Indian Journal of Geo Marine Sciences
Vol. 46 (01), January 2017, pp. 192-195

# Isolation and cellular fatty acid profile analyzation of two marine bioluminescent bacteria

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*Received 11 November 2014; revised 28 November 2014*

Two luminescent bacterial strains KOOS1and KOOS2 isolated from surface mucus of *Octopus* sp. collected from Andaman were identified by their cellular fatty acid composition analyzation with the help of Microbial Identification system (MIDI). SIM indexes obtained for these isolated strains were 0.772 (KOOS1) and 0.754 (KOOS2) respectively and were identified as *Photobacterium damselae* and *Vibrio fischeri*. Major fatty acids found in *Photobacterium damselae* were Saturated: Dodecanoic acid ($C_{12:0}$), Tetradecanoic acid ($C_{14:0}$), Pentadecanoic acid ($C_{15:0}$), Hexadecanoic acid ($C_{16:0}$), Heptadecanoic acid ($C_{17:0}$) and Octadecanoic acid ($C_{18:0}$); and Unsaturated: 3-hydroxy-9-methyl decanoic acid ($C_{11:0}$iso 3OH), 3-hydroxydodecanoic($C_{12:0}$ 3OH), $C_{16:1}\omega5c$, Oleic acid ($C_{18:1}\omega9c$) and $C_{18:1}\omega5c$.In *Vibrio fischeri* Saturated: $C_{12:0}$, Tridecanoic acid ($C_{13:0}$), $C_{15:0}$, $C_{16:0}$, $C_{17:0}$ and $C_{18:0}$; and Unsaturated: $C_{11:0}$iso 3OH,2-hydroxydodecanoic ($C_{12:0}$ 2OH), $C_{12:0}$3OH, $C_{13:0}$iso, $C_{14:0}$iso, $C_{15:0}$iso, $C_{15:0}$anteiso, $C_{16:0}$iso, $C_{17:0}$iso, $C_{16:1}\omega5c$, $C_{15:0}$iso3OH, $C_{17:1}\omega8c$ and $C_{17:1}\omega6c$ were found. Cyclopropane acids have not been detected in both *Photobacterium damselae* and *Vibrio fischeri*.

**[Keywords:** Luminescent bacteria, fatty acids]

## Introduction

Fatty acids are small organic molecules mostly present in cell wall composition, which contain major lipid elements of lipid A, core polysaccharide and an O polysaccharide[1].These fatty acids play an important role in physiological activities andalso help to distinguish the microorganisms based on their fatty acid composition[2]. The major fatty acids found in luminescent bacteria are hexadecenoic, hexadecanoic and octadecenoic acids[3], while some luminescent bacteriastore fatty acids such as poly-β-hydroxybutyrate[4]. Certain fatty acids assist luminescent bacteria to produce luminescence[5,6,7]. The emission of luminescence in *Vibrio salmonicida* was found when exposed to either an aliphatic aldehyde or an autoinducer N-(3-oxo-hexanoyl)-L-homoserine lactone of *Vibrio fischeri*[8].

Despite their role in physiological activities, they are important in characterizing microorganisms, detection of infectious markers and antimicrobial resistance measurement[9]. Earlier studies on the classification, extraction and identification of lipids of different bacteria have showed the importance of lipid analysis[10,11,12]. *Vibrio harveyi*, *V. fischeri* and *Photobacterium damselae* are well-known marine pathogenic luminous bacteria, however paucity of reports on fatty acid profiles of these luminous bacteria has led us to evaluate and identify them based on their cellular fatty acid compounds.

## Materials and Methods

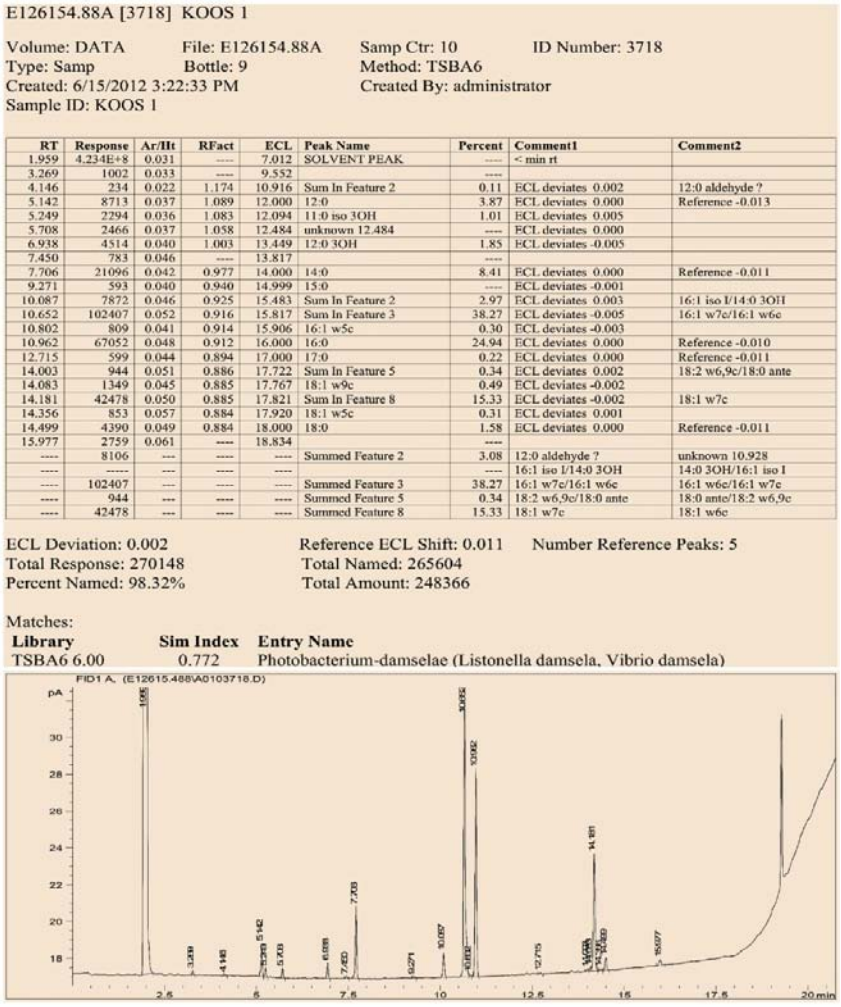
Two bacterial strains (KOOS1 and KOOS2) were isolated fromthe animal *Octopus*sp. that was collected from Kodiyaghat, South Andaman.Collected animal was washed thoroughly with sterile seawater, and its surface was swabbed with sterile cotton bud and it was spread evenly onto the plate containing Luminescent agar media (LA)[13] and incubated at 35$^{o}$C for 24hours.After the incubation period plate was observed in dark room and colonies with high luminescence intensity were picked up with sterile toothpicks. Isolated colonies were restreaked on LA to obtain pure colonies for cellular fatty acid analyzation.

Preparation of fatty acid methyl esters (FAME) from these luminous strains was

performed according to Sasser (1990). Gas Chromatography (GC) analysis was done with Agilent technologies, model 6890N networkwith flame ionisation detector (FID) and high resolution gas chromatography column (Agilent Technologies) capillary with sizes 25m × 200µm × 0.33µm was used in this study. Each sample was maintained for 21 minutes with an injector temperature at 170°C and a detector temperature at 310°C. Carrier gas used was Hydrogen with a flow rate of 30µl/min. Sample size used for GC analysis was 2µl, with a split ratio about 100:1. Quantification and identification of fatty acid methyl esters (FAME) peaks were done witha reporting integrator model Sherlock Microbial Identification System (library: TSBA6;version 6.0B).

## Results and Discussion

The saturated and unsaturated fatty acids found in *Photobacterium damselae*were $C_{12:0}$, $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, $C_{17:0}$, $C_{18:0}$ (Saturated), $C_{11:0}$iso 3OH, $C_{12:0}$ 3OH, $C_{16:1}\omega5c$, $C_{18:1}\omega9c$ and $C_{18:1}\omega5c$ (Unsaturated). Straight chain acids found in *Photobacterium damselae*were $C_{12:0}$, $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, $C_{17:0}$ and$C_{18:0}$ while hydroxy acids found were $C_{11:0}$iso 3OHand$C_{12:0}$ 3OH.Both saturated and unsaturated fatty acids found in *Vibrio fischeri* were $C_{12:0}$, $C_{13:0}$, $C_{15:0}$, $C_{16:0}$, $C_{17:0}$, $C_{18:0}$ (saturated);$C_{11:0}$iso 3OH , $C_{13:0}$iso, $C_{12:0}$ 2OH , $C_{12:0}$ 3OH, $C_{14:0}$iso, $C_{15:0}$iso, $C_{15:0}$ anteiso, $C_{16:0}$iso, $C_{16:1}\omega5c$, $C_{15:0}$ iso 3OH, $C_{17:0}$iso, $C_{17:1}\omega8c$ and $C_{17:1}\omega6c$ (unsaturated). Straight chain acids found in *V. fischeri*were $C_{12:0}$, $C_{13:0}$, $C_{15:0}$, $C_{16:0}$, $C_{17:0}$, $C_{18:0}$, $C_{11:0}$iso 3OH , $C_{13:0}$iso, $C_{12:0}$ 2OH , $C_{12:0}$ 3OH, $C_{14:0}$iso, $C_{15:0}$iso, $C_{15:0}$anteiso, $C_{16:0}$iso, $C_{15:0}$iso 3OH and $C_{17:0}$iso while hydroxy acids present were $C_{11:0}$iso 3OH, $C_{12:0}$ 2OH, $C_{12:0}$ 3OH and $C_{15:0}$iso 3OH. Both the luminescent bacteria did not possess cyclopropane acids. The library matched Sim indexes, summered feature details and gas chromatograms of these strains weregiven in figures (Fig. 1 and 2).



E126154.88A [3718] KOOS 1

Volume: DATA File: E126154.88A Samp Ctr: 10 ID Number: 3718
Type: Samp Bottle: 9 Method: TSBA6
Created: 6/15/2012 3:22:33 PM Created By: administrator
Sample ID: KOOS 1

| RT | Response | Ar/Ht | RFact | ECL | Peak Name | Percent | Comment1 | Comment2 |
|---|---|---|---|---|---|---|---|---|
| 1.959 | 4.234E+8 | 0.031 | ---- | 7.012 | SOLVENT PEAK | ---- | < min rt | |
| 3.269 | 1002 | 0.033 | ---- | 9.552 | | ---- | | |
| 4.146 | 234 | 0.022 | 1.174 | 10.916 | Sum In Feature 2 | 0.11 | ECL deviates 0.002 | 12:0 aldehyde ? |
| 5.142 | 8713 | 0.037 | 1.089 | 12.000 | 12:0 | 3.87 | ECL deviates 0.000 | Reference -0.013 |
| 5.249 | 2294 | 0.036 | 1.083 | 12.094 | 11:0 iso 3OH | 1.01 | ECL deviates 0.005 | |
| 5.708 | 2466 | 0.037 | 1.058 | 12.484 | unknown 12.484 | ---- | ECL deviates 0.000 | |
| 6.938 | 4514 | 0.040 | 1.003 | 13.449 | 12:0 3OH | 1.85 | ECL deviates -0.005 | |
| 7.450 | 783 | 0.046 | ---- | 13.817 | | ---- | | |
| 7.706 | 21096 | 0.042 | 0.977 | 14.000 | 14:0 | 8.41 | ECL deviates 0.000 | Reference -0.011 |
| 9.271 | 593 | 0.040 | 0.940 | 14.999 | 15:0 | ---- | ECL deviates -0.001 | |
| 10.087 | 7872 | 0.046 | 0.925 | 15.483 | Sum In Feature 2 | 2.97 | ECL deviates 0.003 | 16:1 iso I/14:0 3OH |
| 10.652 | 102407 | 0.052 | 0.916 | 15.817 | Sum In Feature 3 | 38.27 | ECL deviates -0.005 | 16:1 w7c/16:1 w6c |
| 10.802 | 809 | 0.041 | 0.914 | 15.906 | 16:1 w5c | 0.30 | ECL deviates -0.003 | |
| 10.962 | 67052 | 0.048 | 0.912 | 16.000 | 16:0 | 24.94 | ECL deviates 0.000 | Reference -0.010 |
| 12.715 | 599 | 0.044 | 0.894 | 17.000 | 17:0 | 0.22 | ECL deviates 0.000 | Reference -0.011 |
| 14.003 | 944 | 0.051 | 0.886 | 17.722 | Sum In Feature 5 | 0.34 | ECL deviates 0.002 | 18:2 w6,9c/18:0 ante |
| 14.083 | 1349 | 0.045 | 0.885 | 17.767 | 18:1 w9c | 0.49 | ECL deviates -0.002 | |
| 14.181 | 42478 | 0.050 | 0.885 | 17.821 | Sum In Feature 8 | 15.33 | ECL deviates -0.002 | 18:1 w7c |
| 14.356 | 853 | 0.057 | 0.884 | 17.920 | 18:1 w5c | 0.31 | ECL deviates 0.001 | |
| 14.499 | 4390 | 0.049 | 0.884 | 18.000 | 18:0 | 1.58 | ECL deviates 0.000 | Reference -0.011 |
| 15.977 | 2759 | 0.061 | ---- | 18.834 | | ---- | | |
| ---- | 8106 | --- | ---- | ---- | Summed Feature 2 | 3.08 | 12:0 aldehyde ? | unknown 10.928 |
| ---- | ---- | --- | ---- | ---- | | ---- | 16:1 iso I/14:0 3OH | 14:0 3OH/16:1 iso I |
| ---- | 102407 | --- | ---- | ---- | Summed Feature 3 | 38.27 | 16:1 w7c/16:1 w6c | 16:1 w6c/16:1 w7c |
| ---- | 944 | --- | ---- | ---- | Summed Feature 5 | 0.34 | 18:2 w6,9c/18:0 ante | 18:0 ante/18:2 w6,9c |
| ---- | 42478 | --- | ---- | ---- | Summed Feature 8 | 15.33 | 18:1 w7c | 18:1 w6c |

ECL Deviation: 0.002 Reference ECL Shift: 0.011 Number Reference Peaks: 5
Total Response: 270148 Total Named: 265604
Percent Named: 98.32% Total Amount: 248366

Matches:

| Library | Sim Index | Entry Name |
|---|---|---|
| TSBA6 6.00 | 0.772 | Photobacterium-damselae (Listonella damsela, Vibrio damsela) |



Fig. 1—showing library matched Sim index, fatty acid summed feature details and Gas chromatogram of *P.damselae* (KOOS1).

Volume: DATA File: E126184.52A Samp Ctr: 3 ID Number: 3721
Type: Samp Bottle: 2 Method: TSBA6
Created: 6/18/2012 11:39:26 AM Created By: administrator
Sample ID: 1

| RT | Response | Ar/Ht | RFact | ECL | Peak Name | Percent | Comment1 | Comment2 |
|---|---|---|---|---|---|---|---|---|
| 1.968 | 4.279E+8 | 0.031 | ---- | 7.012 | SOLVENT PEAK | ---- | < min rt | |
| 2.964 | 156 | 0.020 | ---- | 8.938 | | ---- | < min rt | |
| 3.288 | 686 | 0.034 | ---- | 9.564 | | ---- | | |
| 4.164 | 713 | 0.029 | 1.169 | 10.916 | Sum In Feature 2 | 0.29 | ECL deviates 0.002 | 12:0 aldehyde ? |
| 5.163 | 11764 | 0.037 | 1.087 | 12.000 | 12:0 | 4.37 | ECL deviates 0.000 | Reference -0.006 |
| 5.270 | 2505 | 0.037 | 1.081 | 12.092 | 11:0 iso 3OH | 0.93 | ECL deviates 0.003 | |
| 5.730 | 3303 | 0.038 | 1.056 | 12.482 | unknown 12.484 | ---- | ECL deviates -0.002 | |
| 5.885 | 1667 | 0.034 | 1.048 | 12.615 | 13:0 iso | 0.60 | ECL deviates 0.001 | Reference -0.005 |
| 6.341 | 936 | 0.043 | 1.026 | 13.001 | 13:0 | 0.33 | ECL deviates 0.001 | Reference -0.004 |
| 6.597 | 384 | 0.034 | 1.016 | 13.186 | 12:0 2OH | 0.13 | ECL deviates 0.009 | |
| 6.963 | 7471 | 0.041 | 1.002 | 13.448 | 12:0 3OH | 2.56 | ECL deviates -0.006 | |
| 7.201 | 509 | 0.040 | 0.994 | 13.619 | 14:0 iso | 0.17 | ECL deviates 0.000 | Reference -0.006 |
| 7.471 | 1202 | 0.041 | ---- | 13.812 | | ---- | | |
| 7.732 | 25590 | 0.044 | 0.978 | 13.998 | 14:0 | 8.56 | ECL deviates -0.002 | Reference -0.007 |
| 8.710 | 917 | 0.039 | 0.953 | 14.623 | 15:0 iso | 0.30 | ECL deviates 0.000 | Reference -0.006 |
| 8.852 | 734 | 0.042 | 0.950 | 14.713 | 15:0 anteiso | 0.24 | ECL deviates 0.000 | Reference -0.005 |
| 9.300 | 2405 | 0.045 | 0.941 | 14.999 | 15:0 | ---- | ECL deviates -0.001 | |
| 10.114 | 12423 | 0.047 | 0.927 | 15.481 | Sum In Feature 2 | 3.94 | ECL deviates 0.001 | 16:1 iso I/14:0 3OH |
| 10.361 | 1166 | 0.047 | 0.923 | 15.627 | 16:0 iso | 0.37 | ECL deviates 0.000 | Reference -0.006 |
| 10.680 | 132019 | 0.050 | 0.918 | 15.815 | Sum In Feature 3 | 41.48 | ECL deviates -0.007 | 16:1 w7c/16:1 w6c |
| 10.833 | 909 | 0.045 | 0.916 | 15.906 | 16:1 w5c | 0.28 | ECL deviates -0.003 | |
| 10.990 | 56102 | 0.045 | 0.914 | 15.999 | 16:0 | 17.55 | ECL deviates -0.001 | Reference -0.007 |
| 11.214 | 842 | 0.042 | 0.911 | 16.127 | 15:0 iso 3OH | 0.26 | ECL deviates -0.007 | |
| 12.101 | 865 | 0.041 | 0.902 | 16.633 | 17:0 iso | 0.27 | ECL deviates 0.003 | Reference -0.004 |
| 12.378 | 2732 | 0.048 | 0.900 | 16.790 | 17:1 w8c | 0.84 | ECL deviates -0.002 | |
| 12.505 | 1207 | 0.052 | 0.899 | 16.863 | 17:1 w6c | 0.37 | ECL deviates 0.003 | |
| 12.746 | 1815 | 0.046 | 0.897 | 17.000 | 17:0 | 0.56 | ECL deviates 0.000 | Reference -0.007 |
| 14.210 | 49356 | 0.052 | 0.887 | 17.821 | Sum In Feature 8 | 14.99 | ECL deviates -0.002 | 18:1 w7c |
| 14.528 | 2014 | 0.045 | 0.886 | 17.999 | 18:0 | 0.61 | ECL deviates -0.001 | Reference -0.008 |
| 15.995 | 2505 | 0.063 | ---- | 18.828 | | ---- | | |
| ---- | 13136 | --- | ---- | ---- | Summed Feature 2 | 4.23 | 12:0 aldehyde ? | unknown 10.928 |
| ---- | ----- | --- | ---- | ---- | | ---- | 16:1 iso I/14:0 3OH | 14:0 3OH/16:1 iso I |
| ---- | 132019 | --- | ---- | ---- | Summed Feature 3 | 41.48 | 16:1 w7c/16:1 w6c | 16:1 w6c/16:1 w7c |
| ---- | 49356 | --- | ---- | ---- | Summed Feature 8 | 14.99 | 18:1 w7c | 18:1 w6c |

ECL Deviation: 0.003 Reference ECL Shift: 0.006 Number Reference Peaks: 12
Total Response: 319034 Total Named: 314641
Percent Named: 98.62% Total Amount: 298029

Matches:

| Library | Sim Index | Entry Name |
|---|---|---|
| TSBA6 6.00 | 0.754 | Vibrio-fischeri |

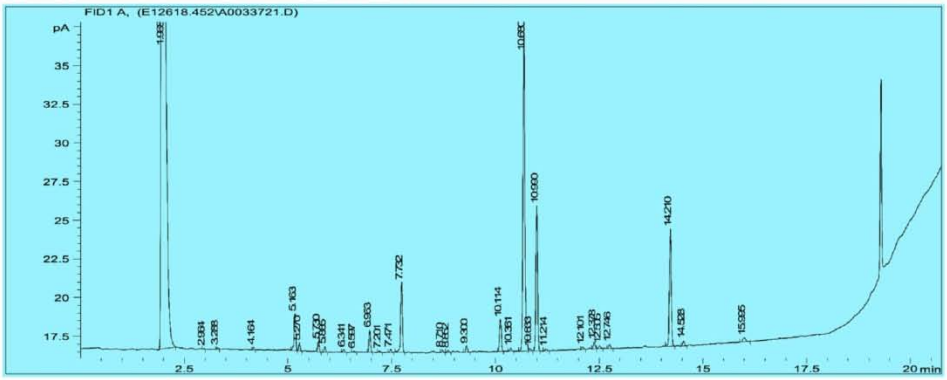



Fig. 2—Showing library matched Sim index, fatty acid summed feature details and Gas chromatogram of *V. fischeri* (KOOS2).

Fatty acids such as $C_{13:0}$ (Saturated); $C_{13:0}$iso, $C_{14:0}$iso, $C_{15:0}$iso, $C_{15:0}$anteiso, $C_{16:0}$ iso, $C_{12:0}$ 2OH, $C_{15:0}$ iso 3OH, $C_{17:0}$iso, $C_{17:1}\omega8c$ and $C_{17:1}\omega6c$ (Unsaturated) found in *V. fischeri* were absent in *P.damselae*, while $C_{14:0}$ found in *P.damselae* was absent in *V. fischeri*. Hydroxy acids $C_{12:0}$ 2OH and $C_{15:0}$ iso 3OHfound in *V. fischeri* were not observed in *P.damselae*. Lambert *et al*. (1983) asserted that fatty acids such as cis-11-hexadecenoic acid (C16:1) help in differentiation of luminescent *Vibrio* species as well as *Photobacterium* species. However such fatty acids have not been detected in both the strains, while$C_{14:0}$, $C_{18:1}\omega9c$ and $C_{18:1}\omega5c$ in *P. damselae* and $C_{12:0}$ 2OH, $C_{13:0}$, $C_{13:0}$iso, $C_{14:0}$iso, $C_{15:0}$iso, $C_{15:0}$anteiso, $C_{16:0}$iso, $C_{17:0}$iso, $C_{15:0}$iso 3OH, $C_{17:1}$ $\omega8c$ and $C_{17:1}\omega6c$ in *V. fischeri* distinguished each other as separate bacterial species.

## Conclusion

It is inferred that FAME analysis has clearly differentiated the luminous bacterial species and it would be helpful in taxonomical discrimination.

**Acknowledgements**

Authors thank Dr. Shanta Achuthankutty, Chief Scientist and Dr. Anas Abdulaziz, Scientist for the laboratory facilities of Marine Microbial Reference Facility of NIO RC,Kochi.The first author grateful to the Department of Science and Technology for the INSPIRE fellowship.

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