

INTERNATIONAL JOURNAL OF

# BIO-SCIENCE AND BIO-TECHNOLOGY





INTERNATIONAL JOURNAL OF

# BIO-SCIENCE AND BIO-TECHNOLOGY



#### Editor-in-Chief of the IJBSBT Journal

Tai-hoon Kim, GVSA and UTAS, Australia

#### General Information of IJBSBT

Bibliographic Information
 ISSN: 2233-7849

· Publisher: SERSC

Science & Engineering Research Support soCiety

#### Contact Information

Science & Engineering Research Support soCiety

· Management Office: 20 Virginia Court, Sandy Bay, Tasmania, Australia

Phone no.: +61-3-9016-9027

Email: ijbsbt@sersc.org

#### Submission Guidelines

- Every submitted paper will be carefully reviewed (blind review) by at least three members of the International Program Committee.
- All accepted papers will be published by the web first, and included in the hard copy journal later.
- Authors should submit paper with about 12 pages ~ 18 pages by using online systems for review.

#### Journal Topics

The goal of IJBSBT is to bring together the researchers from academia and industry as well as practitioners to share ideas, problems and solutions relating to the multifaceted aspects of Bio-Science and Bio-Technology.

#### Basic Topics:

- Bioelectronics and Biorobotics
- Bioinformatics and Bioengineering
- Bioinspired Information Technology
- Biometrics and its Application
- Biomedicine and Medical Informatics
- Hybrid Research between Bio-technology and IT

#### Advisory/Editorial Board

- A. Q. K. Rajpoot, MUET, Pakistan
- · Aishy Amer, Concordia University, USA
- · Apoorva Gaiwak, Medicaps Institute of Technology and Science, India
- Blamah Nachamada Vachaku, University of Jos, Nigeria
- Carlos Juiz, Universitat de les Illes Balears, Spain
- Clare Bates Congdon, University of Southern Maine, USA
- Debnath Bhattacharyya, Heritage Inst. of Technology, India
- Dong-Yup LEE, National University of Singapore, Singapore
- Emilio Corchado, University of Burgos, Spain
- · Farzin Deravi, University of Kent, UK
- Francisco Herrera, University of Granada, Spain
- George A. Gravvanis, Democritus University of Thrace, Greece
- · Guo-Zheng Li, Shanghai University, China
- Janusz Kacprzyk, Polish Academy of Sciences, Poland
- Jason T. L. Wang, New Jersey Institute of Technology, USA
- Jim Torresen, Department of Informatics at the University of Oslo, Norway

- Kenji Mizuguchi, National Institute of Biomedical Innovation, Japan
- · Kevin Daimi, University of Detroit Mercy, USA
- Liangjiang Wang, Clemson University, USA
- Lusheng Wang, City University of Hong Kong
- Maricel Balitanas, University of San Agustin, Philippines
- Md. Al Amin Bhuiyan, Jahagirnagar University, Bangladesh
- Md. Ameer Ali, East West University, Bangladesh
- Meena K. Sakharkar, Nanyang Technological University, Singapore
- Michael E. Schuckers, St. Lawrence Univ., USA
- Mohammad Zahidur Rahman, Jahagirnagar University, Bangladesh
- Pong C. Yuen, Hong Kong Baptist University, Hong Kong
- Prashant Bansod, Shri Govindram Seksaria Institute of Technology & Science, India
- R. Ponalagusamy, National Institute of Technology, India
- Rattikorn Hewett, Texas Tech University, USA
- Rosslin John Robles, University of San Agustin, Philippines
- Saman Halgamuge, University of Melbourne, Australia
- See-Kiong Ng, Institute for Infocomm Research, Singapore
- Sungwon Park, Hannam University, Korea
- Tatsuya Akutsu, Kyoto University, Japan
- Tommaso Mazza, The Microsoft Research University of Trento CoSBi, Italy
- Tughrul Arslan, Engineering and Electronics, Edinburgh University, UK
- Waleed Abdullah, University of Auckland, New Zealand
- Xiaofeng Song, Nanjing University of Aeronautics and Astronautics, China
- Yang-sun Lee, Seokyung Unviersity, Korea
- Zambare Vasudeo, South Dakota School of Mines & Technology, USA

International Journal of Bio-Science and Bio-Technology Vol.7, No.3, (2015)

### IJBSBT is indexed by:

- EBSCO
- ProQuest
- ULRICH
- OpenJ-Gate
- SCOPUS
- Cabell

### **Editorial Secretary**

· Ronnie D. Caytiles

#### Foreword and Editorial

#### International Journal of Bio-Science and Bio-Technology

We are very happy to publish this issue of an International Journal of Bio-Science and Bio-Technology by Science and Engineering Research Support soCiety.

This issue contains 32 articles. Achieving such a high quality of papers would have been impossible without the huge work that was undertaken by the Editorial Board members and External Reviewers. We take this opportunity to thank them for their great support and cooperation.

Paper "Correlations between Medical Students' Self Assessment of Communication Skills and Patient-Physician Interaction Assessed by Standardized Patientsin Clinical Performance Examination" identifies the correlations between medical students' communication skills and patient-physician interaction of clinical performance examination. A total of 36 fourth-year medical students were enrolled in this study and were surveyed concerning communication skills during clinical performance examination. This study demonstrated that medical education of communication skills could not be transferred to improvement of patient-physician interaction in clinical skills assessment.

In the paper "Ex-situ Conservation of Indigenous, Threatened and Ethno-Medicinal Diversity of Forest Species", Madhya Pradesh is rich in plant wealth and endemic flora. As a part of conservation programme, institute has established an arboretum-cum-botanic garden in 1976, covering an area of 7.34 ha. The garden complex includes various sections situated in the campus and nursery. The main forest botanic garden is situated in 4.25 ha area and houses a wide array of forest flora including trees, shrubs, climbers and herbal plant species in various sections. Of the total species planted, over 50% were threatened and ascribed with conservation value. The garden was of scientific and educational utility. The institute provides diploma and degree courses in collaboration with Universities and colleges. The institute forest botanic garden has been registered under the network of Indian Botanic Gardens in 2005. It was one among the 140 Botanic gardens of India registered by Botanic Garden Conservation International under BGCI-Investing in Nature-India programme.

The paper "EEG based Emotion Recognition from Human Brain using Hjorth Parameters and SVM" is presenting the emotion recognition of EEG brain signals using Support Vector Machine (SVM). The emotions were elicited in the subjects using emotion related stimuli. They used the emotional stimuli from the International Affective Picture System (IAPS) database in this research. These stimuli belonged to five types of emotions in our experiment such as, happy, calm, neutral, sad and scared. The raw EEG brain signals were preprocessed to remove the artifacts. They introduced a feature extraction method using Hjorth parameters. The set of features were extracted from preprocessed EEG signals of each subject, separately. The combined feature set of all subjects was processed through SVM.

The thesis "Improved Ventricular Fibrillation/Tachycardia Detection using NEWFM for Automated External Defibrillators" proposes improved VF/VT detection. For our experiments, they use the complete Creighton University Ventricular Tachyarrhythmia Database. Samples are analyzed under the same conditions in intervals of 7 s. Based on this data, they propose a time-delay transform. Then, they extract six shockable features,

#### Table of Contents

Correlations between Medical Students' Self-Assessment of Communication Skills and Patient-Physician Interaction Assessed by Standardized Patients in Clinical Performance Examination 1

Seieun Oh, Woo Jeong Kim and Min Young Kim

Ex-situ Conservation of Indigenous, Threatened and Ethno-Medicinal Diversity of Forest Species 9

O.P. Chaubey, Archana Sharma and G. Krishnamurth

EEG based Emotion Recognition from Human Brain using HjorthParameters and SVM 23

Raja Majid Mehmood and Hyo Jong Lee

Improved Ventricular Fibrillation/Tachycardia Detection using NEWFM for Automated External Defibrillators 33

Xiyu Zhou and Joon S. Lim

WSN Based Wheeze Detection Systems for Diagnosing Disease like Asthma: A Survey 40

Abhinav Hans and Sheetal Kalra

Muscle Activity of Lower Extremities for Normal Adults According to the Type of Chair and Posture during Sit-to-stand Movement 51

Sunghyoun Cho and Haewon Byeon

An Empirical Application of an Information System to Relieve Chronic Obstructive Pulmonary Disease 61

Seong-Ran Lee

International Journal of Bio-Science and Bio-Technology Vol.7, No.3 (2015)

Numerical Simulation of Blood Flow in Centrifugal Heart Pump by Utilizing Meshless Smoothed Particles Hydrodynamic Method 70

ErfanRahmanian. MehdiNavidbakhsh, Mohammad Mohammadzadeh and Hamed Habibi

A Study on Core Stability Training forPostural Control Ability and Respiratory Function in Patients with Chronic Stroke 83

Sung-Pil Chun, Kyung-Yoon Kim, Tae-Gyeong Kang and Gi-Do Kim

Factors That Affect Health Professionals' Preparation of Advanced Directives in Korea 91

Byung Deog Hwang, Ryoung Choi and Jae Woo Park

Camera Calibration Using Direct Mapping and Adaptive Metaheuristics
111

Jaroslav Moravec and Miloslav Hub

Relations of Job Stress, Burnout, Mindfulnessand Job satisfaction of Clinical Nurses 121

Jung Im Choi and Myung Suk Koh

Critical Thinking Disposition, Professional Self-concept and Caring Perception of Nursing Students in Korea 129

Hae Young Woo and Young Ran Tak

Estimation of Respiration Rate from ECG Using Canonical Components Analysis and Ensemble Empirical Mode Decomposition 139

Vineet Kumar and Gurpreet Singh

Nursing students' Experiences of Simulation-based Education on Hypoglycemia 147

In-hye Song and Hyeon-cheol Jeong

Relationship between Job Attitude and Job Performance of Reception Staff at General Hospital in Seoul 155

Yeon Suk Oh, MI Joon Lee and Burn Jeun Seo

The Effectiveness Inhibition Filtrate BacteriocinsLactobacillus acidophilus Toward Contaminants Bacteriafrom Swordfish(Auxis rochei)Stew 163

Rieny Sulistijowati, Jetty Nurhajati and Insawosami Awom

How the Factors of Hospital Choice of Cancer Patients Affect Customer Satisfaction 175

Jong-Hyun Yang and Jeong-Ah Yoon

A Comparison of Cervical Flexion, Pain, and Clinical Depression in Frequency of Smartphone Use 183

> Junhyuk Park, Kwangho Kim, Namkang Kim, Inwon Chol, Sujung Lee, Sajin Tak and Jongeun Yim

Analysis of Force in Human Muscle using EMG in Hot Rolling Mill 191

K. Govindaraju, B. Sasi Kumar, K. Raja and K. Murugabhoopathy

Identifying Symmetry and Contracted Ratio of Lateral Abdominal Muscles in Stroke Survivors 199

Dongkwon Seo, Seungwon Lee and Yeonseop Lee

Research on Size Precision of Implant Fixtures among Four Different Types of Fastening Methods 207

Soo-chul Park, Chang-suk Kim and In-ho Jeong

A Framework for Data Warehouse Using Data Mining and Knowledge Discovery for a Network of Hospitals in Pakistan 217

Muhammad Arif, Asad Khatak and Mehdi Hussain

# Factors Affecting The Spiritual Well-being of Foreign Immigrant Wives in Korea 223

Hye Jin Kim, Eun Kwang Yoo, Eun Sil Jung, Muyeong seak, Yang

Factors Influencing Ego-resilience in Nursing Students 233

Jun Hee Noh, Eun Ju Lim

Integrated Distributed Architecture to Integrate Wireless Sensor Networks (WSN) with Grid for Healthcare 243

Ch. V. Phani Krishna, K. V. D. Kiran and Tai-hoon Kim

Influence Factors on Health and Medical Expense of Public Pension Recipients in South Korea 251

Ryoung Choi and Byung Deog Hwang

Intelligent Precision Improvement on Robot Assisted Minimally Invasive Direct Coronary Artery Bypass 201

> Hossein Davarpanah, Farzin Piltan, Somayeh Jowkar, Mohammad Beheshti and Saman Rahbar

A Study of Emotional Intelligence and Coping Strategies in Baccalaureate Nursing Students 275

Mi-Ran Kim and Su-Jeong Han

Contingent Valuation Method of New and Renewable Energy as a Future Alternative in Korea 283

Woo-Jin Jung

The Study on the Motivation of Sex-Selective Abortion among Indian Immigrants in U.S.A 293

Jill Tucker, Sung Seek Moon, Minkyung Kim and KyungSook Kim

#### Multi-Hop N-Screen Traffic Mechanism for Wearable Health-Monitoring System in Hospital Wireless Networks 301

Kyeong Hur, Won-Sung Sohn and Kil Young Kwon

Effects of Functional Shoes on Joint Moment, Ground Reaction Force, and EMG 315

Chong-hoon Lee

# International Journal of Bio-Science and Bio-Technology

June 2015 Printed June 2015 Published

Publisher: SERSC

Publishing office: Science and Engineering Research Support soCiety

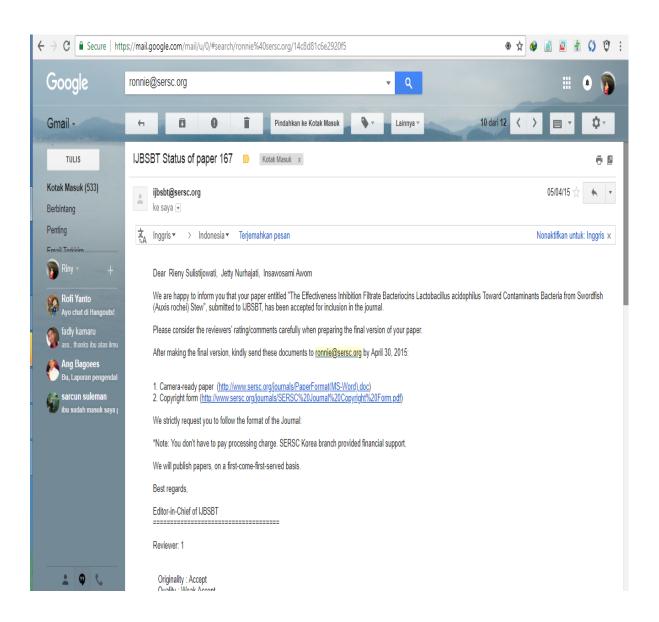
20 Virginia Court, Sandy Bay, Tasmania, Australia

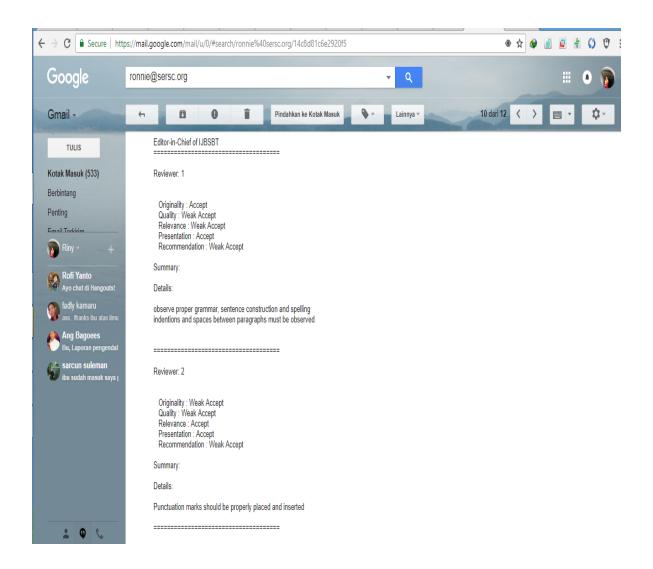
E-mail: sersc@sersc.org; ijbsbt@sersc.org

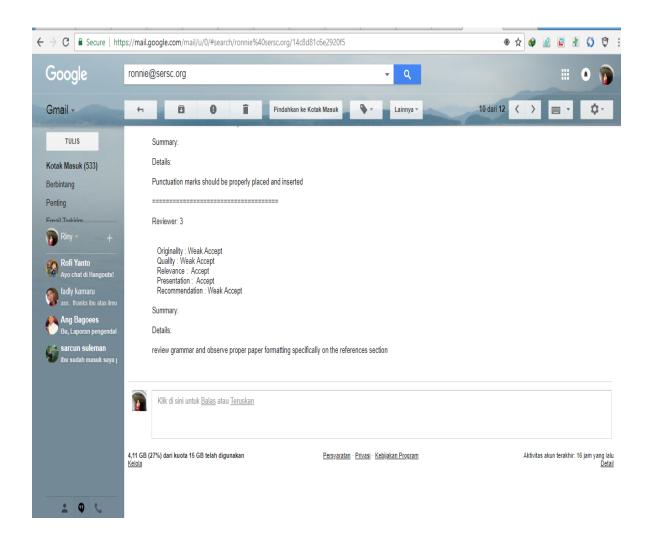
Tel: +61-3-9016-9027

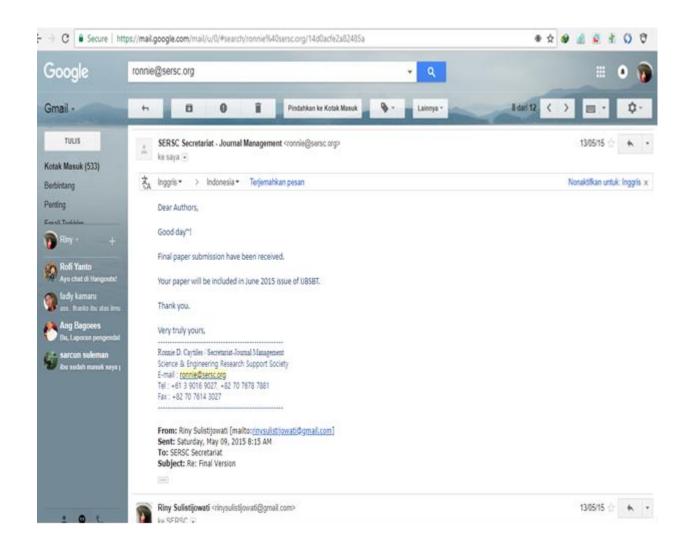
Printing office: Hanall Co. Ltd.

Tel: +82-2-2279-8494









#### The Effectiveness Inhibition Filtrate BacteriocinsLactobacillus acidophilus Toward Contaminants Bacteriafrom Swordfish (Auxis rochei) Stew

Rieny Sulistijowati\* Jetty Nurhajati\*\* Insawosami Awom\*\*

\* Faculty of Marine and Fisheries. Gorontalo State University

\*\*Padjadjaran University

PO BOX 5, Zip Code 96128 Indonesia

rinysulistijowati@gmail.com

#### Abstract

This research was conducted to know the effectiveness of filtrate bacteriocin Lactobacillus acidophilusaccording to concentrations toward zone of growth inhibition contaminant bacteria from swordfish(Auxis tochei)stew. This research was an experimentally research conducted in laboratory of Food Chemical Research Center of LIPI Bandung used completely randomized design with two replications. Species of contaminant bacteria used wereBacillus sp. (RR2) (Da, Te, Amp, Sxt), Staphylococcus aureus(R4) and Staphylococcus aureus (R5) (Da) witha range of concentration the filtrate bacteriocin culture Lacidophilus50, 60, 70, 80, 90 and 100%. The parameters were diameter (mm) of the zones of bacterial growth inhibition. The Results showed that treatment with filtrate of bacteriocin concentration 50 to 100% effective can inhibition the growth of bacterial contaminants. This treatment privide that the filtrate bacteriocin of culture Lacidophilus can be used as bio-preservative in the swordfish stew.

Keywords: Bacteriocins, Lactic Acid Bacteria, Contaminant bacteria, Swordfish

#### 1.Introduction

Wordfish is the object of comercial tuna to be exported as wood fish known as arabushi from Nort Sulawesi Province of Indonesia. The processing of wooden fish (arabushi) includesboiled, curied and drained, then soaked in water and plucked thorns. While immersed in water susceptible bacteria from contaminat water or the hands of workers and the equipment used (BSN,[1]).

Bacterial contaminants often cause spoilage in some foods that have been process (cooked) or raw materials. Bacterial contaminants from fish i.eSerratia, Micrococcus, Bacillus, Achromobacter, Pseudomonas, Staphylococcus, and Flavobacterium (Banwards, [2]). Some strains of the bacteria contaminants are resistant toward antibiotics. It is done by way of preservation of these contaminants destroy bacteria. It can be done by using L'acidophilus bioensiling preservation. The effectiveness of bacteriocins produced by Lacidophilus provide opportunities to reduce or bacterial contaminants safely(Ogunbanwo,[3]). Some of the studies support the implementation of this research are for examples: (Sulistijowati, et al., [4]) state that the treatment of inoculum Lactobacillus acidophilus immersion time 90 minutes gave the best result for absence of E.coli in swordfish stew (Auxis rochei). (Sulistijowati, et al., [5])state that the culture age 18 hours of immersion time in 90 minutes inhibitory Coliform group up to MPN 0 with control MPN 2.63 in comparison without soaking time or 2 log cycle inhibitory Coliform bacteria group. In addition, (Santoso, [6]) the use bacteriocins production of L.Ed plantarum 22 as apreservative in products such as fish and shrimp pasta can reduce bacterial contaminants during storage.

The objective of this research bacterial contaminants from boiled swordfish stew that were resistant to antibiotics. The value of the Minimum Inhibitory Concentration (MIC) of the filtrate Lacidophilus bacteriocins and the extent to which the effectiveness of Lacidophilus bacteriocins by concentration of the filtrate became the choice of study. Identification of this research were: The first whether the bacteria found contaminants of swordfish stew that are resistant to antibiotics; The second how much the value of the Minimum Inhibitory Concentration (MIC) of the filtrate Lacidophilus bacteriocins toward every strain bacteria contaminants of swordfish stew; The third how of effectiveness filtrate Lacidophilus bacteriocins according to the concentration toward contaminant bacterial growth inhibitory zone.

The intent of this study was to determine the effectiveness of the culture filtrate Lacidophilus bacteriocins toward contaminants bacterial of swordfish stew that was resistant to multiple antibiotics. While the purpose of this study is to obtain an effective concentration of the filtrate bacteriocins of L. acidophilus to inhibit bacterial contaminants from boiled swordfish that can be used as an agent biopreservative. The usefulness of this study is to provide information about the benefits of bacteriocins L. acidophilus filtrate to inhibit the growth of bacterial contaminants from boiled sword fish so that it can be used as a new biopreservative.

#### 2. Materials And Methods

#### 2.1 Materials and Equipment

Materials used in this study were: Lacidophilus bacterial isolates available in the laboratory of Chemistry LIPI Bandung. Test bacteria used in this study were isolated from bacterial contaminants stew meat tuna. Medium and chemicals used were alcohol 70 and 95 %, antibiotics, Brain Heart Infusion (BHI) Agar and Broth (Oxoid), sugar cane broth, disinfectants, crystal violet solution, Lugol, oil immersio, Man ROGOSA Sharpe (MRS) broth (Oxoid, CM 359), Mueller Hinton (MH) agar (Oxoid CM 337, Nutrient agar (NA) (Oxoid CM 3B), 0.9 % NaCl physiological, and standardization Mc Farland 1 (3x10<sup>8</sup>Colony Forming units (CFU/ml). Equipment used in this study were:autoclave, incubator, glass equipment, laminary air flow, antibiotic paper, micrometer size millipore

#### 2.2 Research Method Phase I

## Isolation, Identification and Characteristics of Bacterial Contaminants of

The method used a survey method in the laboratory and the results will be discussed in the descriptive customized with previous results. The test resistance patterns using three strains of bacteria contaminants were Bacillus sp. (RR2) Da, Te, Amp, SXT), S. aureus (R4) and S. aureus (R5) (Da) and six antibiotics that Clindamycin (DA.2), Tetracycline (Te.30), Amikacin (AK.30), Ampicillin (AMP.10), Gentamycin (CN.10), Chloramphenicol (C.30), Cotrimoxazole (SXT.25), Amoxicillin Clavulanic Acid (AMC.30). Observations were made by looking at the amount of bacterial growth inhibition zone contaminants.

#### Sterilization Equipment and Materials

Sterilization and autoclaving the medium at 121 ° C and a pressure of 1 atm (15 lbs), a maximum of 1.5 atm for 15 minutes.

#### Isolation of Bacteria Contaminants swordfish stew

The first fish weeded, discarded gills and entrails , then washed with water and then boiled for 30 min at 80° C temperature. Then the fish drained until cool, then put the fish in a container of water while using tweezers plucked prickly, then drained ± 5 hours in a dry place at room temperature. Furthermore, the fish ready to use for the isolation of bacteria . Isolation of bacterial contaminants was conducted using serial dilutions in physiological NaCl solution. A total of 25 g of fish meat samples put in 250 mL of 0.9 % sterile physiological saline, and then 1 mL of sample dilution sampled into 10-2 - 10-9 each containing 9 ml of physiological saline. Three final dilution in each sample plating is done, and then incubated at 37 ° C for 24-48 hours. After the incubation perio, the growth of bacterial colonies observed, and then purified on medium Nutrient Agar (NA), in a petri dish and incubated for 24 hours at 37 ° C (Cappuccino, Sherman[7]).

#### Gram Staining

Gram staining was conducted to determine the properties of the bacteria to a type of staining and for identification. Physiological saline dripped on object glass and then the bacteria in the test loop streaking the glass object. Mixture dried then fixed three times. Mixture is cooled and then the preparations spilled with carbolic gentian violet dyes. Allowed to stand for 30 seconds. Excess dye was washed with water and discarded. Added Lugol (iodine: potassium iodide: distilled water = 1:2:300) as a substance, for 30 seconds. Then washed with water. Preparations were washed with 96% alcohol for 2 seconds fucshin for comparison spilled water for 30 seconds, then washed with water. The results of staining, gram-positive bacteria purple and gram-negative bacteria pink (Cappuccino, Sherman [7]).

#### Biochemistry Test

Test Biochemistry Sugars and Indole , Methyl Red , Voges Proskauer , and Citrate (IMViC ). Bacteria Gram stain results that have been known to be Gram-positive or Gram-negative, further observed by biochemical testing media sugars are glucose , lactose, mannit, maltose and sucrose and peptone water media IMViC ie, methyl red, Voges Proskauer and citrate(Cappuccino, Sherman [7]).

#### SugarTest

Colonies of bacteria to be tested were taken of the loop and then inoculated into the medium of candy in a row on all test media sugars (glucose, lactose, mannit, maltose and sucrose). Then incubated at 37 °C for 24 hours. After an incubation period observed color changes in the medium and the formation of gas in the Durham tube (Cappuccino, Sherman [7]).

#### **IMViCTest**

Colonies of bacteria to be tested were taken of the loop and then grown in medium peptone water, methyl red (MR.), Voges Proskauer (VP) and citrate as planting on slopes. Then the medium was incubated for 24 hours at 37 ° C. After incubation the media spilled reagent is then observed color changes (Cappuccino, Sherman [7]).

#### Rejuvenation Isolates Bacteria Contamination.

Rejuvenation is performed on BHI medium and BHI broth agar incubated for 24 hours at 3.7° C. Colonies that grow then grown into a 10 mL broth medium sugar broth. Bacterial isolates that grow tested in an active state.

#### Test Pattern toward Antibiotic Resistance Bacteria.

Bacterial contaminants that have actively taken 1 mL in 9 mL planted to sugar cane broth, then incubated for 24 hours at 37°C. After incubation, the bacterial suspension was made in sterile physiological saline to a turbidity equivalent to Mc Farland 1 (3x108 CFU/mL), in the suspensions inoculated into a sterile petri dish containing 5 mL of Mueller Hinton medium order and leveled throughout surface. Paper disc was placed on top of a layer of antibiotic agar using sterile forceps that had been dipped in 70 % alcohol and spritus bunsen. Incubated for 24 hours at 37 °C. After the inhibition zone was observed and measured using calipers.

#### 2.3. Research Method Phase II

Determination of Minimum Inhibitory Concentration (MIC)

The testing effectiveness of the filtrate Lacidophilus toward bacterial contaminants from swordfish stew, conducted a preliminary test to determine the MIC values filtrate Lacidophilus bacteriocins. This research was carried out experimentally in the laboratory by using 3 strains of bacterial contaminants, namely Bacillus sp. (RR2) (Da, Te, Amp, SXT), Saureus (R4) and S. aureus(R5) (Da), and various concentrations of filtrate Lacidophilus bacteriocinsie, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 % were namely (C10, C20, C30, C40, C50, C60, C70, C80, C90 and C100). Observations were made descriptively by looking at the growth of bacterial contaminants on NA medium in a petri dish

#### Preparation of L. acidophilus Bacteriocin filtrate (Ogunbanwo et al[3])

One mililiter of the bacterial suspension L.acidophilus who have actively grown in 9 ml of MRS broth and incubated for 18 hour. Then centrifuged at a speed6000 rpm at 4 °C for 15 minutes to separate the cells with the filtrate. Then filtered through millipore membrane size of 0.45 micrometer. Then the filtrate is neutralized to pH 6 with 1 N NaOH was filtered with a Millipore 0.45 to obtain the cell-free supernatant was neutral used as bacteriocins. After the filtrate bacteriocins obtained, then performed concentration of 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 % under exposed in UV light for 40 minutes. Furthermore MIC testing.

#### Rejuvenation Isolates Bacteria Contamination

Rejuvenation was done on medium BHI broth and BHI agar incubated for 24 hours at 37 ° C.

#### Determination of Minimum Inhibitory Concentration (MIC)

MIC conducted to determine the minimum concentration and to determine the concentration of the filtrate bacteriocins L.acidophilus best in inhibiting the growth of bacterial contaminants from swordfish stew. The determined MIC done same procedures, bacterial contaminants which have taken 1 ose active streak in BHI and in order then incubated at 37° C for 24 hours. After an incubation period scraped off and put in a 0.9 % sterile physiological saline and centrifuged 4000 rpm at 4° C for 10 min for the deposition of bacterial cell. Results centrifugation was washed with 0.9 % sterile physiological saline 2 times and centrifuged, which the results are comparable to the turbidity of Mc Farland 1 [3x10<sup>st</sup> CFU/mL]. Subsequently 1 mL of the bacterial suspension was added to a contaminant in 1 mL of filtrate bacteriocins at each concentration was then taken of the loop and in strik on NA medium that had been frozen in a sterile petri dish. Incubation for 24 hours at 37° C. Observations were made by looking at the growth of bacterial contaminants on NA medium in a petri dish.

#### 2.4. Research Method Phase III

# Testing Effectiveness Filtrate Bacteriocins L.acidophilustoward Contaminants Bacteria from Swordfish stew.

The effectiveness of the filtrate bacteriocins Lacidophilus test against bacterial contaminants of swordfish stew. The study was carried out experimentally in the laboratory by using a completely randomized design factorial 3x7 with 2 repetitions. The first factor is the 3 strains of bacterial contaminants, namely Bacillus sp (RR2) (Da, Te, Amp, SXT); Saureus (R4) and S. aureus (R5) (Da). Factor II filtrate bacteriocin concentration were used, namely co (0.9 % physiological saline as a control), c1, c2, c3, c4, c5, and c6 (50, 6, 70, 80, 90 and 100 %) with incubation to 18 hours. Each treatment was repeated 2 times, experimental total 42 units. Parameters were observed to test the effectiveness of bacteriocins was formed inhibition zone diameter (mm). The data were analyzed statistically by ANOVA analysis followed by Duncan 's Multiple Range Test (DMRT) if significantly different.

Equipment and MaterialsSterilization and autoclaving the medium at 121 ° C and a pressure of 1 atm (15 lbs), a maximum of 1.5 atm for 15 minutes.

#### Rejuvenation Isolates Bacteria Contamination

Rejuvenation is done on medium BHI broth and BHI agar incubated for 24 hours at 37  $^{\circ}$  C

Determination of the effectiveness testfiltrate bacteriocins has been done according to method illustrated by Bundesgesundhetsrat,1976 in (Nurhajati [8]). The effectiveness test of the filtrate Bacteriocins Lacidophilus performed by the agar diffusion method (paper disc). Suspension of test contaminants bacteria that has been activated, 1 mL were taken and put in 9 mL of sugar cane brot, incubation at 37 °C for 24 hours. Then 0.1 mL of the bacterial suspension was poured on a petri dish and then 20 mL of sterile MH medium that is poured into sterile petri already containing suspensions were homogenized. After freezing medium, paper disc containing 0.05 mL of the filtrate bacteriocins sterile was placed above medium. Incubated at 37 °C for 24 hours. Observations were made by measuring the inhibition zone diameter of inhibition zone based around the paper discs were formed after the incubation period.

#### 3. Results and Discussion

#### 3.1 The Isolation and Identification of Bacteria Contaminants from swordfish Stew

Based on the results of isolation of bacterial contaminants from swordfish stew obtained 10 isolates of bacterial contaminants and after Gram staining of bacterial contaminants known that the tenth were Gram-positive bacteria. From the results of gram staining selected 4 isolates of bacteria have different cell shape and will further tested antibiotic resistance patterns. These four isolates namely R3(rods, sporulating); R4 (cocci not sporulating); R5 (cocci, not sporulating) and RR2(trunk spora).

Gram staining results showed that the ten isolates of bacterial contaminants isolated from tuna fish stew are Gram-positive. These can be caused by Gram-positive bacteria generally form spores which are relatively resistant to heat. In addition to the Gram-positive bacteria are more resistant to physical interference or mechanical disturbances (such as very high given the pressure) compared with Gram-negative bacteria it is caused Gram-positive bacterial cell wall consists of a very thick peptidoglycan which gives rigidity to maintain cell integrity (Banwart[2]). So the selection of 4 bacterial isolates determined by the form of the bacteria. Three of the ten isolates of bacteria are rod-shaped

and 7 isolates are cocci-shaped, the bacterial isolates are selected rod-shaped bacteria and cocci-shaped bacteria. Four bacterial isolates have been subsequently identifie. The results show that the identification of the four bacterial isolates found two types of bacteria, namely Bacillus sp and Staphylococcus aureus. Based on the test resistance patterns can be seen that the type of Bacillus sp. (RR2) and Bacillus sp. (R3) have similarities in patterns of resistance to antibiotics whereas S. aureus (R3) have the same pattern of resistance to S. aureus (R5) and for S. aureus (R4) have different patterns of resistance to S. aureus (R3) and (R5). It can be seen that there are three different bacterial strains based on resistance patterns to antibiotics that Bacillus sp.(RR2) (Da, Te, Amp, SXT), S. aureus (R4) and S. aureus (R5)(Da).

According (Ogunbanwo et al[3]) stated that some fish pathogenic bacteria resistant to multiple antibiotics. Bacterial resistance to an antibiotic can be caused by drugs (antibiotics) can not reach the place of work in the microbial cell, microbial inactivation and drug itself or due to the transfer of resistant genes have been through conjugation, transformation or transduction of other bacteria. The sensitivity of the bacteria to an antibiotic can be caused due to the mechanism of action of antimicrobial that interfere with the metabolism of cells, inhibiting cell wall synthesis, interfere with cell membrane permeability, inhibits the synthesis of proteins and nucleic acids of microbial cell damage.

#### 3.2 The Minimum Inhibitory Concentration (MIC) Test

Testing was conducted to determine the minimum concentration of the filtrate bacteriocins Lacidophilus that still have antibacterial activity against bacterial contaminants derived from tuna fish meat stew. The based on MIC test against three strains of bacterial contaminants derived from tuna meat stew can be seen that filtrate bacteriocins Lacidophilus have influence in inhibiting the growth of bacterial contaminants and have MIC values different. MIC values filtrate bacteriocins Lacidophilus can be seen in Table 1.

Table1.Minimum Inhibitory Concentration(MIC) Values Filtrate Bacteriocins L.acidophilus

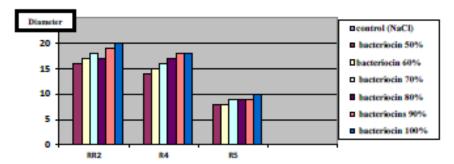
Bakteri	Replication	Concentration bacteriocins(%)					
		50	60	70	80	90	100
Bacillus sp. (RR2) (Da,	1X	+	+	+	+	+	-
Te, Amp, Sxt)	2X	+	-	+	+	+	+
S.aureus (R4)	1X	+	+	+	+	+	+
	2X	+	+	+	+	+	+
S.aureus (R5) (Da)	1X	-	+	+	+	-	-
	2X	+	-	-	-	-	+

(+) = Growth of bacteria (-)= Non growth of bacteria

The based on Table 1 it shows that the value of MIC bacteriocins for Bacillus sp. (RR2) (Da, Te, Amp, SXT), S. aureus (R4) and S. aureus (R5) (Da) is 50 %. Based on these results the determination of MIC values that will be used to test the effectiveness of filtrate bacteriocins Lacidophilus against three strains of bacterial contaminants are at concentrations of 50 %-100 %. Test results of filtrate Bacteriocin effectiveness against bacteria contaminants from swordfish stew. Observations were made by measuring the inhibition zone diameter of bacteriocin filtrate against bacteria contaminants from swordfish (Auxis rochei) stew can be seen in Appendics.

#### 3.3 Effectiveness Filtrate Bacteriocins toward Contaminants Bacteria From Swordfish Stew

Effectiveness filtrate bacteriocins L.acidophilus toward contaminants bacteria Bacillus sp. (RR2) (Da, Te, Amp, SXT), S. aureus (R4) and S. aureus (R5) (Da) from swordfish stew can be seen in Figure 2. The Figure 2. be seen that the effectiveness of the filtrate bacteriocins L.acidophilus concentrations of 50 % - 100 % growth inhibitory regions capable of forming bacteria Bacillus sp contaminants (RR2) (Da, Te, Amp, SXT), S. aureus (R4) and S. aureus (R5) (Da) from swordfish stew. Diameter size at each different concentration. The concentration of the filtrate bacteriocins capable of forming dimeter greater inhibitory regions in the three bacterial contaminant that is 90 % and 100 %. In this figure can also be seen that the higher the concentration of the filtrate bacteriocins diameter greater inhibitory regions. It is influenced by the concentrations of bacteriocins that can affect the content of the filtrate.



RR2= Bacillus sp. (Da, Te, Amp, Sxt) R4 = S.aureus R5= S.aureus (Da)

Figure 2. Effectiveness Filtrate Bacteriocins L. acidophilus Culture Toward Contamination Bacteria From Swordfish Stew (Auxis rochei)

The based on analysis of variance results indicatethat the type of bacterial contaminants and the provision of various concentrations of filtrate bacteriocins Lacidophilusvery real effecton bacterial growth inhibition are a diameter. This means that the formation of bacterial growth inhibition are adiameter various concentrations of culture filtrate Lacidophilus bacteriocins differ for each treatment.

Duncan's multiple rangetest used to determine differences in the effect of the inhibition zone. The results of Duncan's multiple range testof the influence of the type of bacteria to large inhibitory areas are listed in appendics. Based on diameter inhibition zone it is known that the diameter of the bacterial growth inhibitory regions bacteriocins culture filtrate L. acidophilus influenced by the type of bacterial contaminants derived from tuna meat stew.

Largevalue averagediameter of the growthinhibition area between S. aureus bacteria (R4) and Bacillus sp. (RR2) (Da, Te, Amp, SXT) showed highly significant differences are larger than S. aureus (R5) (Da). It can also be seen that the type of bacteria Bacillus sp. (RR2) (Da, Te, Amp, SXT) and S. aureus (R4) showed no difference in effect the formation of bacterial growth inhibition area diameter. This suggests that the growth of bacterial contaminants Bacillus sp. (RR2) (Da, Te, Amp, SXT) and S. aureus (R4) can be inhibited by bacteriocins culture filtrate Lacidophilus with the formation of growth inhibitory

regions. As stated by (Aly, [9])that the bacteriocins produced by Lacidophilus have bacteriostatic and bactericidal effect.

It is also supported by research and (Ibrahim, Desouky [10]) who stated that antimicrobial metabolites produced by Lacidophilus effective against quality tilapia as effective at inhibiting the growth of S. aureus and other bacteria. Based on eppendix it is known that the type of bacteria S. aureus (R5) (Da) showed highly significant differences in the average value of bacterial growth inhibition area diameter smaller than the bacterial species Bacillus sp. (RR2) (Da. Te. Amp. SXT) and S. aureus (R4). This occurs because the activity of antimicrobial compounds Lacidophilus others can be bakteriostatik or bactericidal, this depends on the type and characteristics of microorganisms (Karaoghi, et al.,[11] ) and (Toddorov, Dick [12]). Besides the species of microorganisms showed different susceptibility to an antimicrobial work.

Duncan's multiple range test results on the effect of concentration of filtrate bacteriocins Lacidophilus bacteriocins against large inhibitory areas were listed in appendics. Duncan 's multiple range test results that the formation of the diameter of the area affected by bacterial growth inhibitory concentrations of filtrate bacteriocins Lacidophilus. Additionally bacteriocins culture filtrate concentration Lacidophilus 50 % 100 % indicates no difference in the effect of growth inhibition area diameter forming bacteria, which means that various concentrations of filtrate bacteriocins capable of providing an inhibitory effect on the growth of strains of bacteria contaminant. According (Martiani, et al., [13]) stated that the antimicrobial compounds activity of thebacteriostatic or bactericidalagainst other microorganisms it depends on the types, characteristics and concentration of antimicrobial compounds produced.

Mechanism of action of bacteriocins in inhibiting the bacteria that attach to the cytoplasmic membrane, causing the membrane to become unstable, resulting in decreased cell viability and led to the release of the material contained within the cell nucleus so that the cells become dead (Ko, Ahn [14]) .

#### 4. Conclusion

In this study Effectiveness Inhibition Filtrate Bacteriocins Lactobacillus acidophilus Toward Contaminants Bacteria from Swordfish (Auxis rochei) Stew reveals that

- There Bacterial contaminants of swordfish stewthat has been resistant to someantibioticsthat Clindamicin (DA.2), Tetracycline (Te.30), Ampicillin (AMP.10) and Cotrimoxazole (SXT 25) .
- MIC value for each contaminant different strains of bacteria Bacillus sp (RR2) (Da, Te,Amp,SXT), S.aureus (R4) and S. aureus (R5) (Da) is 50 %.
- Concentration of filtratebacteriocins L.acidophilus from 50 % 100 % effective in inhibiting the growth of bacterial contaminants from swordfish stew.

#### Acknowledgment

The author would like to thank to the Head of LIPI Chemistry Center of Bandung. Indonesia which has provided laboratory facilities during the research.

#### References

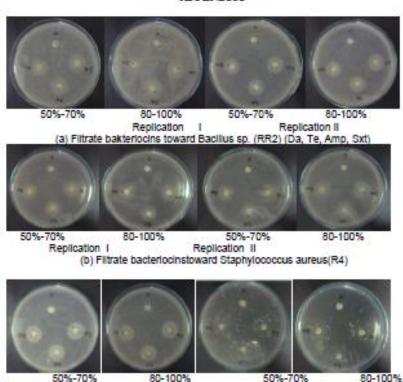
- BSN, "Ikan Kayu Bagian 3: Penanganan dan Pengolahan", SNI2691.3, (2009).
- G.J. Banwart, "Basic Food Microbiology", An AVI Book, (1989), p. 773.
   S.T.Ogunbanwo, A. I. Sanni and A. A. Onilude, "Characterization of bacteriocin produced by Lactobacillus plantarumF1 and Lactobacillus brevisOGI\*, African Journal of Biotechnology, vol.2, no. 8, (2003), pp. 219-227.
- [4] R. Sulistijowati, J. Nurhajati and I. Amalia, "The Influence of Giving Various Concentration and Methode of Inoculum Lactobacillus acidophilus According to Immersion time For Total Escherichia

- coli in Swoodfish Stew (Auxis rochel")", Proceeding International Seminar Biotechnology For Enhancement the Technical Biodiversity, UNPAD Bandung, (2010).
- [5] R. Sulistijowati, "The Influence of Culture Age and Soaking Time Range with Filtrate Lactobacillus acidophilus toward the Number of Coliform Bacteria in Swordfish (Auxis rochei) Stew", Journal of Biology, Agriculture and Care, vol.3, no.4, (2013).
- [6] E. Santoso, "Pemanfantan Bakteriosin Produksi Lactobacillus plantarum Ed 22 Sebagai Pengawet Produk Perikanan", Makalah Bidang Teknik Sumberdaya Alam Pertanian, (2009).

  [7] J.G. Cappuccino and N. Sherman, "MicrobiologyALaboratory Manual", The Benjamin/Cummings
- Publishing Company, Inc. Menlo Park, California, (2005), p. 528.
- [8] J.S.Nurhajati, "Identifikasi Isolat Salmonella Dari Itik dan Lingkungan Sekitar Serta Pengujian Sifatsifat Biologi", [Disertasi] Universitas Padjadjaran, Bandung, (1991).
- E.Aly, "Chromosomal genes-mediated inhibition of intestine and foodborne Lactobacillus acidophilus AA11", Rev Latinoam Microbio, vol.48, no. 1, (2006), pp. 24-30.

  [10] S.M. Ibrahimand S.G. Desouky, "Effect of Antimicrobial Metabolites Produced by Lactic Acid Bacteria
- on Quality Aspect of Frozen Tilignia (Oreochromis niloticus) Filets", World Journal of Fish and Marine Science, vol. 1, no. 1, (2009), pp. 40-45.
  [11] S.A. Karnogle, F. Aydin, S.S. Kilic and A.O. Kilic, "Antimicrobial Activity and Characteristics of
- Bacteriocins Produced by Vaginal Lactobacilli", Journal Medical Science Turk, vol. 33, (2002), pp. 7-
- [12] S.D. Toddorov and L.M.T. Dick, "Bacteriocin Production By Lactobacillus pentosus ST712BZ Isolated From Boza", Journal of Microbiology Bazilian, vol. 38, (2007), pp. 166-172.
- [13] L.M. Martirani, N. G. Varcamontiand M. De Felice, "Purification and partial characterization of Bacillon 490, a novel bacteriocin produced by thermophillic strain of Bacillus licheniformis Microb
- Cell Fact\_vol. 1, no. 1, (2002), p. 1.
  [14] S.H.Ko and C. Ahn, "Bacteriocin production by Lactococcus lactis KCA2386 isolated from White Kimchi", Food Sci. Biotehnol., vol. 9, no. 4, (2000), pp. 263-269.

#### APPENDICS



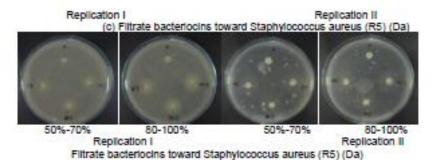


Figure 1. Diameter Inhibition

Table 1. Analysis of Varians Diameters inhibition Bacteriocins Filtrate of Lactobacilus acidophilus Culture toward Bacteria Contaminants Originating from Swordfish Stew

Sour of Varians	ar	Sum of squares	C	Funke	Fuels	
	24:				Feet	Fam
Bacteria	2	524.190	262.095	5.93**	3,467	5.780
Concentration	6	1095.143	182.524	4.13**	2.573	3.812
Interaction Bacteria and concentration	12	94.143	7.845	0.178	2.250	3.173
Galat	21	927.500	44.167			
Total	42	9005.000				

Table 2.Effect Types of Bacterial Contaminants from Swordfish Stew toward Diameter Growth Inhibition Bacteria In The Filtrate Bacteriocin

Bakteria	Diae	meter Inhibition Zone (m	m)
Bacillus sp. (RR2) ( Amp, Sxt)	Da, Te,	15.36 b	
S.aureus (R4)		14.21	
S.aureus (R5) (Da)		7.36	
		a	200
lettersaresimilarto	thevertical	directionindicatesnot	significantly

b : Results oftreatmentthat provides good leverage

Table 3. The Effect of Filtrate Bacteriocin Concentration Toward Diameter Growth Inhibition Contaminan Bacteria

Concentrasi (%)	Diaemeter
	Inhibition Zone (mm)

df=degreesof freedom, SS: sum of squares, Cscentralsquare,
\*\*=Highlysignificant (differ very real) at the level oferrorof 1%according totestAnalysis of Variance

Control (NaCl Phisiologis steril)	0 a
50	13.00 b
60	13.50 b
70	14.17 b
80	14.3 b
90	15.33 b
100	15.83 b

The lettersaresimilarto thevertical directionindicates not significantly differentated when the significantly differentated with the significantly differentiated with the significantly differentiated with the significant significantly differentiated with the significant significant significantly differentiated with the significant significa

#### Authors



Rieny Sulistijowati S, she received her S.Pi (Fish Processing) from Samratulangi University, M.Si (Microbiology Process) from Padjadjaran University, and Dr. (Fish Industri Technology) from Padjadjaran University. Worked as lecture to the Departemen Fishey Technologi, Faculty of Marine and Fisheries Gorontalo State University. Her major research interest is seafood safety, fisheries biotechnology and fisheries fermentation. She has published several Paper in International Journal and Conferences and 4 Books for fish processing.