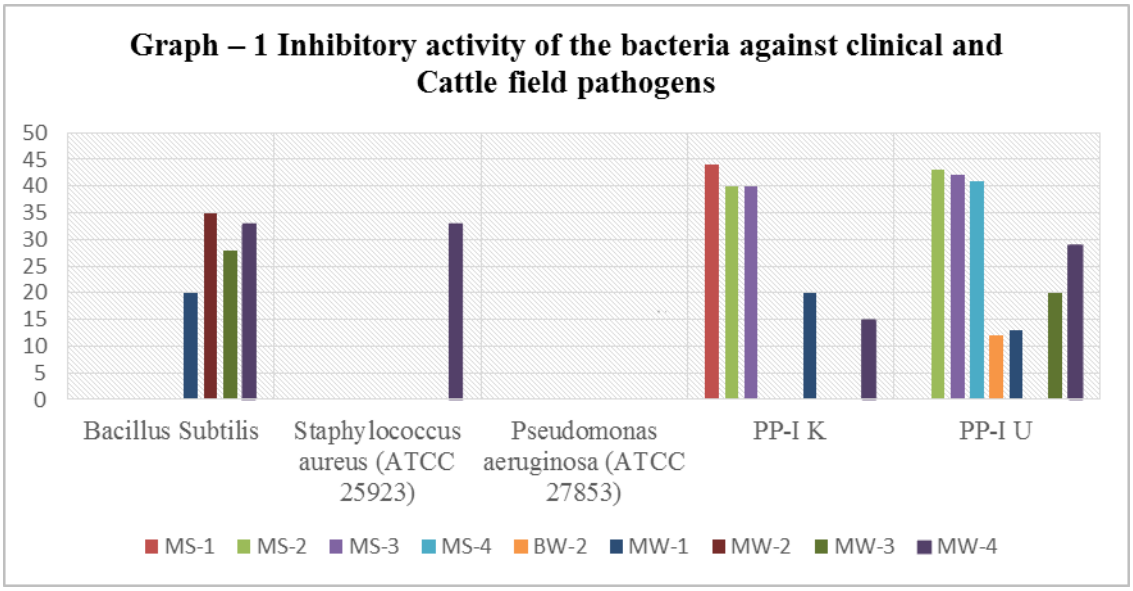


Table – 3: Determination of antibacterial activity by partially purified bacteriocin

Test organisms		Crude Extract (mm)	Partially purified Bacteriocin (mm)
Clinical isolate	*Bacillus Subtilis*	10	*15*
ATCC culture	*Staphylococcus aureus* (ATCC 25923)	15	20
Cattle Field Environmental Pathogen	*Pseudomonas spp.,* PP-I K	14	13
	E.coli PP-I U	11	13

Graph – 2 Determination of antibacterial activity by partially purified bacteriocin

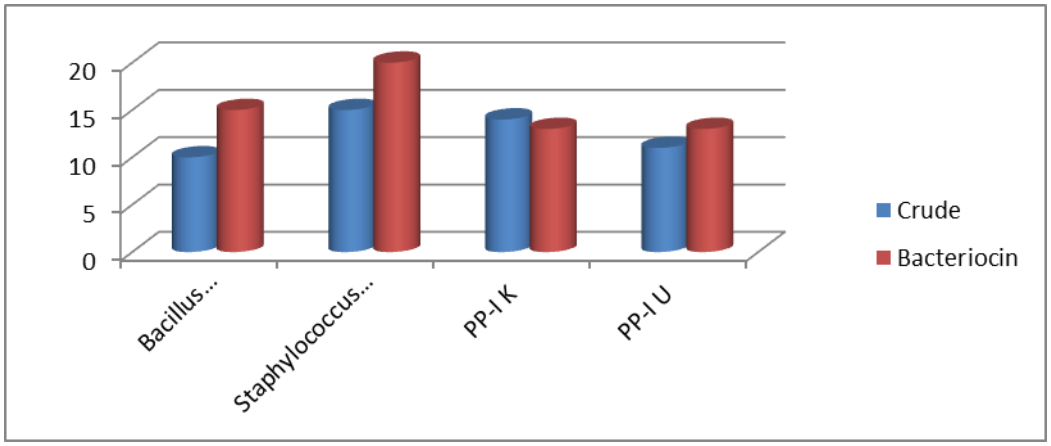


Figure: 1 Determination of antibacterial activity

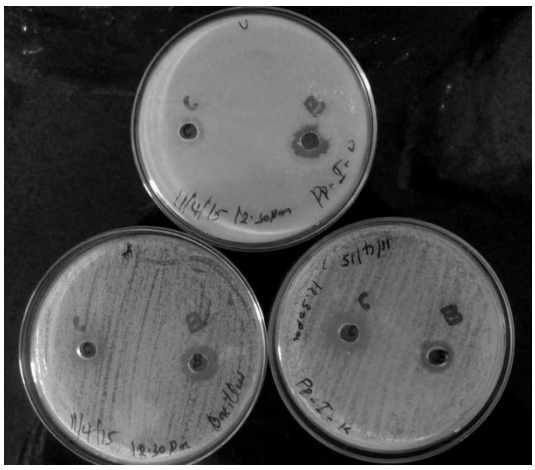


Figure: 2 Protein Profile by SDS PAGE

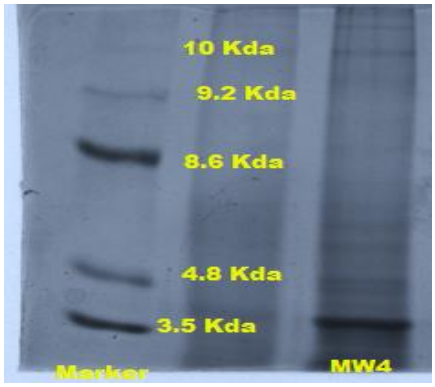


Figure: 3a HPLC Profile of Bacteriocin

Figure: 3b HPLC Profile of purified Bacteriocin

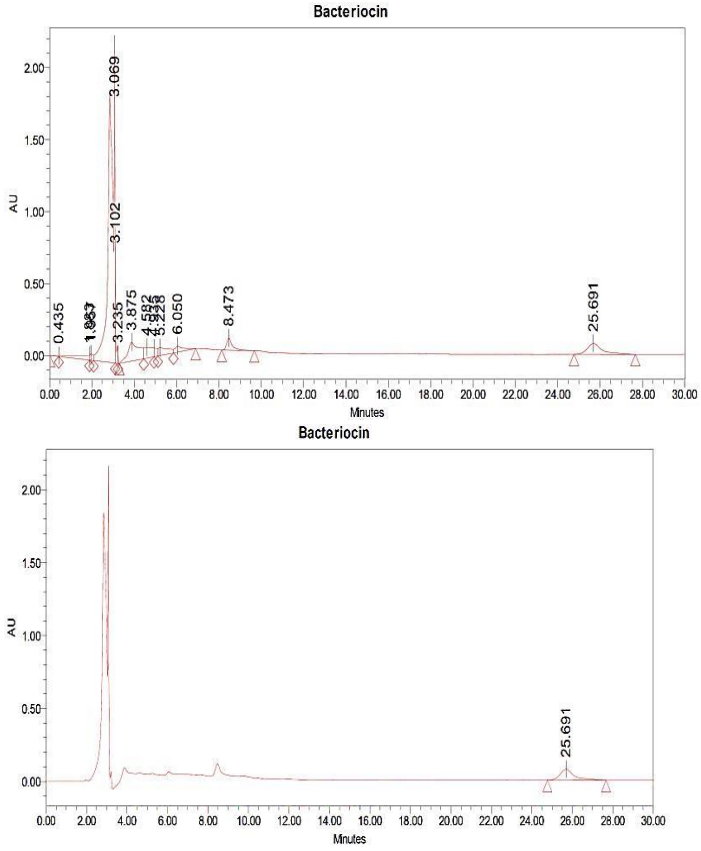


Figure: 4 FTIR Spectral Analysis

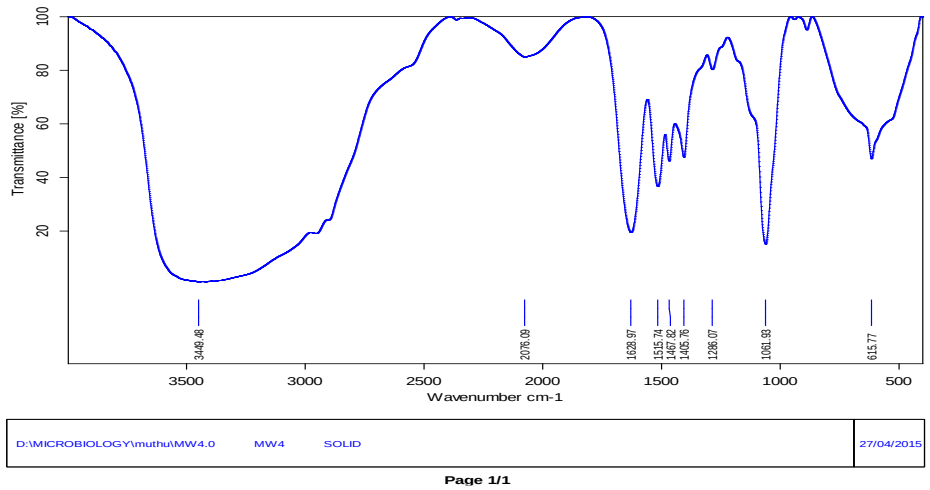
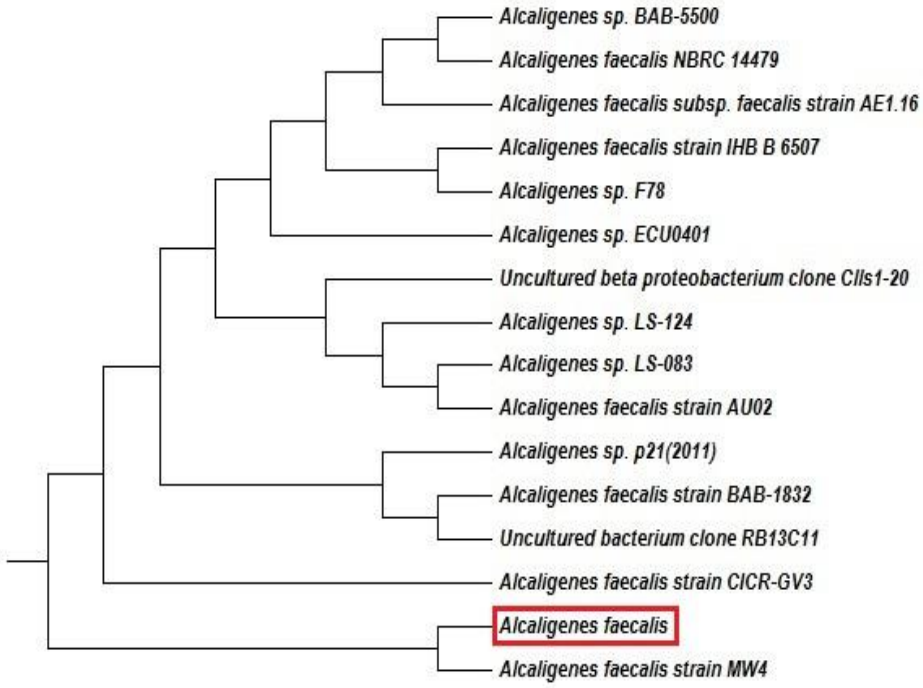


Figure: 5 molecular characterization of 16S rRNA gene sequencing for MW4 strain



References:

a. Al-Thubiani, A. S. A., Maher, Y. A., Fathi, A., Abourehab, M. A. S., Alarjah, M., Khan, M. S. A., & Al-Ghamdi, S. B. (2018). Identification and characterization of a novel antimicrobial peptide compound produced by Bacillus megaterium strain isolated from oral microflora. *Saudi Pharmaceutical Journal*, *26*(8), 1089–1097. https://doi.org/10.1016/j.jsps.2018.05.019

b. Anacarso, I. (2015). Culture Compounds which are Able to Increase the Growth and the Production of Bacteriocins by Two Different LABS. *Journal of Plant Pathology & Microbiology*, *06*(04). https://doi.org/10.4172/2157-7471.1000263

c. Bizani, D., Dominguez, A. P. M., & Brandelli, A. (2005). Purification and partial chemical

characterization of the antimicrobial peptide cerein 8A. *Letters in Applied Microbiology*, *41*(3), 269–273. https://doi.org/10.1111/j.1472-765X.2005.01748.x

d. Djadouni, F., & Kihal, M. (2012). Antimicrobial activity of lactic acid bacteria and the spectrum of their biopeptides against spoiling germs in foods. *Brazilian Archives of Biology and Technology*, *55*(3), 435–444. https://doi.org/10.1590/S1516-89132012000300015

e. Gomashe, A. V, Sharma, A. A., & Wankhede, M. A. (2014). Screening and Evaluation of Antibacterial Activity of Bacteriocin Producing Lab against some Selected Bacteria Causing Food Spoilage. *Int.J.Curr.Microbiol.App.Sci*, *3*(8), 658–665. Retrieved from http://www.ijcmas.com

f. Hassan, M. U., Nayab, H., Rehman, T. U., Williamson, M. P., Haq, K. U., Shafi, N., … De Guía Córdoba, M. (2020). Characterisation of Bacteriocins Produced by Lactobacillus spp. Isolated from the Traditional Pakistani Yoghurt and Their Antimicrobial Activity against Common Foodborne Pathogens. *BioMed Research International*, *2020*. https://doi.org/10.1155/2020/8281623

g. Ilham, Z., Abdellah, H., Wifak, B., Mohammed, I., & Saad, I. (2013). A novel Alcaligenes faecalis antibacterial-producing strain isolated from a Moroccan tannery waste. *African Journal of Microbiology Research*, *7*(47), 5314–5323. https://doi.org/10.5897/ajmr2013.6029

h. Karpiński, T. M., & Szkaradkiewicz, A. K. (2013). Characteristic of bacteriocines and their application. *Polish Journal of Microbiology*. Polish Society of Microbiologists. https://doi.org/10.33073/pjm-2013-030

i. Kaur, B., Balgir, P., Mittu, B., Chauhan, A., & Kumar, B. (2013). Purification and physicochemical characterization of anti-gardnerella vaginalis bacteriocin hv6b produced by Lactobacillus fermentum Isolate from human vaginal ecosystem. *American Journal of Biochemistry and Molecular Biology*, *3*(1), 91–100. https://doi.org/10.3923/ajbmb.2013.91.100

j. Khochamit, N., Siripornadulsil, S., Sukon, P., & Siripornadulsil, W. (2015). Antibacterial activity and genotypic-phenotypic characteristics of bacteriocin-producing Bacillus subtilis KKU213: Potential as a probiotic strain. *Microbiological Research*, *170*, 36–50. https://doi.org/10.1016/j.micres.2014.09.004

k. Menconi, A., Kallapura, G., Latorre, J. D., Morgan, M. J., Pumford, N. R., Hargis, B. M., & Tellez, G. (2014). Identification and characterization of lactic acid bacteria in a commercial probiotic culture. *Bioscience of Microbiota, Food and Health*, *33*(1), 25–30. https://doi.org/10.12938/bmfh.33.25

l. Muhammad, N., Akbar, A., Shah, A., Abbas, G., Hussain, M., & Khan, T. A. (2015). Isolation optimization and characterization of antimicrobial peptide producing bacteria from soil. *Journal of Animal and Plant Sciences*, *25*(4), 1107–1113.

m. Oliveira, R. P. S., Oliveira De Souza De Azevedo, P., Molinari, F., Pinheiro, R., & Oliveira, S. (2018). Importance of the agar-media in the evaluation of bacteriocin activity against the same test-microorganisms. *J. Pharm. Sci*, *54*(1), 17533. https://doi.org/10.1590/s2175-97902018000117533

n. Parada, J. L., Caron, C. R., Medeiros, A. B. P., & Soccol, C. R. (2007). Bacteriocins from lactic acid bacteria: Purification, properties and use as biopreservatives. *Brazilian Archives of Biology and Technology*, *50*(3), 521–542. https://doi.org/10.1590/s1516-89132007000300018

o. Ruiz Rodríguez, L. G., Zamora Gasga, V. M., Pescuma, M., Van Nieuwenhove, C., Mozzi, F., & Sánchez Burgos, J. A. (2021). Fruits and fruit by-products as sources of bioactive compounds.

Benefits and trends of lactic acid fermentation in the development of novel fruit-based functional beverages. *Food Research International*, *140*, 109854. https://doi.org/10.1016/j.foodres.2020.109854

p. Zhang, J., Yang, Y., Yang, H., Bu, Y., Yi, H., Zhang, L., … Ai, L. (2018). Purification and partial characterization of bacteriocin Lac-B23, a novel bacteriocin production by lactobacillus plantarumJ23, isolated from Chinese traditional fermented milk. *Frontiers in Microbiology*, *9*(OCT), 1–7. https://doi.org/10.3389/fmicb.2018.02165