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Microbial degradation of musty odor in aquaculture pond

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Abstract Musty odor due to geosmin and 2-methylisoborneol (MIB) is currently the most serious economic problem faced by aquaculture export industry. They are susceptible to biodegradation with many microorganisms responsible for their removal from water. The study reported the potential microorganism which isolated from 10 Tilapia farming in northern Thailand on the degradation of musty odor. Microbial isolation was done using the media containing geosmin and MIB standard solution. Twenty isolates were selected in order to test their efficiencies on degradation of musty odor. After 48 h. of incubation, isolate TS1 was most efficient to reduce geosmin in the media (92.16%) as analyzed by headspace solid phase micro-extraction (HS-SPME) and gas chromatography–mass spectrometry (GC/MS). Whereas, isolate LS6 had optimum efficiency to reduce MIB (76.61%). Five species (*Achromobacter denitrificans*, *Delftia tsuruhatensis*, *Comamonas* sp., *Acinetobacter calcoaceticus* and *Raoultella ornithinolytica*) with optimal efficiency to reduce geosmin, and MIB were then *in vivo* tested for pathogenesis in fishes. It was shown that all the bacterial species did not cause disease in the fish with survival rate of 96.67-100%. After 48 h. of incubation, *Comamonas* sp. and *Ach. denitrificans* had the highest efficiency to reduce geosmin and MIB (about 40% removal). Microbial cultures in the water with subsequent community profile analysis using 16S rRNA-directed PCR-DGGE identified uncultured bacterial species were grown after 48 h. of incubation. Five bacteria most likely involved in the biodegradation of musty odor within the pond water.

Keywords: Geosmin, MIB, Microbial degradation, Musty odor, Off-flavor

Introduction

Musty odor or off-flavor compounds can reduce the palatability of freshwater fish produced in aquaculture systems. The most common off-flavor are geosmin and 2-methylisoborneol (MIB), produced by many species of cyanobacteria and actinomycetes (Izaguirre *et al*., 1982; Zaitlin and Watson, 2006; Auffret *et al*., 2013). Geosmin and MIB are released into the water and accumulated in fish. Off-flavor is currently the most serious economic problem faced by the fresh fish export industry. This was mainly due to poor management of the ponds. The accumulation of organic matter at the pond bottoms could stimulate the growth of cyanobacteria and actinomycetes in the water at the pond bottoms are growing rapidly. These

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microorganisms can produce compounds that cause musty odor. As a result, musty odors are released into the water and accumulate in the fish.

To solve such problems, treatment techniques to reduce the musty odor in fish could be used to remove geosmin and MIB from water. Conventional treatment techniques such as flocculation, sedimentation and filtration, for the removal of geosmin from water are not effective (Bruce *et al*., 2002). Furthermore, electrochemical, biochemical or photochemical degradation are effective processes but expensive. Therefore, a cost effective method for the treatment of musty odor is required. Biologically active sand filters have recently been recognized as a barrier for the enhanced removal of geosmin and MIB through conventional water treatment (Ho *et al*., 2007 and McDowall *et al*., 2007), degraded geosmin and MIB by bacteria within biofilm **.**The application of biologically active sand filters is favored by water utilities because the process is generally cost effective, requires little maintenance and do not rely on the addition of chemicals which often results in unwanted by-products (McDowall *et al*., 2009).

To date, only a few studies on the microbial degradation of geosmin and MIB in aquaculture system have been reported. The removal of off-flavor compounds in a recirculating aquaculture system (RAS) was optimized by using an adsorption column made of calcium alginate and mucilage biobeads (Alcantar, 2013). It was found that an average 27% reduction of off-flavor compounds was attained after the first cycle. However, subsequent cycles indicate little change in off-flavor compound removal. Application of bioflocs was used to remove geosmin and MIB by interacting with aquaculture waste from RAS (Ma *et al*., 2016). It was found that the decay of these odorous compounds by the bioflocs was mediated by both chemical/physical sorption and biological degradation and it can be used to reuse the nitrogen in fish waste and to remove geosmin and MIB from the culture water efficiently.

The major objective of this study was to evaluate the biodegradation of geosmin and MIB within culture media and aquaculture water as well as to determine the rates of biodegradation of MIB and geosmin. Finally, 16S rRNA gene analysis and generic identification were applied to isolates implicated in the biodegradation of geosmin and MIB.

Materials and methods

Isolation of off-flavor degrading bacteria

Water was collected from 10 Nile Tilapia cultured farms in northern Thailand. Yeast extract and mineral salt medium containing geosmin and MIB standard solution (Sigma Aldrich) were used for microbial isolation. Enrichment of off-flavor-degrading bacteria was modified from Saadoun

and Migdadi (1998). Isolated bacteria ($1x10^8$ CFU/ml) were inoculated in sterile yeast extract (YE) (0.1%) – peptone (0.1%) medium, supplemented with 1 mg/l of geosmin or MIB standard. Then, bacterial cultures were incubated at room temp (27± 2℃ with shaking at 120 rpm for 48 h. Triplicate samples were periodically taken for geosmin and MIB analyses by gas chromatography-mass spectrometry (GC/MS); and bacterial enumeration. Removal efficiency of geosmin and MIB were analyzed (Gutierrez *et al.*, 2013).

Safety testing of off-flavor degrading bacteria

Safety testing of bacterial strains was tested for their applications in the fish pond. Hemolysis of blood (blood agar test) was used to test for the ability to destroy red blood cells. Then, the growth of *Staphylococcus* was tested in mannitol salt agar (MSA) (Bachoon and Dustman, 2008). The presence of *Staphylococcus* indicates the unacceptability of aquaculture products. The export of fish products could not be permitted (Thai Agricultural Standard: TAS 10453-2010, 2010).

In-vivo test in Nile tilapia

Selected bacterial species from the previous methods were tested for pathogenicity. Approximately 15g of Nile tilapia were tested for the incidence of fish disease by measuring the size and weight of fish and monitored the illness and fish death. Bacteria cell suspension at 0.1 ml (1×10^7 cells) was injected subcutaneously in the abdominal area of the fish. The control was injected with sterile normal saline and positive control were those injected with pathogen, *Aeromonas hydrophila*. Pathogenicity was observed as described previously (Verschuere, 2000).

Bacterial identification

Each bacterial strain was identified by using 16S rDNA gene analysis and genus identification was applied to isolates implicated in the biodegradation of geosmin and MIB adjusted from Fukushima *et al.* (2007). Briefly, bacterial DNA was extracted by NucleoSpin® Tissue Kit (Macherey-Nagel GmbH & Co. KG, Germany). 16S rDNA gene was amplified by PCR technique using bacterial specific primer; 27 F and 1492R. 16S rDNA PCR product was sequenced by ABI Prism 3730XL DNA sequencer (Biobasic Inc., Canada). DNA sequences were compared with the database of Gen Bank and aligned by using BioEdit 7.0.

Analysis of geosmin and MIB in culture media and water

Off-flavor analyses were conducted by headspace solid phase micro-extraction (HS-SPME) and gas chromatography–mass spectrometry

(GC/MS) (Gutierrez *et al.,* 2013). A 50/30 μm divinylbenzene/ carboxen/ polydimethylsiloxane SPME fibre (SUPELCO, USA) was extended into the headspace of sample (10 mL culture media or water) placed in a 20-mL straight-sided vial, added with sodium chloride (1.9 g) and a polytetrafluoroethylene (PTFE)-coated stirring bar and sealed with an aluminum crimp cap fitted with a pre-pierced PTFE-faced silicone septum. The sample was then heated to 65°C on a hotplate-stirrer and exposed to SPME fibre for a 12-min. adsorption period while undergoing vigorous agitation. After 12 min., the fibre was withdrawn from the sample and desorbed under a splitless mode at 230°C for 5 min. in the injection port of an HP 6890 N Network gas chromatograph equipped with a 5973 mass selective detector (Agilent Technologies, USA) operated in scanning mode. A Durabond HP-5 capillary column of 30-m length, 0.32-mm i.d. and 0.25-μm film thickness was used with helium carrier gas operated at a rate of 2.5 mL min^{-1}. The oven temperature was programed at 60 ℃ for 1 min., then increased to 220 ℃ with a rate of 15 ℃ $min.^{-1}$ and maintained at 220^{o}C for 8 min. Standard geosmin and MIB from Sigma were used for calibration.

Evaluation on the removal efficiency of geosmin and MIB by active bacteria under in vitro conditions

Isolated bacteria were inoculated in sterile yeast extract (YE) (0.1%) – peptone (0.1%) medium, tilapia aquaculture sterilized and unsterilized water, supplemented with 1 mg/l of geosmin or MIB standard (final culture concentration $1x10^8$ CFU/ml). Each treatment was performed in triplicate. Samples (10 mL) were analyzed daily to determine the concentrations of geosmin and MIB. The initial concentrations of geosmin and MIB in the aqueous phase for each treatment were analyzed immediately after the addition of fresh medium ($C_{initial}$). Samples were analyzed daily to determine the geosmin and MIB concentrations.

The removal efficiency of geosmin and MIB was calculated (%) according to Eq. 1.

$$\text{Removal efficiency} = (C_{inital} - C_t)/ C_{initial} \times 100\% \quad (1)$$

$C_{initial}$ indicates the concentration of geosmin or MIB added. C_t indicates the concentration of geosmin or MIB at the indicated time point.

During enrichment, samples were taken at 24 and 48 h. for geosmin and MIB analysis by GC/MS; triplicate analyses, bacterial enumeration, and community profiling by denaturing gradient gel electrophoresis (DGGE) of the 16S rRNA gene (in triplicate) as described previously (Hoefel *et al.*, 2006) to identify bacteria that became predominant during the enrichment culture.

Data analysis

Analysis of variance (ANOVA) was used to test for difference between means of observed parameters and each treatment. Duncan Multiple Range Test (DMRT) at 95% confidence level was used to analyze the geosmin and MIB concentrations in the aqueous phase of samples with media, the Tilapia aquaculture sterilized as well as those with the unsterilized water.

Results

Preliminary screening of effective bacteria

According to the study to eliminate musty odor, water samples were isolated in the YE medium with addition of geosmin and MIB. Removal efficiency of geosmin and MIB were tested.

Forty four bacterial isolates were picked up. Then, hemolysis of blood was tested by using blood agar with 5% sheep blood. Ten isolates of β-hemolysis and 11 isolates of α-hemolysis were observed. β-hemolysis is a complete lysis of red blood cells in blood agar around and under the colonies: the area appears lightened (yellow) and transparent. When α-hemolysis is present, the agar under the colony is dark or greenish. Some bacterial strains can penetrate into the fish body and blood circulation system. They can decompose red blood cells and fish will die. Therefore, it is necessary to remove these bacteria.

Effective of off-flavor degradation in enrichment culture

Twenty isolates from preliminary screening were tested for off-flavor degradation efficiency. They were cultured in YE medium with 1.00 ppm of geosmin and MIB standard. Samples were periodically taken for geosmin and MIB analysis by GC/MS. Geosmin and MIB degradation efficiencies were shown in Table 1. After 48 h., isolate TS1 had the highest efficiency to reduce geosmin in YE medium (92.16%), followed by PS1 (91.08%) and LY6 (90.96%), respectively. Isolate LS6 had the highest efficiency to reduce MIB in YE medium (76.61%), followed by PS1 (74.78%) and AY4 (73.48%), respectively.

In vivo test in Nile tilapia

Five bacterial isolates which had high efficiency to remove off-flavor: LS6, TS1, PS1, LS4 and KY3, were tested for pathogenicity. The survival rate of LS6, TS1, PS1, LS4, KY3 and control (normal saline injection) were 100.00, 100.00, 96.67, 96.67, 96.67 and 96.67%,

respectively, while the survival rate of positive control (*Aeromonas hydrophila*: AE32) was 70% (Figure 1). From these results, all the five bacterial isolates could not kill fish. The fish death occurred due to injury during transport or caused by the bite of the fish themselves not by infectious pathogens.

Table 1. Geosmin and MIB removal efficiency in YE medium

Isolates	% Geosmin		% MIB	
	24 h.	48 h.	24 h.	48 h.
Control	26.88 ±9.30^{f}	38.79 ±6.53^{j}	21.58 ±9.37^{fg}	27.26 ±5.58^{g}
AY1	82.69 ±1.35^{ab}	87.98 ±3.84^{abc}	45.00 ±0.83^{de}	54.38 ±4.10^{ef}
AY4	65.54 ±1.47^{cd}	81.66 ±2.05^{cde}	49.57 ±1.29^{d}	73.48 ±1.70^{ab}
BS2	85.09 ±2.73^{ab}	73.83 ±3.75^{efg}	49.49 ±0.71^{d}	61.61 ±4.27^{cde}
BY3	49.71 ±3.55^{e}	67.05 ±2.76^{hi}	42.48 ±1.80^{de}	60.79 ±2.00^{cde}
KS3	78.76 ±2.39^{ab}	75.00 ±0.80^{def}	39.68 ±4.10^{d}	69.81 ±0.99^{cde}
KY3	46.72 ±3.28^{e}	76.92 ±0.54^{def}	38.59 ±3.91^{de}	69.97 ±0.70^{abc}
KY4	52.40 ±5.06^{e}	77.00 ±0.28^{def}	49.04 ±3.22^{de}	60.82 ±1.06^{abc}
LS1	54.09 ±4.34^{de}	69.10 ±1.50^{fgh}	31.75 ±4.17^{ef}	60.54 ±3.10^{cde}
LS2	80.23 ±1.86^{ab}	80.15 ±3.89^{cde}	49.04 ±2.82^{d}	56.81 ±5.39^{def}
LS3	87.34 ±1.15^{a}	67.76 ±1.73^{ghi}	51.18 ±3.38^{cd}	59.87 ±4.37^{cde}
LS4	82.68 ±0.92^{ab}	87.75 ±1.14^{abc}	64.88 ±3.94^{abc}	70.30 ±4.35^{abc}
LS6	81.05 ±0.94^{ab}	87.61 ±1.96^{abc}	67.65 ±7.20^{a}	76.61 ±2.21^{a}
LY2	76.48 ±1.82^{abc}	77.52 ±2.04^{def}	66.07 ±3.26^{ab}	65.43 ±1.91^{bcd}
LY3	45.08 ±2.43^{e}	75.54 ±2.71^{def}	29.20 ±4.84^{efg}	67.33 ±3.94^{abc}
LY4	48.35 ±0.58^{e}	63.45 ±1.78^{i}	16.28 ±0.49^{e}	50.02 ±1.01^{f}
LY6	74.85 ±2.53^{abc}	90.96 ±3.19^{ab}	40.45 ±1.57^{de}	59.91 ±3.39^{cde}
NY4	55.00 ±11.33^{de}	75.26 ±1.88^{def}	30.67 ±13.48^{efg}	67.10 ±3.18^{abc}
PS1	85.80 ±1.31^{ab}	91.08 ±0.64^{ab}	52.06 ±2.00^{bcd}	57.65 ±3.36^{def}
TS1	82.57 ±1.54^{ab}	92.16 ±1.17^{a}	44.36 ±1.85^{de}	74.78 ±2.01^{ab}
TY1	73.63 ±0.45^{bc}	83.54 ±0.64^{bcd}	42.22 ±1.22^{de}	62.73 ±1.02^{cde}

*Values in the same column with different superscriptions are significantly different ($p<0.05$)

Bacterial identification

16SrDNA gene analysis of 5 bacterial isolates were found. Approximately 1,400 bp of 16SrDNA nucleotide sequences were analyzed. TS1 isolate was similar to *Achromobacter denitrificans* SM21 (99% similarity) while, KY3, PS1, LS6 and LS4 were similar to *Delftia*

tsuruhatensis (100% similarity), *Comamonas* sp. 6.6 (99% similarity), *Acinetobacter calcoaceticus* B40 (99% similarity) and *Raoultella ornithinolytica* B18 (99% similarity), respectively.

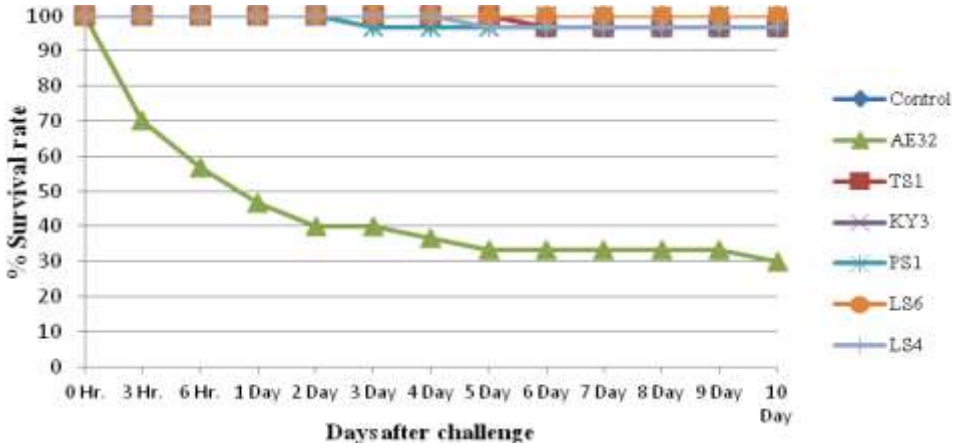

Figure 1. Average cumulative survival rate of Nile tilapia

Geosmin and MIB analysis of Nile Tilapia pond water

Geosmin and MIB in sterilized Tilapia aquaculture water were analyzed by GC/MS. After 48 h, geosmin removal efficiency of *Ach. denitrificans* increased to 82.31 ±0.90%, followed by *Comamonas* sp. (67.44 ±0.46 %) and *D. tsuruhatensis* (66.96±0.38%). MIB removal efficiency of *Ach. denitrificans* increased to 85.00 ±0.19 %, followed by *D. tsuruhatensis* (72.15 ±0.94 %) and *Comamonas* sp. (64.83 ±1.08 %) (Table 2).

Table 2. Geosmin and MIB removal efficiency in sterilized tilapia aquaculture water

	% Geosmin removal		**% MIB removal**	
	24 h.	**48 h.**	**24 h.**	**48 h.**
Control	6.90 ± 3.34 ^{dA}	10.70 ± 3.59 ^{dA}	15.96 ± 6.12 ^{eA}	18.58 ± 6.12 ^{fA}
Ach. denitrificans	72.00 ± 4.65 ^{aB}	82.31 ± 0.90 ^{aA}	81.88 ± 0.19 ^{aB}	85.00 ± 0.19 ^{aA}
D. tsuruhatensis	62.91 ± 0.32 ^{bB}	66.96 ± 0.38 ^{bA}	60.56 ± 0.94 ^{bB}	72.15 ± 0.94 ^{bA}
Comamonas sp.	63.29 ± 4.17 ^{bA}	67.44 ± 0.46 ^{bA}	43.19 ± 1.08 ^{cB}	64.83 ± 1.08 ^{cA}
Aci. calcoaceticus	40.53 ± 1.31 ^{cA}	41.59 ± 1.49 ^{cA}	25.37 ± 2.70 ^{dB}	32.14 ± 2.70 ^{dA}
R. ornithinolytica	40.29 ± 0.99 ^{cB}	44.55 ± 0.81 ^{cA}	21.52 ± 1.35 ^{dA}	24.19 ± 1.35 ^{eA}

*Values in the same column with different superscriptions are significantly different ($p<0.05$)

The off-flavor removal efficiency of non-sterile tilapia cultured water was carried out in order to determine the effectiveness of the 5 bacterial species competed with the existing species in fish culture pond. The effectiveness of geosmin and MIB degradation in Tilapia aquaculture water (non-sterilized water) were analyzed during 168 h. Their efficiencies were shown in Tables 3 and 4. After 168 h, geosmin removal efficiency of all cultures was very high (>80%), also with MIB removal efficiency, above 77 % in pond water decreased. *Comamonas* sp. had the highest geosmin and MIB removal efficacy (83.57 ±0.18 and 79.99 ±1.23%).

Table 3. Geosmin removal efficiency in tilapia aquaculture water (non-sterilized water)

	% Geosmin removal			
	24 h.	**48 h.**	**96 h.**	**168 h.**
Control	6.40 ± 0.55 ^{cD}	19.73 ± 0.16 ^{bC}	55.10 ± 2.45 ^{bB}	68.97 ± 1.99 ^{bA}
Ach. denitrificans	29.58 ± 4.78 ^{aD}	39.44 ± 4.78 ^{aC}	70.22 ± 2.12 ^{aB}	81.78 ± 1.87 ^{aA}
Aci. calcoaceticus	16.53 ± 4.18 ^{bD}	33.36 ± 4.92 ^{aC}	71.76 ± 2.46 ^{aB}	83.49 ± 2.84 ^{aA}
Comamonas sp.	29.91 ± 2.72 ^{aD}	38.73 ± 4.56 ^{aC}	68.21 ± 2.41 ^{aB}	83.57 ± 0.18 ^{aA}
D. tsuruhatensis	17.95 ± 4.54 ^{bD}	37.84 ± 4.54 ^{aC}	67.84 ± 1.56 ^{aB}	82.80 ± 2.40 ^{aA}
R. ornithinolytica	25.86 ± 0.84 ^{aC}	30.31 ± 4.79 ^{aC}	69.22 ± 1.09 ^{aB}	81.15 ± 1.38 ^{aA}

*Values in the same column with different superscriptions are significantly different ($p<0.05$)

Table 4. MIB removal efficiency in tilapia aquaculture water (non-sterilized water)

	% MIB removal			
	24 h.	**48 h.**	**96 h.**	**168 h.**
Control	8.55 ± 0.06 ^{cD}	24.74 ± 2.86 ^{aC}	45.25 ± 2.30 ^{dB}	65.32 ± 0.66 ^{cA}
Ach. denitrificans	23.20 ± 4.93 ^{aD}	33.44 ± 2.54 ^{aC}	68.29 ± 0.94 ^{aB}	79.61 ± 1.62 ^{aA}
Aci. calcoaceticus	8.03 ± 3.15 ^{bcD}	27.93 ± 1.88 ^{aC}	66.04 ± 2.64 ^{abB}	78.12 ± 0.63 ^{abA}
Comamonas sp.	26.88 ± 3.04 ^{aC}	30.23 ± 4.06 ^{aC}	63.97 ± 2.43 ^{bcB}	79.99 ± 1.23 ^{aA}
D. tsuruhatensis	20.24 ± 3.95 ^{abD}	31.02 ± 4.71 ^{aC}	61.07 ± 0.79 ^{cB}	77.18 ± 0.98 ^{bA}
R. ornithinolytica	21.00 ± 2.21 ^{abD}	33.34 ± 1.36 ^{aC}	63.56 ± 1.51 ^{bcB}	79.79 ± 1.35 ^{aA}

*Values in the same column with different superscriptions are significantly different ($p<0.05$)

Examination of the microbial population in the water sample by using 16S rRNA-directed PCR-DGGE, the majority of the microbial population in the water was uncultured bacterial species and *Methylophilus* sp. at 24 h of cultivation, *Aci. calcoaceticus*, *D. tsuruhatensis*, *Ach. denitrificans* and *R. ornithinolytica* were less than the uncultured bacterium

clone E70, except strain *Comamonas* sp. which can thrive with other microorganisms in tilapia culture ponds. *Comamonas* sp. slightly increased when incubated for 24 h (DNA strands on 5-7 lanes). After 48 h, the highest amount of natural microorganisms occurred, while *Comamonas* sp. decreased (Figure 2).

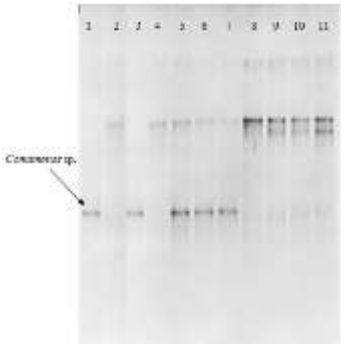

Figure 2. DGGE profile of *Comamonas* sp. cultivation in tilapia aquaculture pond, Lane 1 = *Comamonas* sp. (pure culture), Lane 2 =Control (non- added *Comamonas* sp.; initial time 0 h) , Lane 3 = *Comamonas* sp. (initial time 0 h), Lane 4 = Control (24h), Lane 5 – 7 = *Comamonas* sp. (24 h) , Lane 6-8 = Control (48 h), Lane 9 – 11 = *Comamonas* sp. (48 h)

The DGGE pattern is similar to that of Hoefel *et al.* (2006) who reported the predominance of bacteria from a biologically active sand filter column, *Sphingopyxis alaskensis*, *Novosphingobium stygiae* and *Pseudomonas veronii*, based on 16S rRNA gene sequences detected by DGGE. Subsequent isolation of these organisms revealed that degradation of geosmin, when present as either the sole carbon source (ranged from 40 ng/l to 20 mg/l) or when spiked into sterile reservoir water (37 and 131 ng/l), occurred only when all the three species were present. None of the isolates was shown to be capable of degrading geosmin either individually or in any combination of two species. However, the further work will apply the test with biofilm bioreactor, and also use within aquaculture ponds. Application of the usable microbial product will be studied.

Discussion

Infection of *Staphylococcus* was tested in mannitol salt agar (MSA). This medium distinguishes pathogenic bacteria by encouraging the growth of a group of certain bacteria while inhibiting the growth of others (Bachoon and Dustman, 2008). Two isolates could grow on MSA and produced yellow colonies with yellow zones. They could be defined as *Staphylococcus aureus*, whereas other Staphylococci produce small pink or red colonies with no color change of the medium. It is used for the selective isolation of presumptive pathogen i.e. Staphylococci. Staphylococcal food

poisoning is one of the most prevalent causes of gastroenteritis worldwide, which is caused by the ingestion of food that contains pre-formed toxins (Jablonski and Bohach, 2001). Studies have shown that one of the most common types of food intoxication is caused by certain staphylococcus strains, mainly *Staphylococcus aureus* (Jablonski and Bohach, 2001 and Simon and Sanjeev, 2007).

All species are found in water (fresh and marine) and soils (Spear *et al*., 1988; Khajuria *et al*., 2013; Aundhakar *et al*., 2014). KY3 is similar to *Delftia tsuruhatensis* (100% similarity) found in natural water sources and sludge collected from community wastewater treatment plants in Japan (Shigematsu *et al*., 2003). PS1 is similar to *Comamonas* sp. (99% similarity), found in ponds and wastewater treatment plants. It was isolated from effluent from palm oil extraction plant (Zakaria *et al*., 2008).

Hoefel *et al.* (2006) reported that geosmin-degrading bacteria from a biologically active sand filter column have high efficiency to degrade more than 70% geosmin within 48 h., because of the biofilm inside the sand filter column. Persson *et al*. (2007) used granular activated carbon (GAC) and crushed expanded clay (EC) as filter media in biofiltration and found that both GAC and EC removed geosmin and MIB at low concentration (20 ng/l) by at least 97% at an empty bed contact time of 30 minutes and at 15 °C.

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