Isolation and identification of facultative mixotrophic ammonia-oxidizing bacteria from Bone Regency, Indonesia

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Abstract. Autotrophic ammonia-oxidizing bacteria (AOB) have been widely documented in intensive aquaculture systems, while facultative mixotrophic AOB have been less comprehensively studied. Although facultative mixotrophic bacteria have been isolated from a variety of aquatic environments, their contribution to biogeochemical processes in waters remains neglected. In this study, heterotrophic bacteria were isolated from intensive shrimp ponds and the estuary in Bone Regency, South Sulawesi, Indonesia and then cultivated in a mixotrophic medium containing ammonium and acetic acid. The characterization of the bacteria was performed using morphological features, gram stain, motility, catalase, and other biochemical tests. Results showed the presence of 12 bacteria, among 48 initially heterotrophic isolates. Among these potentially facultative mixotrophic AOB, three isolates were morphologically and biochemically related to Firmicutes (*Bacillus* and *Lactobacillus*), six isolates were affiliated to Actinobacteria (*Acinetobacter, Arthrobacter,* and *Gordonia*), three other isolates were associated with Proteobacteria (*Paracoccus, Pseudomonas, Vibrio*). Pre-screening and screening of these bacteria were conducted to determine the potential for microbial ammonia oxidation.
Key Words: organic pollution, water quality, mixotrophic ammonia oxidizing bacteria, ammonia oxidation.

Introduction. Shrimp farming industries are one of the most important practices worldwide. According to FAO (2012), the world aquaculture production of shrimp are predicted 4,327,520 ton in 2017. Currently, 52% of all shrimp consumed globally comes from aquaculture (FAO 2017). However, recently on a worldwide scale, shrimp disease has grossly affected nearly every shrimp production area (Durai et al 2015). Some studies conclude that the decrease of shrimp culture production is mainly caused by disease attacks triggered by the declining quality of shrimp culture environment (Primavera 2006; Ling et al 2010; Stentiford et al 2012). The accumulation of inorganic nitrogen content, carbonic organic compounds, and sulfides derived from shrimp residues, shrimp manure, and long-term fertilization have a direct impact on the content of NH_3, NO_2, and H_2S compounds that are toxic to shrimp. Ammonia is one of the most toxic substances produced by intensive shrimp farming (Handy & Poxton 1993). Ammonia is toxic and can cause convulsions, coma and death, probably because elevated ammonium (NH_4^+) ions displace potassium (K^+) and depolarizes neurons, causing activation of the N-methyl-d-aspartate (NMDA) type glutamate receptor, which leads to an influx of excessive calcium (Ca_2^+) ions and subsequent cell death in the central nervous system (Randall & Tsui 2002).

Maintaining water quality under optimum conditions for shrimp growth is one of the key factors to successful shrimp cultivation. Lim et al (2014) stated that the use of biological techniques is more efficient compared to mechanical and chemical means. The use of biological techniques by utilizing microorganisms (bioremediation) for the recovery of waters from pollutants and toxic is frequently performed because it is easier and cheaper (Zhang et al 2008).

Utilization of organic decomposing bacteria which is capable of acting as an autotrophic and heterotrophic bacteria or also known as mixotrophic bacteria (Crane &

Grover 2010) provides new hope for solving the problem of organic matter contamination and deterioration of pond water quality and other fish breeding containers. Various commercial products of bioremediation agents in the form of autotrophic and heterotrophic bacteria have been used to cope with contamination of organic matter in ponds. However, from several cases of shrimp disease, it was reported that those bioremediation agents are not effective in improving the quality of the pond environment (Queiroz & Boyd 1998; Gram 2001; Shelby et al 2006; Abd El-Rahman et al 2009; Ferguson et al 2010).

Facultative mixotrophic bacteria are capable of performing the mineralization function of carbonic organic compounds into ammonia and carbon dioxide, degrading ammonia to nitrite and nitrate, and rendering the denitrification of nitrate or nitrite to nitrogen gas. In addition, photosynthetic bacteria that are mixotrophic are able to degrade the hydrogen sulfide compound into sulfate (Kouki et al 2011). Although mixotrophic facultative bacteria have been isolated from a variety of aquatic environments, their contribution to biogeochemical processes in waters remains neglected (Eiler 2006; Kouki et al 2011). Even up to now, the utilization of facultative mixotrophic bacteria for bioremediation has not been optimized. Therefore, the study urgently required to obtain a mixotrophic facultative bacterial species capable of acting as a bioremediation agent to oxidize ammonia and sulfide compounds, which are the main cause of degradation of shrimp pond quality and declining shrimp production. In the first year, the study was aimed to isolate and identify facultative mixotrophic bacteria that can potentially degrade ammonia and sulfides.

Material and Method

Water and sediment collection. Water and sediment were collected in April 2018 from the intensive shrimp farming in Bone Regency of the Province of South Sulawesi, Indonesia. Obtained samples were stored in ice boxes at freezing temperatures until analysed in laboratories.

Isolation and identification of ammonia degrading bacteria. 0.1 mL of obtained samples were plated in triplicate by spread plate method onto SWC (Sea Water Complete) medium and incubated for 24 h at 20 and 30^{o}C to determine the optimum temperature for the growth of facultative mixotrophic ammonia-oxidizing bacteria (AOB). Based on apparent physical features, i.e. the shape and size of colonies, colours, elevations, and edges, each different colony was cultured until the single colony was obtained. The pure isolates were then cultured in a reaction tube containing the SWC agar (slant culture). Putative ammonia-oxidizing bacteria (AOB) were identified by the biochemical method described in Bergey's Manual of Determinative Bacteriology (Holt et al 1994). A variety of biochemical tests were performed, including oxidase, catalase, hydrogen sulfide production, tyrosine hydrolysis, gelatinase test, ornithine decarboxylase, arginine dihydrolase, methyl-red and Voges-Proskauer, casein, starch hydrolysis, citrate utilization, nitrate reduction, TSI agar test, and motility test.

Pre-screening of facultative mixotrophic ammonia oxidizing bacteria. To obtain a mixotrophic bacterial colony, each 0.5 mL pure isolate was cultured on a 0.5 mL TSA (trypticase-soy-agar) medium, and incubated at 25°C for 24-48 h. Subsequently, the colonies grown were sub-cultured on TSB (trypticase-soy-broth) media at 25°C for 24-48 h without shaking. Then 0.5 mL of bacterial culture was inoculated into Erlenmeyer flasks containing 50 mL of mixotrophic medium (Schmidt & Belser 1994), which was enriched with acetic acid with the composition (g L^{-1}) as follows: $(NH_4)2SO_4$ 0.5; $MgSO_4 \cdot 7H_2O$ 0.04; KH_2PO_4 0.203; $CaCl_2 \cdot 2H_2O$ 0.013; iron chelate 0.01; trace elements 0.01 and acetic acid 1. Acetic acid is presented as a source of organic carbon. The pH of the culture medium was maintained at 7.2 using 1 mol L^{-1} NaOH. Incubation was carried out at 25°C for 30 days in the dark with mechanical agitation at 200 rpm, which was performed to adapt the previously heterotrophic bacteria into mixotrophic bacteria.

Screening. Bacterial cells were centrifuged at 4000 rpm for 10 minutes, then re-transferred to mixotrophic media under the same culture conditions as described in the pre-screening stages of mixotrophic bacteria (Kouki et al 2011). The media consisted of 51.3 mg L^{-1} ammonium as a nitrogen source which was used to detect the capacity of bacteria to degrade ammonia in aerobic conditions. Then, aliquots from each culture were used for testing the remaining ammonia levels. A 2 mL of Seignette's salt solution (potassium sodium tartrate) and 2 mL of Nessler reagent (Potassium sodium tartrate) were put in a tube containing 1 mL of bacterial culture. Preparation of standard solutions using concentrated NH_4Cl solution (10 mmol L^{-1}). Ammonia levels were measured using a UV-vis spectrophotometer (Jasco, Tokyo, Japan) at a wavelength of 420 nm.

Statistical analysis. Data were statistically analyzed with SPSS 22 (IBM SPSS Advanced Statistics 22), and the results are shown as the mean value. The differences between mean values were analyzed by one-way analysis of variance (ANOVA). Critical values in all tests were $\alpha=0.05$.

Results and Discussion. Of forty-eight visually different bacterial isolates, 12 bacterial strains were determined by colony morphology, Gram staining, and pigmentation (Table 1).

Table 1

The morphology and physiological characteristic of the putative facultative mixotrophic AOB from intensive shrimp farming, Bone Regency, Indonesia

Strains	*Morphology*	*Gram stain*	*Spore*	*Motility*	*Pigmentation*	*No. of clones (%)*	*Genus*
AOB1	Cocco-bacilli	+	-	+	Pinkish-brown	8.11	*Gordonia* sp.
AOB4	Cocco-bacilli	-	+	+	Translucent	10.14	*Bacillus* sp.
AOB6	Cocci	+	+	+	White	8.11	*Bacillus* sp.
AOB9	Bacilli	-	-	+	Yellow	6.08	*Pseudomonas* sp.
AOB13	Cocci	-	+	-	Pinkish-red	10.14	*Paracoccus* sp.
AOB18	Comma	-	-	+	Yellowish-brown	8.11	*Vibrio* sp.
AOB23	Cocci	+	-	-	Yellowish-white	12.16	*Arthrobacter* sp.
AOB30	Cocci	-	-	-	Yellow	6.08	*Acinetobacter* sp.
AOB32	Cocci	-	-	-	White	4.73	*Acinetobacter* sp.
AOB35	Cocci	-	-	-	White-greyish	8.11	*Acinetobacter* sp.
AOB38	Bacilli	+	-	-	White	10.14	*Lactobacillus* sp.
AOB42	Cocci	+	-	-	Brownish-white	8.11	*Acinetobacter* sp.

The morphological and physiological characteristics of the isolates identified to the genus level showed that gram-negative bacteria dominated the bacterial communities. Specifically, 29.06% of gram-negative cocci isolates were identified as *Paracoccus* and *Acinetobacter*; 10.14% of isolates of gram-negative cocci-bacilli belonged to *Bacillus*; 8.11% of isolates of gram-negative comma were identified as *Vibrio* and 6.08% of isolates of gram-negative bacilli were identified as *Pseudomonas* (Table 1). Non-pigmented isolates belonged to *Bacillus*, *Acinetobacter*, and *Lactobacillus* whereas non-motile isolates were characterized as *Paracoccus*, *Arthrobacter*, *Acinetobacter*, and *Lactobacillus*. Léonard et al (2000) and Michaud et al (2006) reported that *Pseudomonas* and *Acinetobacter* are common inhabitants of various aquatic environments. Naik et al (1982), Rodriguez & Fraga (1999), Diep & Cuc (2013), and Maitra et al (2015) reported that among the bacterial genera with the capacity to oxidize ammonia in aquatic environments one can find *Bacillus, Pseudomonas,* and *Acinetobacter*. Meanwhile, Diep & Cuc (2013) found that *Arthrobacter, Rhodococcus, Bacillus* and *Pseudomonas* are common heterotrophic nitrogen removal bacteria in the sediment and water of striped catfish (*Platydoras costatus*) ponds in the Mekong, Delta, Vietnam. Yang et al (2015) reported that *Paracoccus* sp. was identified as one of the dominant strains in the removal of NH_4^+-N and total nitrogen from industrial wastewater with sewage disposal system.

Table 2

The morphological and biochemical characteristic of AOB isolated from intensive shrimp farming in Bone Regency, South Sulawesi Indonesia

Test	*The AOB strain*											
	AOB1	*AOB4*	*AOB6*	*AOB9*	*AOB13*	*AOB18*	*AOB23*	*AOB30*	*AOB32*	*AOB35*	*AOB38*	*PSB42*
Catalase	+	-	+	+	+	-	+	-	+	-	-	-
Oxidase	+	+	-	-	+	+	+	+	-	+-	-	-
H_2S production	-	-	-	-	-	+-	-	-	-	-		-
Gelatinase	+	+	+	+	+	+	+	+	+	+	+	+
Nitrate reduction	+	+	-	+	-	-	-	-	+	-	-	+
Starch hydrolysis	-	-	+	-	+	+	+	+	+	+	+	+
Citrate utilization	-	-	-	-	-	-	-	-	-	-	-	-
Ornithine		-	-	-	-	-	-	-	+	-		-
Indole	+	-	-	-	-	-	+	+	+	+	+	+
Tyrosine hydrolysis	-	+	-	-	-	+	-	-	-	+	-	+
Arginine dihydrolase	-	-	+	-	-	+	+	-	+	-	-	+
Methyl red	+	+	-	-	+	-	-	-	-	+	-	-
Voges-Proskauer	+	+	+	+	+	+	+	+	+	+	+	+
Urease	+	-	+	-	+	+	-	+	+	-	-	+
Glucose	+	+	-	-	-	+	-	-	-	-	-	+
Fructose	+	+	-	-	-	-	-	-	-	-	-	+
Lactose	-	-	-	-	-	-	-	-	-	-	-	+
Maltose	+	+	-	-	-	-	-	+	-	-	-	+
Sucrose	+	+	-	-	+	+	-	-	-	-	-	+
Xylose	+	-	-	+	-	-	+	+	+	-	-	+
D-Mannitol	+	+	-	-	-	-	-	-	-	-	-	+
D-Arabinose	-	-	-	-	-	-	-	-	-	-	-	-

+ = positive; - = negative; +- = some of the tests show positive, the other is negative.

Out of 12 bacterial strains as shown in Table 1, *Acinetobacter* was represented by 4 different strains, *Bacillus* was represented by 2 species, while the remaining 6 genera, such as *Gordonia, Lactobacillus, Arthrobacter, Vibrio, Paracoccus,* and *Pseudomonas* had only one species each.

Then, the ammonia removal test was used to screen bacteria based on their ammonia removal efficiency (ARE) (Table 3). We noted that all tested isolates were capable of having ARE in the range between 41 and 83%. The higher ARE, the more efficient the bacteria in removing ammonia. Bacterial isolates of the genera *Pseudomonas* (AOB9) and *Arthrobacter* (AOB23) were less efficient in ammonia removal compared to other isolates. The remaining isolates were able to remove ammonia from the mixotrophic culture media with more considerable levels of ARE, while bacterial isolate of the genera *Gordonia* and *Paracoccus* achieved the highest ARE, exceeding 80%. The results suggested that most bacteria obtained in this study have the potential to remove excess ammonia from the media, thus screening based on other characteristics are still required to ensure the potential of the bacteria obtained.

Table 3

Screening of AOB for ammonia removal efficiency (ARE)

Putative AOB bacteria	*Genus*	*Ammonia removal efficiency (ARE, %)*
AOB1	*Gordonia* sp.	81
AOB4	*Bacillus* sp.	58
AOB6	*Bacillus* sp.	54
AOB9	*Pseudomonas* sp.	41
AOB13	*Paracoccus* sp.	83
AOB18	*Vibrio* sp.	62
AOB23	*Arthrobacter* sp.	47
AOB30	*Acinetobacter* sp.	55
AOB32	*Acinetobacter* sp.	67
AOB35	*Acinetobacter* sp	57
AOB38	*Lactobacillus* sp.	78
AOB42	*Acinetobacter* sp.	59

Bacterial isolates belonging to the genera *Bacillus, Pseudomonas*, and *Acinetobacter* were known to help in the mineralization of organic water and in reducing the accumulation of organic loads (Shariff et al 2001). Many studies have reported the use of nitrifying and denitrifying bacteria to control water quality by increasing the removal rate of ammonia in aquaculture ponds (Ma et al 2009; Cardona et al 2016; Qin et al 2016; Shan et al 2016).

In the present study, the most common bacterial genus was *Acinetobacter*. The *Acinetobacter* has been frequently isolated from soils and water. Xin et al (2014) discovered a new bacterial strain that has the ability to tolerate high ammonia nitrogen content, and is a heterotrophic nitrification-aerobic bacteria, based on physiological and biochemical characteristics and molecular identification, and aerobic nitrification-heterotrophic performance, resistance ability to ammonia nitrogen and decontamination ability. According to its physiological and biochemical characteristics and molecular identification, the bacterial strain refers to *Acinetobacter* sp. Members of the genus such as *A. calcoaceticus* have been reported to be involved in a single step nitrogen removal by simultaneous heterotrophic nitrification and aerobic denitrification in wastewaters (Zhao et al 2010; Ardiansyah & Fotedar 2016). The second most common bacteria in our study was *Bacillus*. Kundu et al (2014) and Ardiansyah & Fotedar (2016) reported that the *Bacillus* found in the biofilter of recirculating aquaculture systems (RAS) consisted of obligate aerobes (*B. subtilis*), or facultative anaerobes (*B. licheniformis*), which are known to have a major role in heterotrophic nitrification.

The other 6 genera, such as *Pseudomonas*, *Gordonia, Lactobacillus, Arthrobacter, Vibrio,* and *Paracoccus* were only represented by one species each. Borges et al (2008) reported that the *Pseudomonas* is the most abundant heterotrophic bacteria in marine RAS nitrification biofilters (Borges et al 2008). De Souza et al (2000) stated that *Pseudomonas* can also be found in alkaline and highly saline water environments. Furthermore, Lalucat et al (2006) stated that certain species of *Pseudomonas* have the ability to metabolize an extensive number of organic compounds, whereas others have metabolic properties such as denitrification, degradation of aromatic compounds and nitrogen fixation. Rodriguez & Fraga (1999) reported that the genus *Pseudomonas* was the best strain in dissolving the bonded P from any source of P, and dominant in the mineralization of organic phosphorus. In this study, *Pseudomonas* was represented by *P. mendocina*.

Various types of heterotrophic bacteria have been shown to affect the biological oxidation of nitrogen. Bacteria have been reported to oxidize ammonia to nitrite and nitrate (He et al 2017), for example, the ability of *Arthrobacter arilaitensis* to remove nitrogen from waste-water. About 100% of the ammonia was removed in the nitrogen removal experiment, while the nitrate removal efficiency was 73.3%. All experimental results show that *A. arilaitensis* can remove ammonia, nitrate and nitrite from wastewater, and can also perform nitrification and denitrification simultaneously under aerobic conditions. Abrashev et al (1998) have reported that some *Arthrobacter* species have the ability to produce a number of valuable substances like amino acids, vitamins, enzymes, specific growth factors, and polysaccharides and they possess many advantageous properties and nutritional benefits to be probiotics in aquaculture (Li et al 2006).

Chakravarthy et al (2011) reported that *Paracoccus* was a genus of bacteria that plays a significant role in the process of nitrification and denitrification. During denitrification, *Paracoccus* sp. does not show nitrite accumulation. Increased nitrate concentrations in the medium did not increase the accumulation of nitrite. *Paracoccus* sp. is found to be an efficient denitrifier with an insignificant amount of nitric accumulation, and it can also denitrify nitrate in high quantities up to 2 M. *Paracoccus* was found as an efficient denitrifying bacteria with less amounts of nitrite accumulation and high amounts of nitrate reduction from wastewater. *Paracoccus* sp. is shown to meet this demand and can be a potential organism to effectively remove nitrate.

Gordonia was well known in bioremediation (Chen et al 2012; Kāmpfer et al 2013). *Gordonia* showed significant reduction of ammonia and nitrite. This fact suggests that *Gordonia* was involved in removal of ammonia and conversion of nitrate to gaseous N_2. *Gordonia amarae* is derived from the right-angled mycolata, being one of the most common filamentous bacteria found in the foaming process. *G. amarae* can utilize a large number of organic substrates, both hydrophilic and hydrophobic, and is found capable of taking some substrates under aerobic, anaerobic and anoxic conditions (Carr et al 2006).

Vibrio, a genus from the phylum of Proteobacteria, is a diverse group of heterotrophic bacteria. *Vibrio* is commonly found in the marine environment, particularly in coastal areas (Zhang et al 2018). Kim & Shoda (2002) reported that a heterotrophic marine bacteria, *Vibrio algynoliticus*, can be used to remove high ammonia loads in biofilter where nitrifying bacteria cannot be applied. Ammonia gas was removed efficiently without a period of bacterial acclimatization to ammonia. High removal efficiency of ammonia was maintained even under non-aseptic conditions. Because maintenance of heterotrophic marine bacteria is easier than that of autotrophic bacteria, the use of marine bacteria such as *V. algynoliticus* can provide an alternative method for treating high ammonia concentrations.

Narmadha & Kavitha (2012) reported that the main bacterial species involved in effective wastewater treatments include lactic acid bacteria - *Lactobacillus plantarum*, *L. casei* and *Streptococcus lacti* and photosynthetic bacteria - *Rhodopseudomonas palustrus* and *Rhodobacter spaeroide*. Aly et al (2016) stated that *Lactobacillus* has been used to remove ammonia in the European seabass (*Dicentrarchus labrax*) water tanks. The results obtained showed that the use of bacteria was able to remove ammonia toxicity by 66%.

In addition to being an integral part of the planet's nitrogen cycle, bacterially facilitated nitrification and denitrification are processes of great commercial importance in the wastewater treatment, aquaculture and agronomy industries. Twelve distinct species of heterotrophic bacteria, belonging to the genera *Bacillus, Pediococcus* and *Lactobacillus* were isolated from a commercial water treatment product (Gorsuch et al 2012). Bacteria of the genus *Lactobacillus* synthesise phenyl acetate and this may provide a useful mechanism to control ammonia overspill. Furthermore, Hashimoto (2001) have found that inoculation of *Lactobacillus* could dramatically reduce the accumulation of nitrites.

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All the putative AOB from the present study have been known to play a fundamental role in nitrification-denitrification process in a diverse range of ecosystems (Anand et al 2016). However no information is provided regarding the role of the bacteria in the conversion of ammonia to nitrite and nitrate. Our research suggests that obtained bacterial strains in the study are potential to be the candidate of ammonia-oxidizing bacteria. Further research still needs to be performed to confirm the effectiveness of these mixotrophic bacteria before being used in shrimp farms, including the ability to grow in shrimp ponds and the antagonistic ability of mixotrophic bacteria against shrimp pathogenic bacteria.

Conclusions. The bacterial isolates found in this study are mainly gram-negative cocci isolates and have the ability to remove ammonia more than 40% from culture media. Especially for the gram-negative cocci from the genus *Paracoccus* and gram positive cocco-bacilli from the genus *Gordonia* are capable of removing ammonia by more than 80%. Screening based on other capabilities of the bacteria is still required to confirm the ability of these bacteria in ammonia oxidation, including the ability to inhibit the growth of pathogenic bacteria and the ability of growth in shrimp ponds.

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References

Abd El-Rahman A. M., Khattab Y. A., Shalaby A. M., 2009 *Micrococcus luteus* and *Pseudomonas* species as probiotic for promoting the growth performance and health of Nile tilapia, *Oreochromis niloticus*. Fish and Shellfish Immunology 27(2):175-180.

Abrashev I. R., Genova V. E., Sotirova A. V., Ilieva K. Z., 1998 Purification and partial characterization of neuraminidase from non-pathogenic *Arthrobacter nicotianae*. Enzyme and Microbial Technology 22:142-146.

Aly H. A., Abdel-Rahim M. M., Lotfy A. M., Abdelaty B. S., Sallam G. R., 2016 The applicability of activated carbon, natural zeolites, and probiotics (EM®) and its effects on ammonia removal efficiency and fry performance of European seabass *Dicentrarchus labrax*. Journal of Aquatic Research and Development 7:11.

Anand K., Kumari B., Mallick M. A., 2016 Phosphate solubilizing microbes: an effective and alternative approach as biofertilizers. International Journal of Pharmacy and Pharmaceutical Sciences 8(2):37-40.

Ardiansyah A., Fotedar R., 2016 The abundance and diversity of heterotrophic bacteria as a function of harvesting frequency of duckweed (*Lemna minor* L.) in recirculating aquaculture systems. Letters in Applied Microbiology 63(1):53-59.

Borges M. T., Sousa A., De Marco P., Matos A., Honigova P., Castro P. M., 2008 Aerobic and anoxic growth and nitrate removal capacity of a marine denitrifying bacterium isolated from a recirculation aquaculture system. Microbial Ecology 55(1):107-118.

Cardona E., Gueguen Y., Magré K., Lorgeoux B., Piquemal D., Pierrat F., Noguier F., Saulnier D., 2016 Bacterial community characterization of water and intestine of the shrimp *Litopenaeus stylirostris* in a biofloc system. BMC Microbiology 16(1):157.

Carr E. L., Eales K. L., Seviour R. J., 2006 Substrate uptake by *Gordonia amarae* in activated sludge foams by FISH-MAR. Water Science and Technology 54(1):39-45.

Chakravarthy S. S., Pande S., Kapoor A., Nerurkar A. S., 2011 Comparison of denitrification between *Paracoccus* sp. and *Diaphorobacter* sp.. Applied Biochemistry and Biotechnology 165(1):260-269.

Chen J., Zhan P., Koopman B., Fang G., Shi Y., 2012 Bioaugmentation with *Gordonia* strain JW8 in treatment of pulp and paper wastewater. Clean Technologies and Environmental Policy 14(5):899-904.

Crane K. W., Grover J. P., 2010 Coexistence of mixotrophs, autotrophs and heterotrophs in planktonic microbial communities. Journal of Theoretical Biology 262(3):517-527.

De Souza M. J. B. D., Nair S., Chandramohan D., 2000 Phosphate solubilizing bacteria around Indian peninsula. Indian Journal of Marine Sciences 29:48-51.

Diep C. N., Cuc N. T. K., 2013 Heterotrophic nitrogen removal bacteria in sedimentary and water of striped catfish ponds in the Mekong Delta, Vietnam. American Journal of Life Sciences 1(1):6-13.

Durai V., Gunalan B., Johnson P. M., Maheswaran M. L., Pravinkumar M., 2015 Effect on white gut and white feces disease in semi intensive *Litopenaeus vannamei* shrimp culture system in South Indian state of Tamilnadu. International Journal of Marine Science 5(14):1-5.

Eiler A., 2006 Evidence for the ubiquity of mixotrophic bacteria in the upper ocean: implications and consequences. Applied and Environmental Microbiology 72(12):7431-7437.

FAO (Food Agriculture Organization), 2012 Fishery and aquaculture statistics. Available at: http://www.fao.org/3/a-i3740t.pdf. Accessed: July, 2018.

FAO (Food Agriculture Organization), 2017 FAN FAO Aquaculture Newsletter No. 26. Available at: http://www.fao.org/3/a-i7171e.pdf. Accessed: July, 2018.

Ferguson R. M., Merrifield D. L., Harper G. M., Rawling M. D., Mustafa S., Picchietti S., Balcázar J. L., Davies S. J., 2010 The effect of *Pediococcus acidilactici* on the gut microbiota and immune status of on-growing red tilapia (*Oreochromis niloticus*). Journal of Applied Microbiology 109(3):851-862.

Gram L., 2001 Potential hazards in cold-smoked fish: *Listeria monocytogenes*. Journal of Food Science 66(7):1072-1081.

Gorsuch J. P., Roberts J. J. P., Lenhoff E. A., Showell M. S., 2012 Aerobic nitrification and denitrification among heterotrophic bacterial isolates from a commercial water treatment product (BiOWiSH™ Aqua). 14 pp.

Handy R. D., Poxton M. G., 1993 Nitrogen pollution in mariculture: toxicity and excretion of nitrogenous compounds by marine fish. Reviews in Fish Biology and Fisheries 3(3):205-241.

Hashimoto T., 2001 The cause on the abnormal accumulation of nitrite in pickles of Chinese cabbage (*Brassica pekinesis* Rupr.). Nippon Shokuhin Kagaku Kogaku Kaishi 48(6):409-415.

He T., Xie D., Li Z., Ni J., Sun Q., 2017 Ammonium stimulates nitrate reduction during simultaneous nitrification and denitrification process by *Arthrobacter arilaitensis* Y-10. Bioresource Technology 239:66-73.

Holt J. G., Krieg N. R., Sneath P. H. A., Staley J. T., Wiliam S. T., 1994 Bergey's manual of determinative bacteriology. 9th edition, William & Wilkins, Baltimore, 787 pp.

Kāmpfer P., Martin K., Dott W., 2013 *Gordonia phosphorivorans* sp. nov., isolated from a wastewater bioreactor with phosphorus removal. International Journal of Systematic and Evolutionary Microbiology 63:230-235.

Kim N. J., Shoda M., 2002 Removal of a high load of ammonia by a marine bacterium, *Vibrio alginolyticus* in biofilter. Biotechnology and Bioprocess Engineering 7(5):316-322.

Kouki S., Saidi N., M'hiri F., Nasr H., Cherif H., Ouzari H., Hassen A., 2011 Isolation and characterization of facultative mixotrophic ammonia-oxidizing bacteria from constructed wetlands. Journal of Environmental Sciences (China) 23(10):1699-1708.

Kundu P., Pramanik A., Dasgupta A., Mukherjee S., Mukherjee J., 2014 Simultaneous heterotrophic nitrification and aerobic denitrification by *Chryseobacterium* sp. R31 isolated from abattoir wastewater. BioMed Research International 2014:436056.

Lalucat J., Bennasar A., Bosch R., Garcia-Valdes E., Palleroni N. J., 2006 Biology of *Pseudomonas stutzeri*. Microbiology and Molecular Biology Reviews 70(2):510-547.

Léonard N., Blancheton J. P., Guiraud J. P., 2000 Populations of heterotrophic bacteria in an experimental recirculating aquaculture system. Aquacultural Engineering 22(1-2):109-120.

Li H., Liu Y. H., Luo N., Zhang X. Y., Luan T. G., Hu J. M., Wang Z. Y., Wu P. C., Chen M. J., Lu J. Q., 2006 Biodegradation of benzene and its derivatives by a psychrotolerant and moderately haloalkaliphilic *Planococcus* sp. strain ZD22. Research in Microbiology 157(7):629-636.

Lim M. Y., Shukor M. Y., Wasoh H., 2014 Physical, chemical, and biological methods for the removal of arsenic compounds. BioMed Research International 2014:503784.

Ling T. Y., Buda D., Nyanti L., Norhadi I., Justin J. J. E., 2010 Water quality and loading of pollutants from shrimp ponds during harvesting. Journal of Environmental Science and Engineering 4(6):13-18.

Ma C. W., Cho Y. S., Oh K. H., 2009 Removal of pathogenic bacteria and nitrogens by *Lactobacillus* spp. JK-8 and JK-11. Aquaculture 287:266-270.

Maitra N., Manna S. K, Samanta S., Sarkar K., Debnath D., Bandopadhyay C., Sahu S. K., Sharma A. P., 2015 Ecological significance and phosphorus release potential of phosphate solubilizing bacteria in freshwater ecosystems. Hydrobiologia 745:69-83.

Michaud L., Blancheton J. P., Bruni V., Piedrahita R., 2006 Effect of particulate organic carbon on heterotrophic bacterial populations and nitrification efficiency in biological filters. Aquacultural Engineering 34(3):224-233.

Naik V. M., D'Souza J., Araujo A., 1982 Phosphorus solubilizing yeasts in estuarine environment. Indian Journal of Marine Sciences 11:197-198.

Narmadha D., Kavitha M. S., 2012 Treatment of domestic wastewater using natural flocculants. International Journal of Life Sciences Biotechnology and Pharma Research 1(3):206-213.

Primavera J. H., 2006 Overcoming the impacts of aquaculture on the coastal zone. Ocean and Coastal Management 49(9-10):531-545.

Qin Y., Hou J., Deng M., Liu Q., Wu C., Ji Y., He X., 2016 Bacterial abundance and diversity in pond water supplied with different feeds. Scientific Reports 6:35232.

Queiroz J. F., Boyd C. E., 1998 Effects of a bacterial inoculum in channel catfish ponds. Journal of the World Aquaculture Society 29(1):67-73.

Randall D. J., Tsui T. K., 2002 Ammonia toxicity in fish. Marine Pollution Bulletin 45 (1-12):17-23.

Rodriguez H., Fraga R., 1999 Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnology Advances 17(4-5):319-339.

Schmidt E. L., Belser L. W., 1994 Autotrophic nitrifying bacteria. In: Methods of soil analysis. Part 2: microbiological and biochemical properties. Weaver R., Angle S., Bottomley P. J., Bezdicek D., Smith S. J., Tabatabai A., Wollum A. (eds), SSSA Book Ser. 5, Madison, WI, pp. 159-177.

Shan H. W., Bao W. Y., Ma S., Wei D. P., Gao L., 2016 Ammonia and nitrite nitrogen removal in shrimp culture by *Vibrio alginolyticus* VZ5 immobilized in SA beads. Aquaculture International 24(1):357-372.

Shariff M., Yusoff F. M., Devaraja T. N., Srinivasa Rao P. S., 2001 The effectiveness of a commercial microbial product in poorly prepared tiger shrimp, *Penaeus monodon* (Fabricius), ponds. Aquaculture Research 32(3):181-187.

Shelby R. A., Lim C., Yildirim-Aksoy M., Delaney M. A., 2006 Effects of probiotic diet supplements on disease resistance and immune response of young Nile tilapia, *Oreochromis niloticus*. Journal of Applied Aquaculture 18(2):23-34.

Xin X., Yao L., Lu L., Leng L., Zhou Y. Q., Guo J. Y., 2014 [Identification of a high ammonia nitrogen tolerant and heterotrophic nitrification-aerobic denitrification bacterial strain TN-14 and its nitrogen removal capabilities]. Huan Jing Ke Xue 35(10):3926-3932. [in Chinese]

Yang X., Liu L., Wu B., Liu S., Chen F., 2015 [Screening and ammoxidation characteristics of an ammonium oxidizing bacteria group]. Wei Sheng Wu Xue Bao 55(12):1608-1618. [in Chinese]

Zhang K., Xu Y. Y., Hua X. F., Han H. L., Wang J., Wang J., Liu Y., Liu Z., 2008 An intensified degradation of phenanthrene with macroporous alginate-lignin beads immobilized *Phanerochaete chrysosporium*. Biochemical Engineering Journal 41(3):251-257.

Zhang X., Lin H., Wang X. L., Austin B., 2018 Significance of *Vibrio* species in the marine organic carbon cycle - a review. Science China Earth Sciences 61(10):1357-1368.

Zhao B., He Y. L., Hughes J., Zhang X. F., 2010 Heterotrophic nitrogen removal by a newly isolated *Acinetobacter calcoaceticus* HNR. Bioresource Technology 101(14): 5194-5200.

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