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

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Two luminescent bacterial strains KOOS1 and 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 (C12:0), Tetradecanoic acid (C14:0), Pentadecanoic acid (C15:0), Hexadecanoic acid (C16:0), Heptadecanoic acid (C17:0) and Octadecanoic acid (C18:0); and Unsaturated: 3-hydroxy-9-methyl decanoic acid (C11:0 isoo 3OH), 3-hydroxydodecanoic(C12:0 3OH), 3-hydroxydodecanoic(C12:0 3OH), C16:0iso, Oleic acid (C18:1 ω9c) and C18:0iso. In Vibrio fischeri Saturated: C12:0, Tridecanoic acid (C13:0), C15:0, C16:0, C17:0 and C18:0 and Unsaturated: C11:0 iso 3OH, 2-hydroxydodecanoic (C12:0 2OH), C12:0, C14:0iso, C15:0iso, C15:0anteiso, C16:0iso, C17:0iso, C16:0iso, C15:0iso3OH, C17:1 ω8c and C17:1 ω6c 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. These fatty acids play an important role in physiological activities and also help to distinguish the microorganisms based on their fatty acid composition. The major fatty acids found in luminescent bacteria are hexadecenoic, hexadecanoic and octadecenoic acids, while some luminescent bacteriastore fatty acids such as poly-β-hydroxybutyrate. Certain fatty acids assist luminescent bacteria to produce luminescence. 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. Despite their role in physiological activities, they are important in characterizing microorganisms, detection of infectious markers and antimicrobial resistance measurement. Earlier studies on the classification, extraction and identification of lipids of different bacteria have showed the importance of lipid analysis. 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 from the 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) and incubated at 35°C for 24 hours. 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

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performed according to Sasser (1990). Gas Chromatography (GC) analysis was done with Agilent technologies, model 6890N network with 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 with 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 C12:0, C14:0, C15:0, C16:0, C17:0, C18:0 (Saturated), C11:0iso 3OH, C12:0 3OH, C16:1ω5c, C18:1ω9c and C18:1ω5c (Unsaturated). Straight chain acids found in Photobacterium damselae were C12:0, C14:0, C15:0, C16:0, C17:0, C18:0 while hydroxy acids found were C11:0iso 3OH, C12:0 2OH, C14:0, C16:0iso, C16:1ω5c, C17:0iso and C17:1ω6c. Both saturated and unsaturated fatty acids found in Vibrio fischeri were C12:0, C13:0iso, C15:0, C16:0, C17:0, C18:0iso, C13:0iso, C14:0iso, C15:0iso, C16:0iso, C16:1ω5c, C17:0iso, C17:1ω6c (unsaturated). Straight chain acids found in V. fischeri were C12:0, C13:0iso, C15:0, C16:0, C17:0, C18:0iso, C13:0iso, C14:0iso, C15:0iso, C16:0iso, C16:1ω5c, C17:0iso while hydroxy acids present were C11:0iso 3OH, C12:0 2OH, C12:0 3OH and C15:0iso 3OH. Both the luminescent bacteria did not possess cyclopropane acids. The library matched Sim indexes, summed feature details and gas chromatograms of these strains were given in figures (Fig. 1 and 2).

Fig. 1—showing library matched Sim index, fatty acid summed feature details and Gas chromatogram of P.damselae (KOOS1).
Fatty acids such as C_{13:0} (Saturated); C_{13:0iso}, C_{14:0}, C_{15:0iso}, C_{15:0anteiso}, C_{16:0iso}, C_{17:0} 2OH, C_{15:0iso} 3OH, C_{17:0iso}, C_{17:1} \omega8c and C_{17:1} \omega6c (Unsaturated) found in *V. fischeri* were absent in *P. damsela*, while C_{14:0} found in *P. damsela* was absent in *V. fischeri*. Hydroxy acids C_{12:0} 2OH and C_{15:0 iso} 3OH found in *V. fischeri* were not observed in *P. damsela*. 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. damsela* and C_{12:0} 2OH, C_{13:0}, C_{14:0}, C_{15:0iso}, C_{15:0anteiso}, C_{16:0iso}, C_{17:0iso}, C_{15:0iso} 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.
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References