The threat of foodborne disease from raw seafood: isolation and molecular identification of bacteria from the gut of Portunus pelagicus

by Ana Hidayati Mukaromah

The threat of foodborne disease from raw seafood: isolation and molecular identification of bacteria from the gut of *Portunus pelagicus*

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Abstract. *Portunus pelagicus* is a marine commodity that is in great demand by consumers, especially coastal communities. *Portunus pelagicus* habitat in the sea allows contamination from microorganisms and marine waste. Proper cleaning and processing of *Portunus pelagicus* can cause foodborne disease. One part of Portunus *pelagicus* that contains a lot of contaminants is the gut. The process of isolation and molecular identification of bacteria from the gut of *Portunus pelagicus* is important. *Portunus pelagicus* samples that have been taken from the gut and extracted. *Portunus pelagicus* gut extract was put in NA media and continued into BAP media. Pure bacterial cultures were isolated using CIAA phenol DNA method and amplified using 16S rRNA followed by sequencing. Four bacterial isolates were obtained from the gut of *Portunus pelagicus*, namely PorTRJ6, PorTRJ8, PorTRJ9, PorTRJ10. PorTRJ6 are β-hemolytic bacteria and PorTRJ8, PorTRJ9, PorTRJ10 are α-hemolytic bacteria. Based on the results of sequencing the bacterial isolates had similarity with *Vibrio parahaemolyticus*, Uncultured bacterium clone RS-E27, *Staphylococcus haemolyticus, Staphylococcus* sp. Some bacteria found in the gut of *Portunus pelagicus* can cause foodborne disease. *Portunus pelagicus* is a high-protein seafood that can be toxic if it is not processed cleanly and properly.

1. Introduction

Portunus pelagicus is one of the fishery commodities that is the mainstay of exports in Indonesia, from the local market as well as the export market. The commodity *Portunus pelagicus* is ranked third or fourth of the total export value of Indonesian fishery products after shrimp, tuna and seaweed. Until 2013 the fulfillment of *Portunus pelagicus* raw materials still depended on catches in nature [1].

Portunus pelagicus is a fishery product which is generally perishable food. Organs in *Portunus pelagicus* will rot if they are not processed and handled post-harvest. The nature of *Portunus pelagicus* which is easy to decay can cause problems in its distribution. The decrease in quality in *Portunus pelagicus* is caused by the activity of enzymes and bacteria biochemically and microbiologically. Biochemical damage is caused by the presence of enzymes and biochemical reactions that are still ongoing in the body of fresh fish, while microbiological damage is caused by microbial activity, especially bacteria [2].

Decomposition of *Portunus pelagicus* due to microbes can have negative effects on the host. Pathogenic bacteria such as *Aeromonas* sp. , *Pseudomonas* sp. , and *Vibrio* sp. , with examples of species that often cause disease by Crustacean class animals (such as shrimp, mangrove crabs and *Portunus pelagicus*) [3]. The source of *Portunus pelagicus* contamination comes from the stomach and haemolymph [4,5]. Pollution of pathogenic bacteria in seafood causes cases of extraordinary events. An increase in the incidence of outbreaks has been identified as being caused by the consumption of raw *Portunus pelagicus* contaminated by *Vibrio* sp. [6].

In general, pathogenic bacteria can be identified by conventional methods (phenotype) can be done by testing on BAP (Blood Agar Plate) media. BAP media is an enriched and differential selective medium that can distinguish pathogenic bacteria based on the ability to hemolyze erythrocytes [7], but it takes a long time and does not provide enough clear information in distinguishing intraspecies and interspecies strains. This causes errors in distinguishing bacterial species due to the presence of characters that are not static and can change along with changes in the environment, so it is necessary to identify genotype based on genes encoding Ribosome Ribonucleic Acid (rRNA).

Analysis of the 16S rRNA gene sequence has been widely used to identify bacterial species and carry out taxonomic studies [8]. The 16S rRNA gene has several regions with conservative base sequences as well as regions with highly variable base sequences. Conservative base sequence comparisons are useful for constructing universal phylogenetic trees, while varied base sequences can be used to track diversity and locate strains within a species. Deoxyribonucleic Acid (DNA) obtained from the isolation will be used as a template in the amplification stage by Polymerase Chain Reaction (PCR). The primer used in PCR is a universal 16S rRNA primer measuring about 1500 bp, such as 27F and 1492R so that it can amplify the 16S rRNA region of all bacteria [9].

The purpose of this study was to obtain isolates and identity of bacterial contamination from the gut of *Portunus pelagicus* which leads to foodborne disease. Information about the types of bacterial contamination from the gut of *Portunus pelagicus* obtained at the traditional market of Demak, Indonesia is still very limited so that molecular-based research is needed. Information on contamination of *Portunus pelagicus* provides awareness to the public regarding clean and correct processing methods for *Portunus pelagicus* so that it does not cause disease.

2. Materials and methods

2.1 Sample collection

The sample of this study used *Portunus pelagicus* which was obtained from a marine fish seller at the Sayung market, Demak.

2.2 Bacteria isolation procedure

5 *Portunus pelagicus* samples were ground using a mortar, diluted in 5 ml Physiological NaCl, then samples were inoculated on Nutrient Agar (NA) media and incubated for 24 hours at 37^0 C, the next day, bacterial colonies were purified by subculture to obtain pure colonies.

2.3 Bacterial isolation purification process

The purification process was carried out by taking colonies from NA media. One eye of the colony inoculum was taken, then replanted on NA media and incubated at 37^0 C for 24 hours. The growing colonies were observed for morphology. Bacterial purification should be carried out 3 times or until one type of pure colony is consistent and not mixed with other bacterial colonies.

2.4 Pathogenicity test

Bacteria from NA media were inoculated on BAP media and incubated for 24 hours at 37^0 C. Growing colonies were observed. In BAP media, colonies with high pathogenicity were selected, namely colonies that were able to completely hemolyze red blood cells. Colonies on BAP media were transferred to slanted HIA media as pure isolates.

2.5 Gram stain

Colonies of bacteria that are capable of complete hemolysis are taken with the eye socket, placed on a glass object that has been given 1 drop of physiological NaCl. Furthermore, the preparation is passed on a fire which aims to kill bacteria.

The first staining stage was inundated with Gram A containing crystal violet and left for 1 minute then rinsed with running water. The second stage is dripped with 1 drop of Lugol and left for 1 minute, washed with running water. The third stage is dripping with 96% alcohol and left for 10-20 seconds and then washed with running water. The fourth stage, the preparation was flooded using carbol fuchsin for 1 minute and then rinsed with water, observed under a microscope with an objective lens magnification of 100x. If the bacteria is stained purple then the bacteria are declared Gram positive bacteria, if the bacteria is stained red then the bacteria are Gram negative.

2.6 Genomic DNA isolation

Bacterial isolates from *Portunus pelagicus* samples were grown on 15 ml BHI media and incubated for 2x24 hours at 37^0 C. The inoculation results were centrifuged at 6000 rpm for 15 minutes at 4^0 C. Gram positive supernatant was discarded and the cells were suspended in 480 μl 50 mM EDTA, added 120μl of lyzozyme enzyme, incubated at 37^0 C for 45 minutes. The solution was centrifuged for 4 minutes with an acceleration of 6000 rpm and the supernatant was discarded. For the lysis of Gram-positive and negative bacteria, 750 μl of vortex lysis buffer was added for a few seconds. 20μl proteinase K was added to the solution, then shaken vigorously for 15 minutes, then incubated at 55^0 C for 30 minutes (every 10 minutes homogenized).

The solution was centrifuged at 3000 rpm for 15 minutes at 4^0 C. The supernatant was transferred to a 1.5 ml microtube and 700 μl of CIAA phenol was added, then gently shaken for 30 minutes. The solution was centrifuged at 12000 rpm for 10 minutes at 4^0 C. The aqueous layer was transferred to a microtube, then cold ethanol 96% was added in a ratio of 1:1. The solution is mixed slowly until precipitation is formed like fine threads. DNA strands were taken, then transferred into a tube. 500 μl of 70% ethanol was added as a DNA wash, then centrifuged at 12000 rpm for 10 minutes at 4^0 C (3 times). The supernatant was discarded, the pellet was dried. The DNA pellet was added with 200 μl of TE (Tris EDTA) to dissolve the DNA. DNA concentration and purity were measured using nanodrop® (NP-80 Implant).

2.7 DNA amplification with 16S rRNA gene

Nuclease Free Water was inserted into the PCR tube as much as 5.5 μl, Primers 27F 5'-AGAGTTGATCCTGGCTCAG-3' and 1492R 5'-GGTTACCTTGTTACGACTT-3' were added 2 μl each to the mixture. 12.5 μl of the master mix was added, and 3 μl of DNA template was added. The PCR mix mixture was put in a thermocycler machine with pre-denaturation program at 95°C for 3 minutes, denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 2 minutes. Final extension at 72°C for 10 minutes. And cooling down at 4°C for 10 minutes. The PCR results were electrophoresed using 2% agarose gel. The PCR results will then be sequenced.

2.8 Phylogenetic tree analysis

The results of the sequencing were analyzed using DNA Baser, Blast, and Mega X.

3. Results and discussion

3.1 Bacterial isolate culture from the stomach of Portunus pelagicus on BAP media

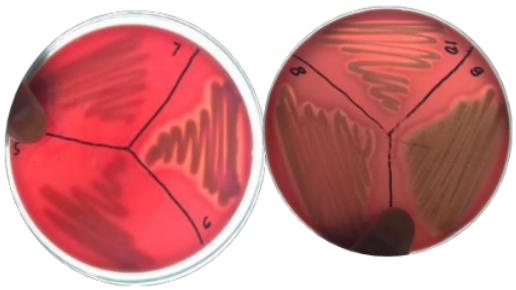


Figure 1. Bacterial isolate culture from the stomach of *Portunus pelagicus* on BAP media

Isolation of bacteria from the stomach of *Portunus pelagicus* obtained 4 pure isolates namely PorTRJ6, PorTRJ8, PorTRJ9, PorTRJ10. The four pure isolates were cultured on BAP media to determine their pathogenicity. The results of BAP media culture showed that PorTRJ6 isolates were β-hemolytic bacteria (Figure 1) and PorTRJ8, PorTRJ9, PorTRJ10 were α-hemolytic bacteria. Hemolysin is a toxin that can form a hemolysis zone around bacterial colonies. Hemolysin consists of α-hemolysin, β-hemolysin, and γ -hemolysis. Those that produce α -hemolysin will form a light zone around the colony, those that produce β -hemolysin will form a slightly clear dark zone around the colony and those that produce γ- hemolysin do not form hemolysis [10].

3.2 Analysis of the bacterial isolate phylogeny tree from the gut of Portunus pelagicus

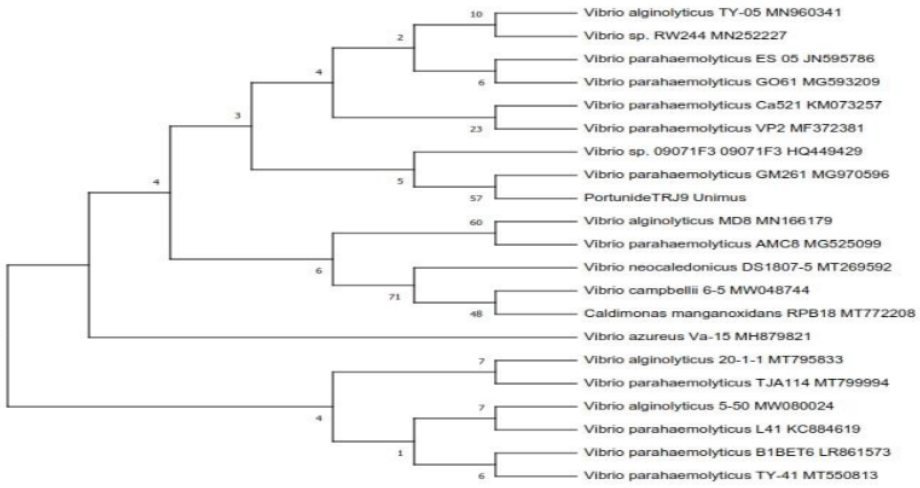


Figure 2. Phylogeny tree analysis of PorTRJ6 isolate bacterial

Analysis of the phylogeny tree showed that the isolate PorTRJ6 had similarity with the bacterium *Vibrio parahaemolyticus* (95.79%). *V. parahaemolyticus V. alginolyticus, V. ordalii, V. harveyi* were found in mangrove crabs taken from haemolymph in the Demak area, Indonesia.

These bacteria cause the death of mud crabs [4]. The family Vibrionaceae includes species that cause infections in the intestinal tract of humans and animals when food is not cooked properly [11]. The genus Vibrio is Gram negative rods that are evenly distributed in the waters and marine environment. Several members of the genus have major health implications from water or seafood that cause gastrointestinal infections in humans including *V. cholera, V. parahaemolyticus, V. mimicus, V. algynolyticu, and V. hollisae*. Vibrio species are widespread and increase in pathogenic strains of marine vertebrates and invertebrates [12]. *Vibrio* spp. form biofilms on the surface of seafood which causes health complications. *V. harveyi* produces extracellular products, namely salmonids, which are pathogenic to humans. Several Vibrios that have been isolated from lobsters and crabs are *V. vulnifucus, V. alginolyticus, V. mimicus, V. parahaemolyticus, and V. harveyi* [13–15]. *V. harveyi* can be removed by heating when consuming Portunus so as not to cause foodborne disease [15]. Family Vibrionaceae is not only pathogenic in humans when ingested, but also causes death in seafood [16–21]. *Staphylococcus epidermidis, V. harveyi, V. parahaemolyticus, Micrococcus luteus and Pseudoalteromonas piscicida* [14] were found in the gut of *P. pelagicus* and were able to kill *P. pelagicus* larvae. The most toxic bacteria causing the death of most marine animals is *V. harveyi* [18]. Most of *P. pelagicus* were able to survive even though they contained pathogenic bacteria in their stomach because they were able to produce haemocyanins. Haemocyanin is an antimicrobial protein found in the haemolymph of flower crab (*P. pelagicus*). Haemocyanin was able to inhibit the biofilm activity of 5 Gram-negative bifilm-forming bacteria, namely *V. alginolyticus, V. herveyi, V. parahaemolyticus, Pseudomonas aeruginosa, and Proteus vulgaris* [22].

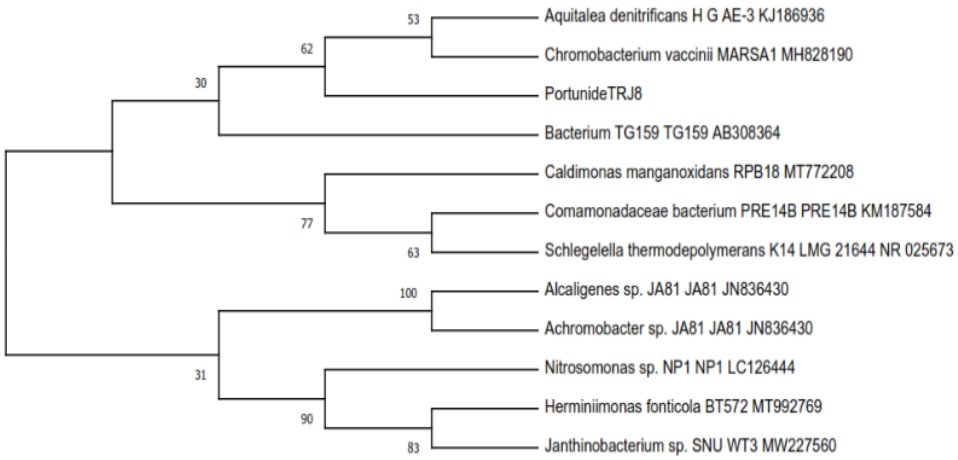
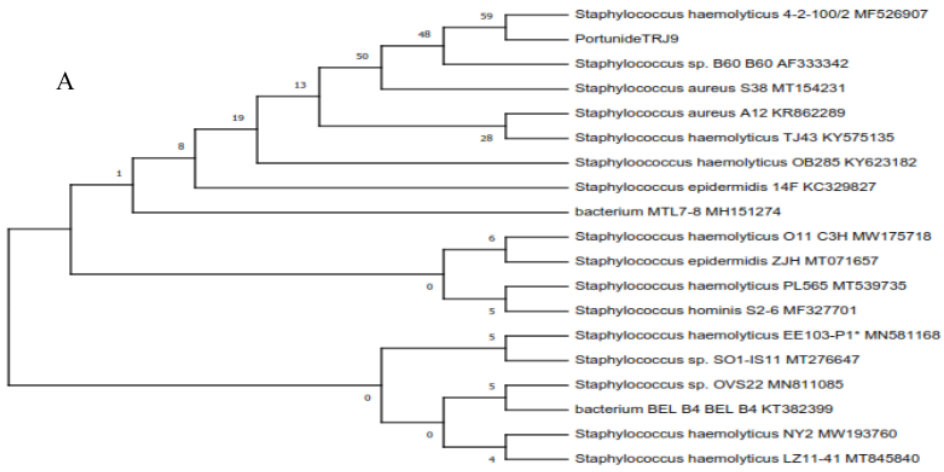


Figure 3. Phylogeny tree analysis of PorTRJ8 isolate bacterial

PorTRJ8 isolate had similarity with Uncultured bacterium clone RS-E27 (94.44%). Uncultured bacterium clone RS-E27 is a bacterium found in the marine environment in the basaltic section which reflects the diversity of horizontal distribution. Other species including nitrospirae, aminicenantes, calescamantes, and chloroflexi are unique types of bacteria found on the seabed [23]. The microbial community is dominated by Gammaproteobacteria, followed by deltaproteobacteria, planctomycetes, acidobacteria, and alphabacteria [24]. The phylogenetic tree analysis showed that PorTRJ8 had similarity with *Chromobacterium vaccinia* MARSA MH828190 and *Aquitalea denitrificans*. Microbial communities dominated by denitrifican bacteria were also

found to be responsible for cleaning up heavy metal pollutants on the seabed. Heavy metals are the biggest source of pollutants in the aquatic environment. Heavy metals are essential elements in small amounts, but in excess they can cause toxic effects. Aquatic organisms often respond to heavy metal concentrations in the waters, which can cause tolerance. Microorganisms are important mediators in aquatic environmental processes, including self-purification and nutrient recycling. Microorganisms have an important role in the tolerance to heavy metal pollution in the waters. Microbial communities that cannot tolerate heavy metal contamination may cause the community to disappear. There are three communities in the waters, namely planktonic [25], sedimentary, and epilithic which have different reactions to heavy metal stress. Epilithic bacteria, developed to adapt to high concentrations of heavy metals [26]. The PorTRJ8 isolate found in the gut of *Portunus pelagicus* could be used for the tolerance process of *Portunus pelagicus* in order to survive in waters polluted by heavy metals. In addition to heavy metal pollution in waters, this group of bacteria is also found in wastewater in China. Livestock waste water is the main source of water affected by pollution in China's Nitrosomonas group [27–31].

The PorTRJ8 isolate was also identical to the bacteria found in the roots of rice plants. These bacteria are active and involved in the denitrification process. Species found on the roots of rice plants include neisseriales, rhodocyclales, burkholderiales [32]. Apart from rice, PorTRJ8 isolate was also found in symbiosis with citrus roots. This group of bacteria has the ability to increase the productive capacity and sustainability of the agro-ecosystem. 39 isolates were found to be related to mineral nutrition, phosphate solubilization, siderophore production, nitrogen fixation, development (indole acetic acid (IAA) synthesis). Bacteria that fall into this group are *Paenibacillus validus, Lysinibacillus fusiformis, Bacillus licheniformis, Pseudomonas putida, Microbacterium oleivorans,* and *Serratia plymutica* [33].



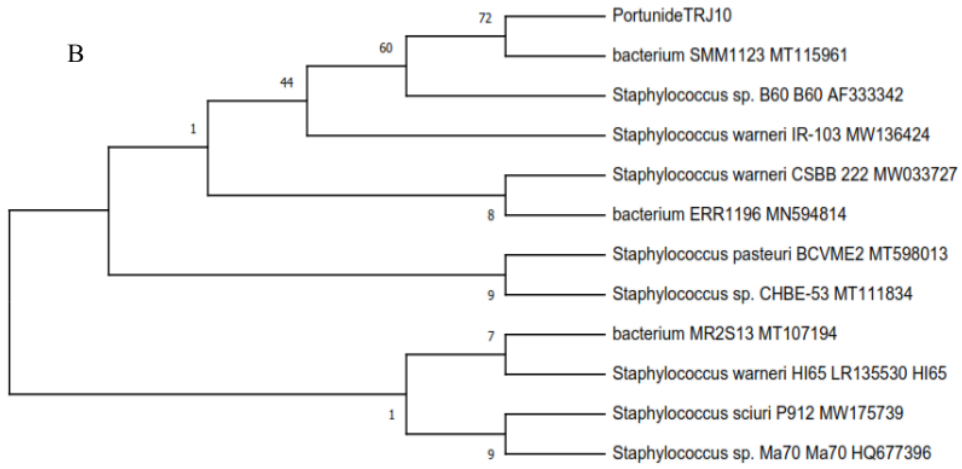


Figure 4. Phylogeny tree analysis (A) isolate PorTRJ9 and (B) PorTRJ10

Phylogenetic tree analysis showed that isolate PorTRJ9 was identical to *Staphylococcus haemolyticus* (94.85%) and isolate PorTRJ10 to *Staphylococcus* sp. (86.74%). The genus Staphylococcus is frequently found in marine environments [18,19]. *Staphylococcus haemolyticus* is found in fish and is methicillin resistant [34]. The *Staphylococcus aureus* species is associated with food, which is the 16nsmission of fish and tilapia. *Staphylococcus aureus* contaminating food occurs at various stages of the food chain, starting from primary production, processing, distribution, marketing, if not paying attention to hygiene [35]. Staphylococcus was also found to have resistance to MRSA and MRSE antibiotics in ready-to-eat fish [36–38]. There are oxacillin-resistant *Stapylococcus* spp. in freshwater fish and fish market environments in Northern Greece [39]. Besides being able to cause death in larvae and marine organisms [8,17–19], they can also cause disease in humans. *Staphylococcus haemolyticus* was found in hospital laboratories causing nosocomial infections [7,36]. *Staphylococcus aureus* causes atopic dermatitis. *Staphylococcus epidermidis* was also significantly increased during flares. Staphylococcus is often found in lesions and non-lesions of atopic dermatitis [40,41].

4. Conclusions and Suggestions

PorTRJ6, PorTRJ8, PorTRJ9, PorTRJ10 isolates isolated from the stomach of *Portunus pelagicus* are pathogenic bacteria in humans so that the processing of *Portunus pelagicus* must be clean by cooking until cooked. Processing of *Portunus pelagicus* that is not clean and cooked can cause foodborne disease because the bacteria content in crabs is pathogenic.

References

[1] Setiyowati D 2016 Kajian Stok Rajungan (Portunus pelagicus) di Perairan Laut Jawa, Kabupaten Jepara [Stock Study of Crayfish (Portunus pelagicus) in Java Sea Waters, Jepara Regency] *J Disportex* **7** (1) pp 84–97

[2] Mudaningrat A, Ramdan K, Salsabila M, Aisyah S and Umami M 2020 Kerupuk lemi Portunus pelagicus sebagai solusi pengelolaan limbah rajungan di wilayah Cirebon [Portunus pelagicus lemi crackers as a solution for crab waste management in the Cirebon area] *ProsSeminNas Pendidik Biol*

[3] Linggarjati K F and Djunaedi A 2013 Uji Penggunaan *Bacillus sp.* sebagai Kandidat Probiotik untuk Pemeliharaan Rajungan (*Portunus sp* .) [Test Use of Bacillus sp. as a Probiotic Candidate

for Rearing Crayfish (Portunus sp.)] *Journal of Marine Research* **2**(2) pp 1–6
[4] Burhan M and Sarjito I S 2014 Agensia Penyebab Penyakit Bakteri pada Kepiting Bakau (*Scylla paramamosain*) yang Berasal dari Demak [Agents Causing Bacterial Disease in Mangrove Crab (Scylla paramamosain) Derived from Demak] *J Aquac Manag Technol* **3**(2) pp 33–43
[5] Purushothaman G and Jayaprakash J 2020 Screening and Optimization of Protease Producing Bacterial Isolate from The Gut of *Portunus pelagicus* and Its Prospecting Potential in Detergent Industry
[6] Syamsir E 2011 *Kasus Vibrio parahaemolyticus di dalam Seafood [Cases of Vibrio parahaemolyticus in Seafood]* (Bogor: FATETA IPB) pp 3–5
[7] Ashour A and Jasim SA 2020 Phylogenetic Tree of Some Nosocomial Bacteria Isolated from Ramadi Phylogenetic Tree of Some Nosocomial Bacteria Isolated from Ramadi Teaching Hospital *International Journal for Pharmaceutical Research Scholar* **12** (4) pp 2650-61
[8] Chakravorty S, Helb D, Burday M, Connell N and A lland D 2007 A Detailed Analysis of 16S Ribosomal RNA Gene Segments for the Diagnosis of Pathogenic Bacteria *J Microbiol Methods* **69**(2) pp 330–9
[9] Sekuensing A and Mikrobiologi B 2011 Analisis Sekuensing 16S rRNA Di Bidang Mikrobiologi pp 172–7
[10] Rahmi Y, Darmawi D, Abrar M, Jamin F, Fakhrurrazi F and Fahrimal Y 2015 Identifikasi Bakteri *Staphylococcus aureus* pada Preputium dan Vagina Kuda (*Equus caballus*) *J Med Vet* **9**(2)
[11] Lee SKY, Wang HZ, Law SHW, Wu RSS and Kong RYC 2002 Analysis of the 16S – 23S rDNA intergenic spacers (IGSs) of marine vibrios for species-specific signature DNA sequences *Marine pollution Buletin* **44** pp 412–20
[12] Liu W, Ye D, Luo Z, Yu Y and Gong T 2013 Isolation and Characterization of Five Lytic Bacteriophages Infecting a *Vibrio* Strain Closely Related to *Vibrio owensii* **348** pp 112–9
[13] Franco PF and Hedreyda CT 2006 Amplification and Sequence Analysis of the Full Length ToxR Gene in *Vibrio harveyi* **52** pp 281–7
[14] Govindasamy C and Srinivasan R 2012 Association of Bioluminescent Bacteria from Blue Swimmer Crab *Portunus pelagicus* (Linneaus , 1758) *Asian Pacific J Trop Dis* **2** pp 699–702
[15] Mahin B, Iqbal M, Rajendiran S and Vasudevan S 2018 Isolation, Identification and Characterization of the Bioluminescent Bacteria Isolated from the Blue Swimmer Crab *Portunus pelagicus* along Thondi Coast and Virulence Studies at High Temperatures *Microb Pathog*
[16] Regev Y, Davidovich N, Berzak R, Lau SCK and Scheinin AP 2020 Molecular Identification and Characterization of Vibrio species and Mycobacterium species in Wild and Cultured Marine Fish from the Eastern Mediterranean Sea *Microorganisms* **8**(863) pp 1–17
[17] Dad A, Ikhwanuddin M, Danish M, Abdullah D and Ambok A 2013 Indigenous *Lactobacillus Plantarum* as Probiotic for Larviculture of Blue Swimming Crab, *Portunus pelagicus* (Linnaeus , 1758) Effects on Survival , Digestive Enzyme Activities and Water Quality *Aquaculture* **417** pp 173–8
[18] Allah M and Talpur D 2016 A Novel of Gut Pathogenic Bacteria of Blue Swimming Crab *Portunus pelagicus* (Linneaus , 1758) and Pathogenicity of *Vibrio harveyi* a Transmission Agent in Larval Culture under Hatchery Condition *Research Journal of Applied Sciences* **6**(2) pp 116-27
[19] Allah M, Talpur D, Karachi D, Ikhwanuddin M and Danish-daniel M 2012 Effects of Lactobacillus Probiotics on the Enhancement of Larval Survival of Portunus pelagicus (Linnaeus , 175) Fed Via Bioencapsulated in Live Feed *World Journal of Fish and Marine Sciences* **4**(1) pp 42-9
[20] Allah M and Talpur D 2012 First isolation of *Vibrio probioticus* from the gut of *Portunus pelagicus* (Linnaeus 1758) and its probiotic competency against shellfish pathogens *Pure Appl*

Bio **1**(2) pp 28-32
[21] Shen GM and Shi CY 2017 Isolation , identification and pathogenicity of Vibrio harveyi , the causal agent of skin ulcer disease in juvenile hybrid groupers Epinephelus fuscoguttatus 3 Epinephelus lanceolatus *Journal of Fish Diseases* pp 1–12
[22] Ramasubramanian V, Alharbi NS, Khaled JM, Mohammed N and Benelli G 2018 Bio-mining drugs from the sea: High antibiofilm properties of haemocyanin purified from the haemolymph of flower crab Portunus pelagicus (L.) (Decapoda: Portunidae). aquaculture
[23] Jungbluth SP, Bowers RM, Lin H, Cowen JP and Rappé MS 2016 Novel microbial assemblages inhabiting crustal fluids within mid-ocean ridge flank subsurface basalt *the ISME jurnal* **4**(20) pp 2033–47
[24] Román S, Ortiz-álvarez R, Romano C and Casamayor EO 2019 Microbial Community Structure and Functionality in the Deep Sea Floor : Evaluating the Causes of Spatial Heterogeneity in a Submarine Canyon System NW Mediterranean *Frontier in Marine Science* (6) pp 8–18
[25] Kojima H, Watanabe T, Iwata T and Fukui M 2014 Identification of Major Planktonic Sulfur Oxidizers in Stratified Freshwater Lake *Plosone* **9**(4) pp 1–7
[26] Dean-ross D and Mills AL 2009 Bacterial Community Structure and Function along a Heavy Metal Gradient *Applied and Environmental Microbiology* **55**(8) pp 2002–9
[27] Guan T, Lin Y, Ou M and Chen K 2019 Isolation and Diversity of Sediment Bacteria in the Hypersaline Aiding Lake *PLoS ONE* **15** (7)
[28] Liu J, Qin K, Wu C, Fu K, Yu X and Zhou L 2021 De Novo Sequencing Provides Insights Into the Pathogenicity of Foodborne Vibrio parahaemolyticus Bacteria and Growth Conditions *Frontiers in Cellular and Infection Microbiology* (11) pp 1–21
[29] Liu LEI, Ma C, Xu Q, Cheng L and Xiao L 2014 A rapid nested polymerase chain reaction method to detect circulating cancer cells in breast cancer patients using multiple marker genes *Spandidos Pubication* (4) pp 2192–8
[30] Chen Q, Ni J, Ma T, Liu T and Zheng M 2015 Bioaugmentation treatment of municipal wastewater with heterotrophic-aerobic nitrogen removal bacteria in a pilot-scale SBR *Bioresour Technol*
[31] Cheng R, Liu YJ, Cui JW, Yang M, Liu XL and Li P 2017 Aspirin regulation of c-myc and cyclinD1 proteins to overcome tamoxifen resistance in estrogen receptor-positive breast cancer cells. *Oncotarget* **28**(18) pp 30252–64
[32] Crop N 2012 Identification of Active Denitrifiers in Rice Paddy Soil by DNA- and RNA-Based Analyses *Microbes and Environments* **27**(4) pp 456–61
[33] Trivedi P, Spann T and Wang N 2011 Isolation and Characterization of Beneficial Bacteria Associated with Citrus Roots in Florida *Microb Ecol* **62**(3) pp 324–36
[34] Sivaraman GK, Muneeb KH, Sudha S, Shome B, Holmes M and Cole J 2021 Fish-borne methicillin resistant Staphylococcus haemolyticus carrying atypical staphylococcal cassette chromosome mec (SCCmec) elements Gene Reports Fish-borne methicillin resistant Staphylococcus haemolyticus carrying atypical staphylococcal cassette chromosome mec (SCC mec) elements *Gene Reports* (22) pp 100982
[35] Dos C, Lerma MU, Villada L De, Cayaco-puerto C and Dos C 2020 Food Safety and Fish Production the Case of *Staphylococcus aureus A Review*
[36] Hammad AM, Watanabe W, Fujii T and Shimamoto T 2012 Occurrence and characteristics of methicillin-resistant and -susceptible Staphylococcus aureus and methicillin-resistant coagulase-negative staphylococci from Japanese retail ready-to-eat raw fish *Int J Food Microbiol* **156**(3) pp 286–9
[37] Sergelidis D, Abrahim A, Papadopoulos T, Soultos N, Martziou E and Koulourida V 2014 Isolation of methicillin-resistant *Staphylococcus spp* . from ready-to-eat fish products *Letters in Applied Microbiology* **59** pp 500-6
[38] Kumar LRG, Kasim AK, Lekshmi M, Nayak BB and Kumar S 2016 Incidence of Methicillin-Resistant Staphylococci in Fresh Seafood *Advances in Microbiiology* **6** pp 399-06

[39] Abrahim A, Sergelidis D and Kirkoudis I 2010 Isolation and Antimicrobial Resistance of Staphylococcus spp . in Freshwater Fish and Greek Marketplaces *Journal of Aquatic Food Product* **19** pp 93-102
[40] Kong HH, Oh J, Deming C, Conlan S, Grice EA, Beatson MA, et al 2012 Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis *Genome Research* **22** pp 850–9
[41] Oh J, Freeman AF, Comparative N, Program S, Park M, Sokolic R, et al 2013 The altered landscape of the human skin microbiome in patients with primary immunodeficiencies *Genome Research* **23** pp 2103–14

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