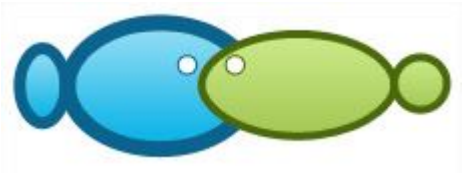

# Cellulolytic bacteria isolated from agar waste as candidate seaweed fermentation agents in rabbitfish (*Siganus guttatus*) feed bioprocessing

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**Abstract**. This study aimed to isolate, characterize and identify cellulolytic bacteria from solid agar waste as seaweed fermentation agents for rabbitfish (*Siganus guttatus*) feed bioprocessing. Bacteria were isolated using a spread plate method on a carboxymethyl cellulose (CMC) medium. Characterization included morphology, Gram staining, cellulolytic index, CMCase activity and bacterial pathogenicity. Bacteria with a high cellulolytic index and CMCase activity were selected as fermentation agent candidates. The pathogenicity test used an intramuscular injection method. Selected bacterial isolates (density $10^6$ CFU $mL^{-1}$) were injected into healthy juvenile rabbitfish (10 fish $container^{-1}$) with a saline solution as control. The juvenile fish were monitored for 10 days; parameters observed were survival rate, disease symptoms, fish behaviour and leucocyte counts. Bacterial identification was carried out by BLAST analysis of bacteria DNA sequences. Data on bacteria characteristic and identity were analysed descriptively, while data on survival rate and leucocyte counts were analysed using analysis of variance (ANOVA). Nineteen bacteria were isolated of which 9 were selected as candidate fermentation agents including 9 Gram-negative bacteria. SIM91 had the highest cellulolytic index (3.03) and CMCase activity (1.2 U $mL^{-1}$). Eight isolates were not pathogenic and 1 isolate (SIM103) was pathogenic with symptoms including swollen eyes and pale gills. Survival rate and leucocyte counts of juvenile rabbitfish were not significantly different between injected fish and the control ($p > 0.05$). BLAST analysis of bacteria DNA identified four species of bacteria (*Pseudomonas stutzeri*, *P. chengduensis, P. aeruginosa,* and *P. songnenensis*) with 97-99% similarity. This study resulted in the identification of bacteria with potential as fermentation agents in rabbitfish (*S. guttatus*) feed bioprocessing.
**Key Words**: cellulose, seaweed, aquafeed, rabbitfish, bacterial fermentation.

**Introduction**. The rabbitfish (*Siganus guttatus*) is an economically valuable herbivorous fish. Rabbitfish aquaculture has good prospects for development because this species is relatively easy to cultivate at high densities (Saoud et al 2007, 2008). However, the development of rabbitfish farming requires innovations in feed provision to support this development, in particular sustainably produced local raw materials for feed production. One raw material that could be an alternative rabbitfish feed ingredient is seaweed. Supporting factors for the use of seaweeds as rabbitfish feed ingredients (Xu et al 2011) include their high nutritional content as well as the fact that they are abundant and sustainable production methods could ensure raw material availability.

Seaweeds are one of the links in the aquatic food chain, and are a direct and indirect source of food for fish in the wild, and therefore have potential as raw materials for cultured fish feed. Studies on the use of seaweed as fish feed include the use of *Gracilaria lemaneiformis* as feed for rabbitfish (*S. canaliculatus*) (Xu et al 2011), *Gracilaria arcuata* for catfish (*Clarias gariepinus*) (Al-Asgah et al 2016), *Ulva lactuca* for catfish (*C. gariepinus*) (Abdel-Warith et al 2016), *Hypnea cornuta* and *Hypnea musciformis* for Nile tilapia (*Oreochromis niloticus*) (Arori et al 2019), *Gracilaria* spp., *Ulva* spp. and *Fucus* spp. for sea bream (*Dicentrarchus labrax*) (Peixoto et al 2016), *U.*

*lactuca* for shrimp (*Litopenaeus vannamei*) and freshwater shrimp (*Macrobrachium rosenbergii*) (Felix & Brindo 2014).

Several species of seaweed have high protein and amino acid contents (Dawczynski et al 2007; Biancarosa et al 2017). However, the fibre or non starch polysaccharides (NSP) content in seaweeds is generally high, so that bioprocessing is required in order to optimize their utilization as feed ingredients. Bhuiyan et al (2016) reported that seaweed generally has a fibre content of 10-12%. The NSP content in aquafeeds can reduce digestibility, making it necessary to carry out enzymatic hydrolysis processes (Horn 2017).

Cellulase enzymes are a group of enzymes that work to hydrolyse cellulose into glucose. There are three groups of cellulase enzymes that work together to hydrolyse cellulose, namely: exoglucanases, endoglucanases and β-glucosidases. Cellulase enzymatic hydrolysis works by breaking the β-1,4 glycoside bonds in the molecular structure of cellulose (Jayasekara & Ratnayake 2012). Cellulase can be obtained from microorganisms such as fungal, bacteria and actinomycetes. Cellulase is broadly utilized in many industries such as food, feed, pharmaceutical, paper pulp and biofuel industries. In the feed industry, cellulase is used to reduce the cellulose content in feed ingredients. Cellulolytic microorganisms can be isolated from several sources such as ruminal digestive tracts (Weimer et al 1991), insect digestive tracts (Sheng et al 2012), fish digestive tracts (Sumathi et al 2011), seaweeds (Comba-González et al 2018) and agar waste (Munifah et al 2015).

Fermentation processes to improve the quality of feed raw materials mostly use commercial microorganisms, while there has been much less attention to developing the use of microorganisms isolated from specific substrates. Agar waste is one cellulolytic microorganism source that can be used as a fermentation activator in seaweed bioprocessing. Munifah et al (2015) reported the use of cellulolytic bacteria isolated from agar waste to produce biofuel through seaweed bioprocessing. The production of agar and carrageenan also produces seaweed fibre residue as a waste product. This fibre residue can be used as a medium for cellulolytic bacteria. Hessami et al (2020) reported that the by-products of agar (from *Gracilaria* sp.) and carrageenan (from *Kappaphycus alvarezii*) production still contain 13% of cellulose, while other studies indicate a fibre content in seaweed waste of 6.95% (Jumaidin et al 2017).

The cellulose content in agar and carrageenan waste is a good medium for cellulolytic bacteria. This study aimed to isolate, characterize and identify cellulolytic bacteria from agar waste which could potentially be used as a seaweed fermentation agent in rabbitfish (*S. guttatus*) feed bioprocessing.

## Material and Method

***Study site and materials***. This study was conducted at Research Institute for Brackish Water Aquaculture and Fisheries Extension (RIBAFE), Maros, South Sulawesi, Indonesia from May 7 to August 14, 2020. Solid agar waste was obtained from an agar producing industrial company and transported to the RIBAFE laboratory using a cool box. The waste was ground using a porcelain mortar and stored at 4°C until used.

***Bacterial isolation***. An aliquot of 1 g agar waste was dissolved in 9 mL of physiological solution (NaCl 0.85%) and homogenized at room temperature. A nine series dilution process (10-$10^9$) was used to isolate cellulolytic bacteria. An aliquot of 0.1 mL of the suspension was inoculated in 1% sodium Carboxy Methyl Cellulose (CMC) medium according to Sheng et al (2012) with modification, as follows: 10 g of CMC; 1.6 of KCl; 1.43 g of NaCl; 1.9 g of $KH_2PO_4$; 0.94 g of $K_2HPO_4$, 0.15 g of $NH_4Cl$, 0.037 g of $MgSO_4.7H_2O$; 0.017 g $CaCl_2.H_2O$; 10 g of bacto agar and 5 g of yeast extract. The inoculant was incubated at 37°C for 48 hours. A single colony of bacteria was cultured on CMC medium and was then stored at 4°C for further study.

***Bacterial characterization***

*Morphology.* The morphological characteristics of isolated bacteria (colony shape, margin shape, elevation shape and Gram staining characteristic) were observed under a stereomicroscope.

*Cellulolytic index.* The cellulolytic index was determined by CMC clear zone measurement with 4 replicates for each bacterial isolate. Bacterial isolates were inoculated on 1% CMC medium and incubated for 5-6 days, then stained using 0.1% Congo red dye and allowed to stand for 30-60 minutes. The inoculant was then rinsed 2-3 times using 1 M NaCl, then allowed to stand for 15 minutes. Bacterial isolates which provided a clear zone around the colony were cellulolytic bacteria, as the clear zone indicated that they could produce cellulase enzymes. Bacteria isolates which had a high cellulolytic index were selected as fermentation promoter candidates. The cellulolytic index was calculated using the following formula:

$$\text{Cellulolytic index (CI)} = \frac{\text{Ø clear zone - Ø isolate}}{\text{Ø isolate}}$$

*Cellulase activity (CMCase).* Bacterial isolate was inoculated onto 1% CMC medium, and then incubated at 37°C for 48 hours. Two loops of isolate were then inoculated into 100 mL of 1% liquid CMC medium and incubated in a shaker for 24 hours. The suspension was centrifuged at 9000 x g at 4°C for 10 minutes. CMCase activity was determined by the reaction of 0.5 mL of supernatant (cellulase crude extract) and 0.5 mL of 1% CMC (in 0.1 M phosphate citrate buffer, pH = 5). The solution was vortexed and incubated at 37°C for 30 minutes. The reaction was terminated by boiling the solution at 100°C for 15 minutes. An aliquot of 1 mL of the solution was added to 1 mL DNSA (dinitro salicylic acid) and boiled at 100°C for 15 minutes. The absorbance of the solution was measured using a UV-Vis spectrophotometer at $\lambda$ = 550 nm using glucose as standard (Munifah et al 2015). Enzyme activity was calculated using the following formula:

$$\text{Cellulase activity} = \frac{\text{Glucose concentration}}{\text{Volume x incubation time x molecular weight of glucose (180)}}$$

***Bacterial pathogenicity test***. The pathogenicity test aimed to evaluate the pathogenicity of 9 isolates to rabbitfish. Juvenile rabbitfish (50.13±5.22 g) were acclimated in aerated fibre tanks, and reared for one week prior to the pathogenicity test. During the acclimation period, the juvenile rabbitfish were fed commercial feed four times a day and the water in the fibre tank was changed daily. After a week, the juveniles were placed in 10 aerated fibre tanks with a density of 10 fish $tank^{-1}$. Each selected isolate ($10^6$ CFU $mL^{-1}$) was injected into 10 juvenile rabbitfish, while saline solution was used as control. The fish were observed every day during the 10 day pathogenicity test. Parameters observed were survival rate, symptoms of disease, and fish behaviour. At the end of the pathogenicity test, leucocyte and erythrocyte counts were observed under a microscope with three replicates. Data on survival rate and the percentage of leucocytes and erythrocytes under each treatment were analysed statistically using a one-way analysis of variance (ANOVA).

***Bacterial identification***. The isolates were identified using 16S-rDNA sequencing according to Sheng et al (2012) with modification. DNA was extracted from each of the 8 selected isolates using a Presto Mini gDNA Bacteria Kit (Geneaid), following the manufacturer's protocol. Polymerase chain reaction (PCR) was performed with 30 cycles at annealing temperature T = 55°C using a universal 16S-rRNA primer pair, MyTaq HS Red Mix (Bioline) and GeneRuler 1 kb Plus DNA ladder (thermoscientific) as marker in a 50 µL reaction mixture. The PCR product was sequenced and the result was analysed using the NCBI BLASTn (Basic Local Alignment Sequencing Tools) routine (https://blast.ncbi.nlm.nih.gov).

## Results

***Bacterial isolation***. During this study we obtained 19 bacterial isolates which had different cellulolytic index values (0.62-3.03). Nine isolates which had high cellulolytic index values were selected as fermenter candidates. Among these nine isolates, the highest cellulolytic index came from isolate SIM91 (3.03±0.53) and the lowest from isolate SIM915 (1.45±0.70) (Figure 1). The nine selected isolates were then used for further study.

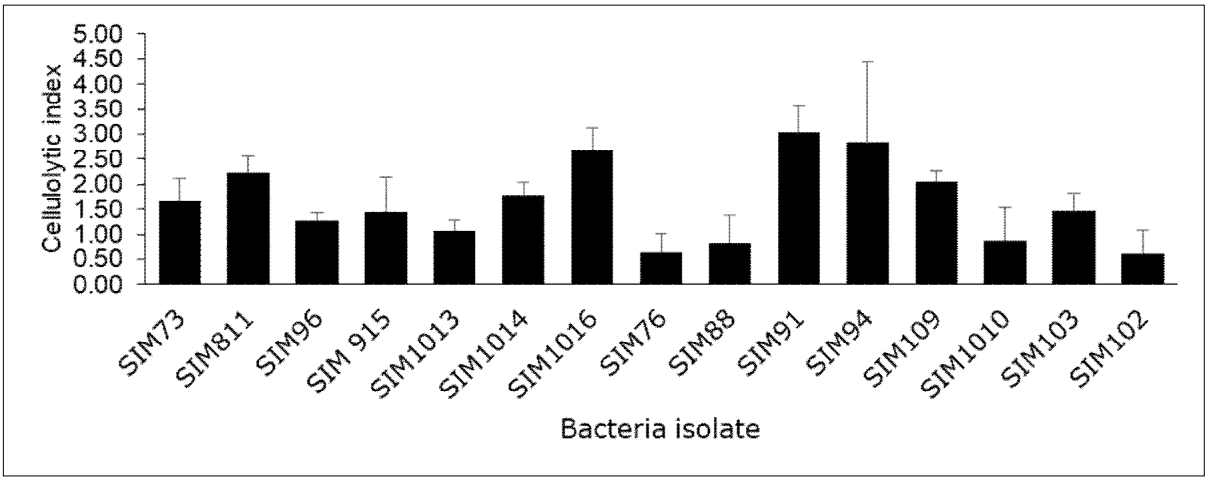


Figure 1. Cellulolytic index of 19 bacterial isolates from agar waste. The bars represent mean values; the whiskers show the standard deviation (n = 4).

### *Bacterial characteristics*

*Morphology.* The morphology and Gram-staining characteristics observed for nine selected isolates are shown in Table 1. The nine isolates had various morphological traits, with two forms (cocobacilli and bacilli) and all isolates were Gram-negative.

Table 1

Morphological and Gram staining characteristics of nine cellulolytic bacterial isolates from agar waste

| *Bacterial isolate* | *Elevation* | *Margin* | *Form* | *Gram (+/-)* |
|---|---|---|---|---|
| SIM91 | Convex | Flat | Cocobacilli | - |
| SIM811 | Flat | Flat | Bacilli | - |
| SIM1016 | Flat | Flat | Bacilli | - |
| SIM73 | Convex | Flat | Cocobacilli | - |
| SIM915 | Convex | Wavy | Cocobacilli | - |
| SIM94 | Convex | Flat | Bacilli | - |
| SIM103 | Convex | Flat | Bacilli | - |
| SIM109 | Convex | Flat | Bacilli | - |
| SIM1014 | Flat | Flat | Bacilli | - |

*Cellulolytic index.* Nineteen isolates had various cellulolytic index values (0.62-3.03) as shown in Figure 1. The nine isolates with the highest cellulolytic index values were: SIM91 (3.03±0.53); SIM94 (2.84±1.61); SIM1016 (2.68±0.44); SIM811 (2.24±0.32); SIM109 (2.06±0.20); SIM1014 (1.79±0.26); SIM73 (1.67±0.44); SIM103 (1.46±0.36) and SIM915 (1.45±0.70). These isolates were selected as potential fermentation agents.

*Cellulolytic activity (CMCase).* The isolate showing the highest CMCase activity was SIM91 (1.20±0.07 U $mL^{-1}$) and the lowest was SIM915 (0.4±0.04 U $mL^{-1}$) (Figure 2).

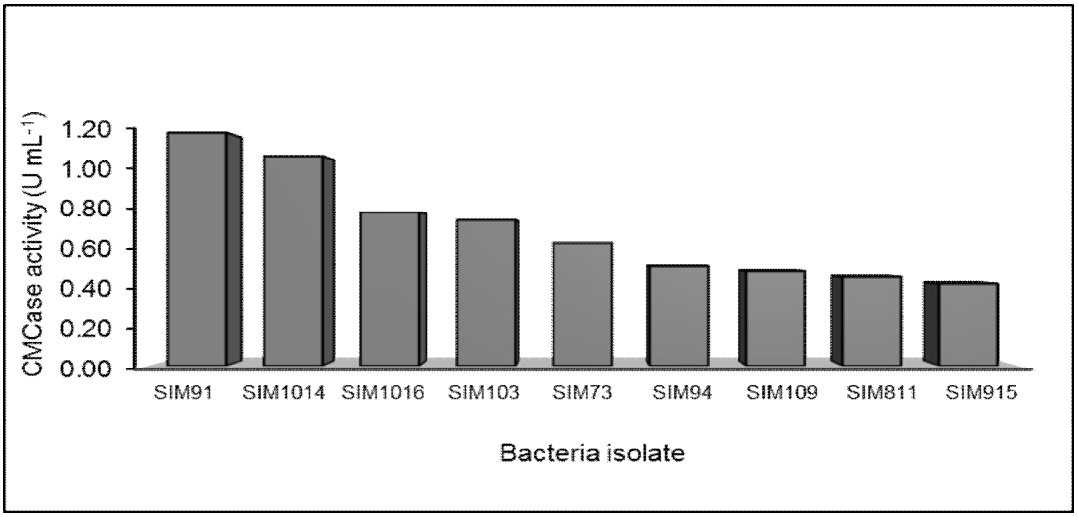

Figure 2. CMCase activity of nine bacterial isolates from agar waste.

***Bacterial pathogenicity***. The survival rate of juvenile rabbitfish (*S. guttatus*) over the 10 days pathogenicity test was 100% for all treatments, with no significant difference between treatments ($p > 0.05$). We observed active juveniles in all treatments during the pathogenicity test, and there were no changes in fish behaviour during the tests. Eight isolates (SIM73, SIM91, SIM94, SIM109, SIM811, SIM915, SIM1014 and SIM1016) had no visible effect on the juveniles; however, one isolate (SIM103) seemed to have a negative effect on the juvenile rabbitfish (Table 2).

Table 2

Survival rate, disease symptoms and behaviour of injected juvenile rabbitfish (*S. guttatus*) during a 10 day pathogenicity test

| *Injected isolate* | *Survival rate (%)* | *Symptoms of disease* | *Fish behaviour* |
|---|---|---|---|
| Control* | 100 | 0 | Normal |
| SIM91 | 100 | 0 | Normal |
| SIM1014 | 100 | 0 | Normal |
| SIM1016 | 100 | 0 | Normal |
| SIM103 | 100 | Swollen eyes (50%); Pale gills (20% of fish with swollen eyes). | Normal |
| SIM73 | 100 | 0 | Normal |
| SIM94 | 100 | 0 | Normal |
| SIM109 | 100 | 0 | Normal |
| SIM811 | 100 | 0 | Normal |
| SIM915 | 100 | 0 | Normal |

Note: Control* = non injected-saline solution.

Fifty percent of juveniles injected with SIM103 exhibited swollen eye symptoms 24 hours after the injection. At the end of pathogenicity test we found 20% of the juveniles with swollen eyes also had pale gills (Figure 3). However the symptoms did not result in any mortality.

The leucocyte counts of the juvenile rabbitfishes were (2.3-6.6) x $10^4$ cell $mL^{-1}$ in range, meanwhile erythrocyte counts were (2.0-8.9) x $10^3$ cell $mL^{-1}$ in range. The percentage of leukocytes and erythrocytes of juvenile rabbitfish under each treatment are shown in Figure 4. Statistical analysis (ANOVA) showed no significant differences between treatments ($p > 0.05$).

A B

Figure 3. Juvenile rabbitfish infected by bacteria isolate (SIM103) with swollen eyes and pale gills (arrows) (A) compared to control (B).

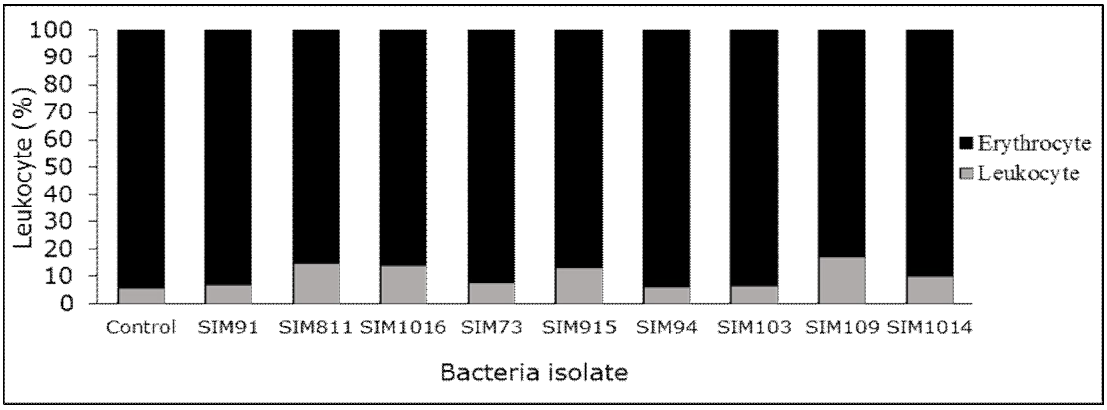


Figure 4. Leukocyte and erythrocyte profiles of juvenile rabbitfish injected with bacterial isolates identified as potential fermentation agents.

***Bacterial identification***. The eight non-pathogenic isolates (SIM73, SIM91, SIM94, SIM109, SIM811, SIM915, SIM1014 and SIM1016) were identified using 16S-rDNA sequencing. The PCR product obtained for each of the bacterial isolates had a 1500 bp DNA band (Figure 5). The BLAST analysis of the DNA sequences identified four species of the genus *Pseudomonas* with 97-99% of similarity, namely *Pseudomonas stutzeri*, *P. songnenensis*, *P. aeruginosa* and *P. chengduensis* (Table 3).

Table 3

BLAST analysis of eight selected bacterial isolate DNA sequences

| *Code* | *Species* | *Similarity (%)* |
|---|---|---|
| SIM91 | *Pseudomonas stutzeri* | 98 |
| SIM811 | *Pseudomonas songnenensis* | 98 |
| SIM1016 | *Pseudomonas aeruginosa* | 97 |
| SIM73 | *Pseudomonas stutzeri* | 99 |
| SIM915 | *Pseudomonas stutzeri* | 97 |
| SIM94 | *Pseudomonas chengduensis* | 98 |
| SIM109 | *Pseudomonas chengduensis* | 99 |
| SIM1014 | *Pseudomonas aeruginosa* | 98 |

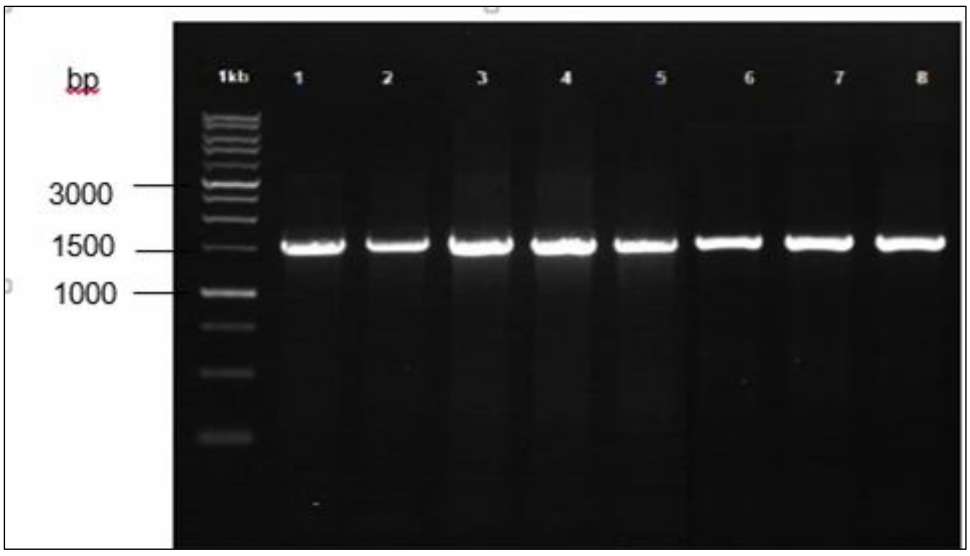


Figure 5. PCR product (1500 bp) of bacterial isolates: 1 = SIM73; 2 = SIM91; 3 = SIM94; 4 = SIM109; 5 = SIM915; 6 = SIM1014; 7 = SIM1016; 8 = SIM811.

**Discussion**. A total of 19 bacterial isolates were obtained with different cellulolytic index values. Bacteria clear zone measurement on CMC media is an early screening to find out whether these bacteria have cellulolytic abilities or not. This difference in the cellulolytic index reflects the differences in the ability of the bacteria to degrade cellulose (Huang et al 2012). The range of cellulolytic index values obtained in this study was 0.62-3.0, which is quite high compared to the results of several previous studies. Mulyasari et al (2015) reported cellulolytic index values of bacterial isolates from seaweed in the range 0.4-2.5, while (Ferbiyanto et al 2015) reported the cellulolytic index of bacterial isolates from termite digestive tracts in the range of 0.75-2.5. Isolate SIM91 had the highest cellulolytic index (3.0±0.53) with a ratio of the clear zone diameter to the isolate diameter of 4.03±0.53. According to Li et al (2016), bacteria with a ratio clear zone diameter (D) to colony diameter (d) of 5 > D/d ≥ 3 were classified as bacteria with moderate cellulolytic ability.

Quantitative cellulase enzyme activity (CMCase) was in the 0.4-1.2 U $mL^{-1}$ range. This CMCase activity range was higher than that obtained by Munifah et al (2015) who obtained cellulase enzyme activity in bacteria isolated from industrial agar waste of 0.15-0.2 U $mL^{-1}$. The difference in enzyme activity was most likely due to the different types of bacteria obtained from previous studies. Apart from being influenced by temperature, pH, and substrate concentration, the activity of cellulolytic enzymes is also influenced by the types of carbon and nitrogen sources present in the media (Sheng et al 2012).

The pathogenicity test of bacterial isolates did not result in mortality of juvenile rabbitfish (100% survival rate). However, one isolate (SIM103) did cause symptoms of disease (swollen eyes) in 50% of the tested animals; however only 20% of the fish with swollen eyes also had pale gills. During the observation period, there was no change in fish behaviour, with all juvenile fish remaining active and feeding normally. There was no significant difference in the percentage of leukocytes and erythrocytes between treatments ($p > 0.05$), this indicates that bacterial isolates did not affect the fish blood. Red blood cells (RBC) play a major role in oxygen transport, while white blood cells (WBC) play an important role in stimulating the immune system by producing antibodies (Movahed et al 2016; Shen et al 2018). The pathogenicity test indicated that eight bacterial isolates were safe to be used as fermentation agents in fish feed. However, to ensure the safety of the bioprocessed feed, the isolate SIM103 was not selected as a fermentation candidate.

The identification using DNA barcoding found that *Pseudomon*as was the only genus isolated in the present study. Four species were identified, namely: *P. stutzeri, P. songnenensis, P. aeurigonsa* and *P. chengduensis.* This result is similar that reported by some other studies. For example, Talia et al (2012) also obtained the genus

*Pseudomonas* on a medium enriched with CMC, while Huang et al (2012) reported *Pseudomonas* as a genus that dominated the group of cellulolytic bacteria from the digestive tract of termite larvae. Bacteria of the genus *Pseudomonas* are cellulose-degrading bacteria that are found in soil and have been widely used to treat agricultural solid waste (Sun et al 2020). Pahlawi et al (2019) reported *Pseudomonas* sp. was not pathogenic bacteria for white shrimp (*Litopenaeus vannamei*) and it could be a probiotic candidate.

**Conclusions**. This study produced 8 bacterial isolates with cellulolytic potential as seaweed fermentation agents for rabbitfish feed bioprocessing which were not pathogenic to juvenile rabbitfish. *Pseudomonas* was the only genus found in the cellulolytic bacterial community in agar waste. The four species identified from DNA barcoding using the BLAST routine were *P. sutzeri*, *P. songnenensis*, *P. aeruginosa* and *P. chengduensis*.

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## References

Abdel-Warith A. W. A., Younis E. S. M. I., Al-Asgah N. A., 2016 Potential use of green macroalgae *Ulva lactuca* as a feed supplement in diets on growth performance, feed utilization and body composition of the African catfish, *Clarias gariepinus*. Saudi Journal of Biological Sciences 23(3):404-409.

Al-Asgah N. A., Younis E. S. M., Abdel-Warith A. W. A., Shamlol F. S., 2016 Evaluation of red seaweed *Gracilaria arcuata* as dietary ingredient in African catfish, *Clarias gariepinus*. Saudi Journal of Biological Sciences 23(2):205-210.

Arori M. K., Muthumbi A. W. N., Mutia G. M, Nyonje B., 2019 Potential of seaweeds (*Hypnea cornuta* and *Hypnea musciformis*) in Nile tilapia (*Oreochromis niloticus*) fingerlings diets. International Journal of Fisheries and Aquatic Studies 7(2):103-107.

Bhuiyan M. K. A., Qureshi S., Kamal A. H. M., AftabUddin S., Siddique M. A., 2016 Proximate chemical composition of sea grapes *Caulerpa racemosa* (J. Agardh, 1873) collected from a sub-tropical coast. Virology and Mycology 5(2):158.

Biancarosa I., Espe M., Bruckner C. G., Heesch S., Liland N., Waagbø R., Torstensen B., Lock E. J., 2017 Amino acid composition, protein content, and nitrogen-to-protein conversion factors of 21 seaweed species from Norwegian waters. Journal of Applied Phycology 29(2):1001-1009.

Comba-González N., Ruiz-Toquica J., López-Kleine L., Montoya-Castaño D., 2018 Epiphytic bacteria of macroalgae of the genus *Ulva* and their potential in producing enzymes having biotechnological interest. Journal of Marine Biology and Oceanography 5:2.

Dawczynski C., Schubert R., Jahreis G., 2007 Amino acids, fatty acids, and dietary fibre in edible seaweed products. Food Chemistry 103(3):891-899.

Felix N., Brindo R. A., 2014 Evaluation of raw and fermented seaweed, *Ulva lactuca* as feed ingredient in giant freshwater prawn *Macrobrachium rosenbergii*. International Journal of Fisheries and Aquatic Studies 1(3):199-204.

Ferbiyanto A., Rusmana I., Raffiudin R., 2015 Characterization and identification of cellulolytic bacteria from gut of worker *Macrotermes gilvus*. HAYATI Journal of Biosciences 22(4):197-200.

Hessami M. J., Salleh A., Phang S. M., 2020 Bioethanol a by-product of agar and carrageenan production industry from the tropical red seaweeds, *Gracilaria manilaensis* and *Kappaphycus alvarezii.* Iranian Journal of Fisheries Sciences 19(2):942-960.

Horn S. J., 2017 Bioprocessing of seaweed to fish feed. Foods of Norway: WP1: development of novel feeds and processing. Norwegian University of Life Sciences, Faculty of Chemistry, Biotechnology and Food Science, 17 pp.

Huang S., Sheng P., Zhang H., 2012 Isolation and identification of cellulolytic bacteria from the gut of *Holotrichia parallela larvae* (Coleoptera: Scarabaeidae). International Journal of Molecular Sciences 13(3):2563-2577.
Jayasekara S., Ratnayake R., 2012 Microbial celluases: an overview and applications. IntechOpen 38.
Jumaidin R., Sapuan S. M., Jawaid M., Ishak M. R., Sahari J., 2017 Characteristics of *Eucheuma cottonii* waste from East Malaysia: physical, thermal and chemical composition. European Journal of Phycology 52(2):200-207.
Li H., Wu S., Wirth S., Hao Y., Wang W., Zou H., Li W., Wang G., 2016 Diversity and activity of cellulolytic bacteria, isolated from the gut contents of grass carp (*Ctenopharyngodon idellus*) (Valenciennes) fed on Sudan grass (*Sorghum sudanense*) or artificial feedstuffs. Aquaculture Research 47(1):153-164.
Movahed R., Khara H., Ahmadnezhad M., Sayadboorani M., 2016 Hematological characteristics associated with parasitism in pikeperch *Sander lucioperca* (Percidae) from Anzali Wetland. Journal of Parasitic Diseases 40(4):1337-1341.
Mulyasari, Melati I., Sunarno T. D., 2015 [Isolation, selection and identification of cellulolytic bacteria from seaweed as a candidate of crude fibre degradation]. Jurnal Riset Akuakultur 10(1):51-60. [in Indonesian]
Munifah I., Sunarti T. C., Irianto H. E., Meryandini A., 2015 Biodiversity of cellulolytic bacteria isolated from the solid waste of agar seaweed processing industry. Squalen Bulletin of Marine & Fisheries Postharvest and Biotechnology 10(3):129-139.
Pahlawi I. M. H., Satyantini W. H., Sudarno, 2019 [Pathogenicity test of *Pseudomonas* sp. in white shrimp (*Litopenaeus vannamei*) as a probiotic candidate]. Journal of Aquaculture and Fish Health 8(2):92-98. [in Indonesian]
Peixoto M. J., Salas-Leitón E., Pereira L. F., Queiroz A., Magalhães F., Pereira R., et al, 2016 Role of dietary seaweed supplementation on growth performance, digestive capacity and immune and stress responsiveness in European seabass (*Dicentrarchus labrax*). Aquaculture Reports 3:189-197.
Saoud I. P., Kreydiyyeh S., Chalfoun A., Fakih M., 2007 Influence of salinity on survival, growth, plasma osmolality and gill $Na^+$-$K^+$-ATPase activity in the rabbitfish *Siganus rivulatus*. Journal of Experimental Marine Biology and Ecology 348(1-2):183-190.
Saoud I. P., Ghanawi J., Lebbos N., 2008 Effects of stocking density on the survival, growth, size variation and condition index of juvenile rabbitfish *Siganus rivulatus*. Aquaculture International 16(2):109.
Shen Y., Wang D., Zhao J., Chen X., 2018 Fish red blood cells express immune genes and responses. Aquaculture and Fisheries 3(1):14-21.
Sheng P., Huang S., Wang Q., Wang A., Zhang H., 2012 Isolation, screening, and optimization of the fermentation conditions of highly cellulolytic bacteria from the hindgut of *Holotrichia parallela larvae* (Coleoptera: Scarabaeidae). Applied Biochemistry and Biotechnology 167(2):270-284.
Sumathi C., Priya D. M., Babu V. D., Sekaran G., 2011 Analysis of enzyme activities of the gut bacterial communities in *Labeo rohita* fed differentially treated animal fleshing diets. Journal of Microbial & Biochemical Technology 3(5):112-120.
Sun S., Zhang Y., Liu K., Chen X., Jiang C., Huang M., Zang H., Li C., 2020 Insight into biodegradation of cellulose by psychrotrophic bacterium *Pseudomonas* sp. LKR-1 from the cold region of China: optimization of cold-active cellulase production and the associated degradation pathways. Cellulose 27(1):315-333.
Talia P., Sede S. M., Campos E., Rorig M., Principi D., Tosto D., et al, 2012 Biodiversity characterization of cellulolytic bacteria present on native Chaco soil by comparison of ribosomal RNA genes. Research in Microbiology 163(3):221-232.
Weimer P. J., French A. D., Calamari T. A., 1991 Differential fermentation of cellulose allomorphs by ruminal cellulolytic bacteria. Applied and Environmental Microbiology 57(11):3101-3106.
Xu S., Zhang L., Wu Q., Liu X., Wang S., You C., Li Y., 2011 Evaluation of dried seaweed *Gracilaria lemaneiformis* as an ingredient in diets for teleost fish *Siganus canaliculatus*. Aquaculture International 19(5):1007-1018.

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