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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.

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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 & Engineering Research Support soCiety.

This issue contains 34 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.

The study "Factors Affecting Nurses' Customer Orientation, Abstract", aimed to identify the factors affecting nurses' customer orientation. A survey was conducted on 440 nurses working for general hospitals in South Korea from November 22 through December 15, 2012, and this study analyzed the data from the questionnaire with SPSS Windows 18.0. Data were analyzed using t-test, analysis of variance, Pearson's correlation, and multiple regression. All factors had a significant correlation with customer orientation. Multiple multiple regression analysis revealed that self-leadership and deep acting was the factor positively influencing nurses' customer orientation. These factors explained 54.0% of customer orientation. It has important implications in that it suggests a customer orientation prediction model that hospital managers can use as baseline data for nursing human resource management.

The paper "Development of an Acceleration Plethysmogram based Cardioid Graph Biometric Identification" presented the development of an Acceleration Plethysmogram (APG) based Cardioid graph biometric identification. A total of 10 Photoplethysmogram (PPG) data from MIMIC II Waveform Database (MIMIC2WDB) with sampling frequency of 125 Hz were obtained. The datasets are later converted to APG signal by the second order differentiation and preprocessed with Butterworth filter. Then, Cardioid based graph of APG signal was generated. Its centroid and Euclidean distance are calculated. Finally, classification is done by applying these extracted features to Multilayer Perceptron (MLP) and Naïve Bayes neural networks classifiers.

The study "Emotional Intelligence, Stress Coping, and Adjustment to College Life in Nursing Students" desires to suggest base line data to develop program of adjustment to college life and intervention program for improvement of emotional intelligence and stress coping of nursing students in the future, by understanding emotional intelligence, stress coping, and adjustment to college life of nursing students, and establishing the relationship between them. Subjects were 227 associate nursing students (1st and 2nd grade) in Korea. The data were collected using self-report questionnaire from september 16 to 22, 2015. Data were analyzed by frequencies, independent t-test, ANOVA, Pearson's correlation coefficients, multiple regression using SPSS Win 23.0. Significant correlations were found between emotional intelligence, stress coping, and adjustment to college life in nursing students.

The purpose of the study "Development of Nurse-led Home Visit Intervention Program for Holistic Healthcare of Multi-cultural Couples" was to develop nurse-led home visit intervention program in order to maintain and promote holistic healthcare of multi-cultural couples in farm and fishery areas. The period of research was from March 2014 to July 2014. The method was literature reviews and interviews. Firstly, through literature

absence of an existing system, the service data, and fitness exercise equipment Recommend.

In the paper "Studies on the Effect of Molecular Weight on Biodegradable Polymer Membrane", Pla and PLGA have been extensively used for controlled drug delivery and used to fabricate device for tissue engineering. The objective of the present study was to know the effect of different molecular weight of PLLA and PLLGA. PLLA and PLLGA membrane was prepared using a solvent-casting method. In vitro degradation of the blank membrane was characterized by techniques including NMR, SEM, BET. And examined the degradation of PLA-PGA copolymer at different temperature range. The lower molecular weight, the more porosity and the smaller pore size. The degradation ratio of membrane increased with increasing test temperature.

Paper "Automated Detection of Exudates for Diabetic Retinopathy Screening in Fundus Images Using CS-ACO Optimization Approach" states that Diabetic Retinopathy (DR) is a disease that creates some changes in the retinal blood vessels. Blood vessels leaks fats or lipids in the yellowish color which is a cause of blindness and the aggregate yellowish color fat or lipid on the eye is known as exudates. In order to help the ophthalmologists for accurate detection of exudates hybrid CS-ACO is applied on the online dataset HEI-MED consists 169 images. Hybrid approach works in three steps first hybrid CS-ACO is performed to enhance the image second DWT is used to reduce the time elapsed in image enhancement and finally ACO is used for the detection of exudates. The performance of CS-ACO is better than ACO in the detection of exudates. The proposed model has attained mean values of 99.6%, 98.7% and 98.6% for sensitivity, specificity and accuracy respectively on online database.

The study "Development of Web-based Reproductive Health Program" was done to develop an education program for reproductive health management of unmarried women over 35. In order to investigate the education demand of the subjects, documentation research and learner analysis were conducted to develop an education program, and Delphi investigation was conducted against 7 experts. Through the web-based reproductive health management program for unmarried women over age 35 developed in this study, the knowledge on reproductive health can be enhanced and a positive attitude can be cultivated, so as to increase health activity practice rate.

June, 2016

Tai-Hoon Kim, GVSA and UTAS, Australia

Editor of the June Issue on
International Journal of Bio-Science and Bio-Technology

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Dear Rieny Sulistijowati, Lukman Mile

We are happy to inform you that your paper entitled "Identification Of Lactic Acid Bacteria Isolates From Intestine Of Milkfish (Chanos-Chanos) Potential Activity Against Pathogen Bacteria Used PCR 18s Rrna Methode", submitted to IJBSBT, has been accepted for inclusion in the journal.

Please consider the reviewers' rating/comments carefully when preparing the final version of your paper.

After making the final version, kindly send these documents to ronnie@sersc.org by April 25, 2016:

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Quality : Weak Accept

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Presentation : Accept
Recommendation : Weak Accept

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Reviewer: 2

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Quality : Weak Accept
Relevance : Accept
Presentation : Accept
Recommendation : Weak Accept

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review grammar and observe proper paper
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Reviewer: 3

Originality : Weak Accept
Quality : Weak Accept
Relevance : Weak Accept
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Dear Authors,

Good day~!

Final paper submission have been received.

Your paper will be included in June 2016 issue of IJBSBT.

Thank you.

Very truly yours,

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Identification of Lactic Acid Bacteria Isolates from Intestine of Milkfish (Chanos-Chanos) Potential Activity against Pathogen Bacteria Used PCR 18s Rrna Methode

Rieny Sulistijowati S* and Lukman Mile*

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Abstract

The research aimed to identify the lacted acid bacteria from intestinal of milkfish (Chanos chanos) potential acvity pathogen anbacteria used PCR 18s Rrna Methode. The lactic acid bacteria were isolated using selective media MRS agar for lactic acid bacteria. Identification isolates PCR 18S rRNA and sequencing method that are DNA isolation, DNA amplification use PCR, DNA visualizasion from amplification with electroforesis, Basic Local Alignment Search Tool (BLAST) and Tree view program. In view of PCR analysis and tree view program, LAB is Lactobacillus acidophilus strain ATTC 4796.

***Keywords**: Lactic Acid Bacteria, Milkfish, PCR*

1. Introduction

Lactic acid bacteria (LAB) constitute a group of bacteria that have morphological, metabolical and physiological similarities, and they are also relatively and closely related phylogenetically. They are gram-positive, non-sporulating, non-respiring cocci or rods, which ferments carbohydrate to produce lactic acid as their major end product (Dike,Sanni, [1]). Lactic acid bacteria are widely distributed in the nature. Representatives of the genus Lactobacillus, Lactococcus, Pediococcus and Leuconostoc are be largs this group. According to many reports, lactic acid bacteria are normal flora in gastrointestinal (GI) tract of healthy animals like mammals and aquaculture animals (Nikoskelainen, et al, [2]) with no harmful effects(Ringo, et al, [3]).

The milkfish that breed in brackish waters has slim bodied characteristics, fin forked, fleshy scales like glass and white. It has a uniqueness, that his mouth is toothless and seaweed base food consumtion. In addition, the intestinal length of the milkfish 9 times longer than the body length (Murtidjo [4]). In the long intestine, there are many different types of bacteria including lactic acid bacteria (LAB), which helps the food digestion process. LAB also function as antagonistic bacteria against pathogenic bacteria. Lactic acid bacteria can be isolated and tested its antagonistic activity against pathogenic bacteria and can be developed as a new antibiotic.

Lactic acid bacteria (LAB) are known as microorganisms that have probiotic properties. They can produce inhibitory compound such as lactic acid, hydrogen peroxide, diacetyl, acetal dehyde and bacteriocin. These compounds are able to inhibit the growth of harmful microorganisms (Ringo, Gatesoupe [5]), (Gatesoupe [6]). Lactic acid bacteria are widely distributed in the nature. Representatives of the genus Lactobacillus, Lactococcus, Pediococcus and Leuconostoc are be largs this group.

The research was an experimentally conducted that treatment with filtrate lactic acid of Lactobacillus concentration 50 to 100% effective can inhibition the growth of bacterial pathogen were Bacillus cereus, Staphilococcus aureus, Salmonella paratyphi and E.coli.

The acid lactic from culture Lactobacillus can be used as bio-preservative isolate from intestinal of milkfish (Sulistijowati, Mile, [7]).

The determination of lactic acid bacteria genus as potential biopreservative was conducted performed according to their morphological, cultural, physiological and biochemical characteristics as described in Bergey's Manual. The rusults after characterization, based on Bergey's manual, seven of the isolates (RS1,RS2,RS3,RS4,RS5,RS8 and RS10) were determined as representatives of refferred to genus *Leoconostoc* were cocci, Gram +, gas production, hetero/homo fermented and others (RS6, RS7 and RS9) were representatives of reffered to genus *Lactobacillus* were rod, Gram +, gas production, hetero fermented (Sulistijowati, [8]).

The 16S rRNA gene sequence is a molecular technique to identify microorganisms up to species level. This method has been considered as one of the advanced tool at molecular level for identifying isolated bacteria. 16S rRNA analysis has facilitated the study of microbial populations without cultivation which has made quantitative assessment of microbial diversity now conceivable (Subramani, Aalbersberg [9]).

The aim of this study was to develop PCR 18s Rrna in order to simultaneously detect Lactobacillus species in single reaction.

The polymerase chain reaction (PCR) is a technology in molecular biology used to amplify a single copy or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence. Developed in 1983 by Kary Mullis,[1][2] PCR is now a common and often indispensable technique used in medical and biological research labs for a variety of applications.[3][4] These include DNA cloning for sequencing, DNA-based phylogeny, or functional analysis of genes; the diagnosis of hereditary diseases; the identification of genetic fingerprints (used in forensic sciences and DNA paternity testing); and the detection and diagnosis of infectious diseases. In 1993, Mullis was awarded the Nobel Prize in Chemistry along with Michael Smith for his work on PCR.[5]

The method relies on thermal cycling, consisting of cycles of repeated heating and cooling of the reaction for DNA melting and enzymatic replication of the DNA. Primers (short DNA fragments) containing sequences complementary to the target region along with a DNA polymerase, which the method is named after, are key components to enable selective and repeated amplification. As PCR progresses, the DNA generated was itself used as a template for replication, setting in motion a chain reaction in which the DNA template exponentially amplified. PCR can be extensively modified to perform a wide array of genetic manipulations.

2. Material and Methodes

2.1. Materials and Equipment

This research used lactic acid bacteria isolate potential biopreservative from Pohuwato District were cultivated in deMan Rogosa Sharp (MRS) Agar at 35°C for 24 hours, reagen PCR: *deoxyribonucleotide triphosphate* (dNTP) mix, larutan *buffer*, LA Taq, *primer* 1141R dan 765R , dH_2O, *Big Dye Teminator*, gel agarosa, *buffer* TAE (1x) Merck 106023), DF *buffer* (Merck.71340), *wash buffer*, *lotion buffer*, SyBr *safe*, EDTA (Merck.324503), natrium asetat (Merck. 106268), etanol (Merck.104025), alkohol 70% (Merck.117946). The equipment machine PCR *Thermal cycler* Takara, machin elektroforesis, UV *Transluminator* Gel Doc Sys-Vilber Loumart, sequensing ABI 3130 XL *Genetic Analyzer*

2.2. Reasearch Methodes

The determination of Lactobacillus species was permormed DNA genom total isolation, DNA amplification, electrophoresis, gel electrophoresis purification, PCR cycle

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sequencing, cycle sequencing purification, sequencing and Basic Local Aligment Search Tool (BLAST) and Tree view program (Maniatis, et al,[10]).

DNA Genom Total Isolation use PrepMan Ultra Reagent

Samples were inserted into the tube was added 20 µL PrepMan Ultra then homogenized using vortex for 10-30 seconds. Furthermore incubated at a temperature of 100°C for 10 minutes on the digital heatblock, then centrifuged at 16,000 x g for 3 min. A total of 12 µL of the supernatant was transferred into 1.5 mL tube and stored at a temperature of 40°C.

DNA Amplification

Total genomic DNA that has been obtained from the isolation was amplified using the Polymerase Chain Reaction (PCR) Thermal cycler in the area Internal Transcribe Spacer (ITS). The total gene DNA used as a template and added with another reagent-reagent with the following composition: 25 mM $MgCl_2$ 5 mL, 2.5 µM dNTPs mix 4 µL, 10 X buffer free Mg 2+ 5 µL, LA Tag 0.25 µL , template 1 µL , 10 µM primer 1141R (base sequence 5'-TCC GTA GGT GAA CCT GCG G-3 ') 2 µL , 10 µM R 765 primer (base sequence 5' TCC GCT TAT TGA TAT GC-3') 2 µL and 30.75 µL dH_2O. The total volume of the reaction mixture of 50 µL, then put in a PCR machine with the program: Heating beginning of 94^{0}C for 3 minutes, denaturation at temperature 940C for 30 seconds, annealing at a temperature 55^{0}C for 20 seconds, elongation of DNA at atemperature 720C for 1 min. After PCR 30 cycles were left at a temperature of 72^{0}C for 7 minutes later the temperature was lowered to 4^{0}C.

Electrophoresis

Done the 1% agarose gel, 0.25 g dissolved in 25 agarose TAE buffer (1x) and then heated to dissolve agarose. Cooled to at temperature 450C was added 0.25 µL of SyBr safe then poured into molds and allowed to stand until the gel hardens.

Electrophoresis; PCR products were subsequently examined by electrophoresis. Electrophoresis was carried out in TAE buffer (1x). A total of 50 µL PCR product and 6x loading dye 10 µL homogenised by means of repeated pipette 2-3 times and then put in a pipette and gel well. Loading dye serves to increase the density of the sample so it is not out of the well and the samples be colored to facilitate observation of the current migration process. Also incorporated into the gel other wells as a comparison (marker) 4µL 1 Kb DNA Ladder Mix fermentas GeneRuler DNA ladder to facilitate the determination of the size of the DNA. Electrophoresis is run at a voltage of 100 volts for 25 minutes, then the gel electrophoresis results checked over transluminator UV and photographed for documentation. Then the DNA bands appear at 600 base pairs was cut to enter the stage of purification.

Purification

Purifying Gel Electrophoresis with GeneAid Gel / PCR DNA Fragments Extraction Kit DF buffer gel added Pieces of 300 µL, incubated at 60^{0}C for 15 minutes on a heat block until later dispindown soluble gel and then inserted into a filter column and centrifuged at 13000 x g for 1 minute. Then the supernatant was discarded, and then added 600 µL wash buffer into the filter column is then centrifuged at 13000 xg for 1 minute, remove the supernatant and centrifugation in an empty column to remove residual wash buffer at 13000 xg for 3 minutes and then move the filter column into eppendorf new and added a solution of 40 µL lotion buffer and incubated for 5 min at room temperature. Then was centrifuged at 13000 x g for 2 minutes. Remove the filter column. Store the supernatant at temperature 40C.

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PCR Cycle sequencing

Cycle Sequencing PCR was performed use a primer, ie 1141R and 765R. PCR tube inserted in the Big Dye Terminator 2 mL , 4 mL 5x sequencing buffer , 1.6 pmol / µL primer 1141R much as 4 µL, 6 µL DNA template, and 4 µL dH_2O. Mixture reaction volume 20 uL, then put in a PCR machine with the program: Preheating 96^{0}C for 10 seconds , annealing (annealing) at temperature 50^{0}C for 5 seconds, and DNA elongation at temperature 60^{0}C for 4 minutes. After 25 cycles of PCR then the temperature lowered to 4^{0}C. Furthermore, do the same thing on the primer 765R.

Purification Cycle Sequencing

A total of 20 mL samples of cycle sequencing equivalent dH2O was added (20 µL), then transferred to 1.5 mL tube. EDTA then added 4 µL, 4 µL of sodium acetate, and 100 µL of absolute ethanol, incubated at room temperature for 15 minutes in a state covered with aluminum foil. Then centrifuged at 5000 x g for 30 minutes, then the supernatant removed and added to 140 mL of 70% alcohol, followed by centrifugation at 3000 x g for 15 minutes. Subsequently the supernatant was discarded, spindown and dried with a tissue, and then use a vacuum desiccator for 10 minutes. Then added 16 µL of dH2O and spindown. The last heated use a heat block at temperature 52^{0}C for 10 minutes. Samples ready to prepare sequenced.

Sequencing and Basic Local Aligment Search Tool (BLAST) Analize

Cycle Sequencing DNA purified and then sequenced used a sequencing ABI 3130 Genetic XL analyzer to determine the nucleotide sequence of the DNA fragment. In this sequencing a lengthening or extension cords of DNA which begins at specific sites on the DNA template using short oligonucleotides, called primers. Primer used 1141R as 765 as forward and reverse. Sequencing was run for 1 hour. Furthermore, the results of sequencing the DNA sequences do assembly, namely the incorporation of bases of reading both directions (forward and reverse). Furthermore, DNA sequences that have been in the assembly copied to the notepad in the form of FASTA for purposes of analysis Basic Local Alignment Search Tool (BLAST). The analysis performed using the program's website: http: /www.ncbi.nih.nlm.gov/BLAST/ by copying the DNA sequence that will be in BLAST, and then pasted into the field that the DNA sequences in the BLAST window then the window appears on the monitor that contains the results of BLAST similarity diagram sequences that we enter with sequences in the gene bank. To determine the kinship of bacteria identified, selected sequences which have a percentage of similarity (Query) high, then the sequence data stored on a notepad in the form FASTA. Analysis of the sequence data using Clustal-X program and GeneDoc to edit sequences. The results of the analysis will be obtained in the form of family tree (phylogenetic tree) that can be opened with the program Tree View. From the family tree can be known species and strains of bacteria were identified.

3. Results and Discussion

DNA Isolation

Total genomic DNA has been isolated from BAL isolates that possess antibacterial activity on pathogenic bacteria. Total genomic DNA isolation used Ultra PrepMan Reagents.

DNA Amplification

Total genomic DNA isolates BAL amplification technique Polymerase Chain Reaction (PCR) used PCR Thermal Cycler. DNA amplification process consists of three stages, namely DNA denaturation, primer extension or DNA template and primer elongation by

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the enzyme DNA polymerase. LAB isolates DNA amplification using primers 1141R and 765R produce DNA fragments were very clear with a size of about 600 base pairs.

Electroforesis

The results of DNA amplification used primer 1141R and 765R produce DNA fragments very clearly with a size of about 597 base pairs checked by electrophoresis which one of the main techniques in molecular biology.

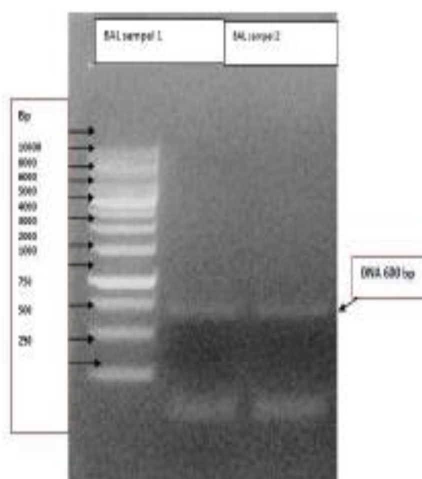

Figure 1. Electroforesis

Sequensing and Basic Local Aligment Search Tool (BLAST) Analize

Cycle Sequencing DNA tool was run on the ABI 3130 Genetic XL analyzer to determine the nucleotide sequence of the DNA fragment. In this sequencing a lengthening or extension cords of DNA which begins at specific sites on the DNA template using the primer 765R 1141R as a forward and as reverse. Sequencing running for one hour. Here was the results of assembly, was read from two directions (forward and reverse) the DNA sequences of the isolates BAL (597 bp) which has antibacterial activity.

Sequence isolate LAB

Lactobacillus acidophilus 30 SC

CCTGCCCCATAGTCTGGGATACCACTTGGAA
ACAGGTGCTAATACCGGATAAGAAAGCAGAT
CGCATGATCAGCTTATAAAAGGCGGCGTAAG
CTGTCGCTATGGNNTGGCCCCGCGATTGGTG
CATTAGCTAGTTGGTAAAGTCGAGCGAGCTG
AACCAACAGATTCACTTCGGTGATGACGTTG
GGAACGCGAGCGGCGGATGGGTGAGTAACA
CGTGGGGAACCTGCCCCATAGTCTGGGATAC
CACTTGGAAACAGGTGCTAATACCGGATAAG
AAAGCAGATCGCATGATCAGCTTATAAAAGG
CGGCGTAAGCTGTCGCTATGGGATGGCCCCG
CGGTGCATTAGCTAGTTGGTAGGGTAACGGC
CTACCAAGGCAATGATGCATAGCCGAGTTGA
GAGACTGATCGGCCACATTGGGACTGAGACA
CGGCCCAAACTCCTACGGGAGGCAGCAGTAG
GGAATCTTCCACAATGGACGAAAGTCTGATG
GAGCAACGCCGCGTGAGTGAAGAAGGTTTTC

Lactobacillus acidophilus ATCC 4796

AAAAACGAGAGTTTGATCCTGGCTCAGGACG
AACGCTGGCGGCGTGCCTAATACATGCAAGT
CGAGCGAGCTGAACCAACAGATTCACTTCGG
TGATGACGTTGGGAACGCGAGCGGCGGATGG
GTGAGTAACACGTGGGGAACCTGCCCCATAG
TCTGGGATACCACTTGGAAACAGGTGCTAAT
ACCGGATAAGAAAGCAGATCGCATGATCAGC
TTATAAAAGGCGGCGTAAGCTGTCGCTATGG
GATGGCCCCGCGGTGCATTAGCTAGTTGGTA
GGGTAACGGCCTACCAAGGCAATGATGCATA
GCCGAGTTGAGAGACTGATCGGCCACATTGG
GACTGAGACACGGCCCAAACTCCTACGGGAG
GCAGCAGTAGGGAATCTTCCACAATGGACGA
AAGTCTGATGGAGCAACGCCGCGTGAGTGAA
GAAGGTTTTCGGATCGTAAAGCTCTGTTGTG
GTGAAGAAGGATAGAGGTAGTAACTGGCCTT
TATTTGACGGTAATCAACCAGAAAGTCACGG

DNA sequences that have been copied to the notepad in the form FASTA used for the analysis (BLAST) using the program on the website http: /www.ncbi.nih.nlm.gov/BLAST/, phylogeny of fungi identified, selected from a sequence that has a percentage high similarity> 90% and in the program tree view derived phylogenies (filogenetic tree), it was found that the species and strains of bacteria. The philogenetic tree diagram of the LAB isolate is Lactobacillus acidophilus ATTC 4796 on figure 2.

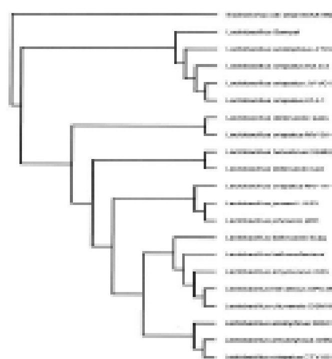

Figure 2. Philogenetic Similarity Lacidophilus

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Based on philogenetic tree diagram known that LAB isolate resemblance> 98% with bacterial species Lactobacillus acidophilus ATTC4796.

Tacsonomy of Lactobacillus acidophilus :

Kingdom	: Bacteria
Divisio	: Firmicutes
Classis	: Bacilli
Ordo	: Lactobacillales
Familia	: Lactobacillaceae
Genus	: *Lactobacillus*
Species	: *Lactobacillus acidophilus* (Moro 1900)
Strain	: ATTC 4796

4. Conclusion

As a conclusion this study had successfully identified based on 16S rRNA gene sequence analysis of the Lactic Acid Bacteria isolate from intestinal milkfish as Lactobacillus acidophilus ATTC4796 (98% similarity).

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