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ISOLATION AND SCREENING OF INDIGENOUS MICROORGANISMS STRAINS FROM FATTY ACIDS METHYL ESTER ANALYSIS (FAME) PROFILES FOR COMPOST PRODUCTION

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The present study was conducted to identify the indigenous bacteria isolated from various indigenous microorganisms (IMO) sources using phenotypic methods (FAMEs). Data of fatty acid analysis showed the presence of 30 different fatty acids in 18 bacterial strains examined in the study. A 12:0 fatty acid was present in all strains, while 17:0 cyclo, 17:0 and 19:0cyclo w8c were only present in T1 stain and 12:0 2OH, 12:0 3OH, 17:1 anteiso in T2 strain from kitchen waste (KW) source. Bacterial strains of T6, T8, T10, T12, T13, T15, T17, and T19 had similar fatty acid profiles which contain 12:0, 15:0 iso, 16:0 iso and 16:0 with concentrations between 2.63 and 43.13%. From the 18 strains, only five are classified as beneficial strains mainly; *B. cereus*, *B. sphaericus*, *B. megaterium*, *Acinetobacter calcoaceticus* and *Microbacteriumbarkeri* from different IMO sources. The quantitative data from FAMEs profiles are highly reproducible showing that FAMEs are very useful in bacterial species identification.

Keywords:
FAME, bacteria identification, indigenous microorganisms (IMO)

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ABSTRACT

Microorganism is one of the important factors that controlled composting processes and commonly effective microorganisms (EM) is used to promote compost quality. However, low income farmers generally have difficulties in using it due to its availability and cost. Thus identification of local microbial sources which are cheap and sensible is needed for the production of compost. The present study was conducted to identify the indigenous bacteria isolated from three sources (forest under bamboo tree, kitchen waste, and aerated fish pond water) using phenotypic methods (FAMEs). Data of fatty acid analysis showed the presence of 30 different fatty acids in 18 bacterial strains examined in the study. A 12:0 fatty acid was present in all strains, while 17:0 cyclo, 17:0 and 19:0cyclo w8c were only present in T1 stain and 12:0 2OH, 12:0 3OH, 17:1 anteiso in T2 strain from kitchen waste (KW) source. Bacterial strains of T4,T5, T6, T7, T8, T10, T12, T13, T14, T15, T17, T18 and T19 had similar fatty acid profiles which contain 12:0, 15:0 iso, 16:0 iso and 16:0 with concentrations between 2.63 and 43.13%. From the 18 strains, only five are classified as beneficial strains mainly; *B. cereus*, *B. sphaericus*, *B. megaterium*, *Acinetobacter calcoaceticus* and *Microbacterium barkeri* from different IMO sources. The quantitative data from FAMEs profiles are highly reproducible showing that FAMEs are very useful in bacterial species identification

Key words: FAMEs, bacteria identification, indigenous microorganisms (IMO)

1. INTRODUCTION

According to Slabbinck *et al.* (2009), more than 300 fatty acids have been identified in bacteria but different in chain length, positions of double bonds and the binding of functional groups. The useful taxonomy markers by chemotaxonomic technique, gas chromatographic whole-cell fatty acid methyl ester (FAME) analysis with commercial identification systems, Sherlock Microbial Identification System (MIS, Microbial ID Inc. (MIDI), Newark, DE, USA) is a standard method for identification of microorganisms (Yousef *et al.*, 2012; Slabbinck *et al.*, 2009; Buyer, 2006). Buyer (2006) and Buyer (2002a,b) reported that the entire fatty acid cells are converted to methyl esters and analyzed by gas chromatography (GC), and the fatty acid composition of the unknown is compared to a library of known organisms in order to find the closest match. Yousef *et al.* (2012) reported the FAME analysis using GC to distinguish among different microorganisms through an overall analysis of the types of fatty acids present in the samples and the fatty acids composition in organisms which

may vary due to environmental variables such as growth substrate, incubation time, and incubation temperature.

According to Yousef *et al.* (2012), fatty acids profiling is a more cost-effective, rapid, and less technically difficult method compared to molecular methods. Lipids are major cellular components of all living organisms, and include a wide range of structurally and functionally diverse fatty acids (Zelles 1999a,b; Macnaughton *et al.*, 1997; Welch, 1991). The variation in fatty acid composition among microorganisms creates a unique lipid fingerprint for each organism, which can identify organisms in pure culture (Miller *et al.*, 1999; Zelles 1999b; Welch 1991). Yousef *et al.* (2012) mentioned that fatty acid compositions have been widely applied in soil microbial ecology studies to: (i) determine structures of functional microbial groups in soil (Nazih *et al.*, 2001; Schutter and Dick, 2000); (ii) to characterize, differentiate and identify genera, species and strains of bacteria in pure culture (Cox *et al.*, 2006; El Manyawi *et al.*, 2000; Stahl and Klug, 1999); (iii) measure the response of specific groups of microorganisms to soil management practices (Frostegaard *et al.*, 1993). In part the aims of this study were (1) to determine the reliability of the MIDI Microbial Identification System as a means of identifying unknown bacteria isolated from different IMO sources and (2) to evaluate the feasibility of this approach for identification of large numbers of natural isolates.

2. MATERIALS AND METHODS

Bacterial strains from different sources

An isolate from three sites, i) undisturbed area under bamboo trees collected using steamed white rice (SWR), ii) aerated fish pond water (AFPW) from UPM fish pond and iii) kitchen wastes (KW) from UPM food stores were used as indigenous microorganisms (IMO) sources. The growth media used for the experiments were, i) nutrient agar and ii) trypticase soy agar. Cultures were routinely maintained on trypticase soy agar at 28°C.

Analysis of cellular FAME

Whole cell fatty acids of bacterial strains were extracted and analysed as methyl ester derivatives (FAME) according to the method described by the manufacturer's manual (Sherlock Microbial Identification System version 4.0, MIDI, Inc., Newark, DE, USA) (Miller and Berger, 1985; Roy, 1988). Bacterial strains were streaked on trypticase soy agar at 28°C. After 24 hours of growth, on average 4 mm (approx. 40 mg) bacterial cells were harvested in the overlapping region of third quadrant of the streaked plates as this area typically yields the most stable fatty acid compositions since the inoculums have been diluted enough to result in abundant growth of colonies without limiting nutrient supply.

The bacterial cells were transferred into clean 13 mm x 100 mm culture tubes with Teflon-coated screw caps containing 1 mL of saponification reagent (3.25 M NaOH in methanol). The tubes were then vortexed and placed in a 100°C water bath for 30 min and mixed every 10 min. The samples were cooled for 10 min in a cold-water bath, and methylated by adding 2 mL of methylating reagent (3.25 M HCL in methanol). Subsequently, the samples were heated for 10 min in an 80°C water bath and allowed to cool to room temperature prior to the extraction of FAME using 1.3 mL of lipid extraction reagent (1:1, methyl tert-butyl ether:hexane). The samples were gently vortexed every 2 min over 10 min to mix the aqueous and organic layers, after which the top organic phase was transferred into clean culture tubes using glass pipettes and washed with 3 mL of 0.1 M NaOH. Finally, the 'cleaned' organic phase was transferred to a crimp top GC glass vial for analysis by GC. Samples that were not immediately analyzed were stored at -20°C (Sasser, 2001).

3. RESULTS AND DISCUSSION

Fatty acids analysis

From the 18 unknown bacterial isolates studied by FAME analysis (Table 1) 13 strains were Gram-positive and 5 Gram-negative. The isolated 18 bacterial were identified as *Escherichia fergusonii*, *Acinetobacter calcoaceticus*, *Microbacterium* spp., 3 strains as *M. barkeri*, *M. chocolatum*, *M. liquefaciens*, *Corynebacterium pseudodiphtheriticum*, *Bacillus* spp., 5 strains as *B. cereus*, *B. sphaericus*, *B. atrophaeus*, *B. megaterium*, *B. alcalophilus*, *Cellulosimicrobium cellulans*, *Kocuria rhizophila*, *Enterobacter hormaechei*, *Staphylococcus cohnii-cohnii*, *Aeromonas ichthiosm* and 2 strains thermophiles bacteria were *Brochothrix thermosphacta* and *Geobacillus stearothermophilus*.

The characterization studies based on FAME analysis showed that a total of 30 different FAMEs were present in 18 bacterial strains tested in the present study (Table 1). A 12:0 fatty acid (FA) appeared in all strains. However, 17:0 cyclo, 17:0 and 19:0cyclo w8c fatty acids were only present in T1 stain and 12:0 2OH, 12:0 3OH and 17:1 anteiso in T2 strain from kitchen waste (KW) source. Bacterial strains of T6, T8, T10, T12, T13, T15, T17 and T19 had similar fatty acid profiles which contained 12:0, 15:0 iso, 16:0 iso and 16:0 with the concentration between 2.63 and 43.13%. These strains were identified as *Bacillus* spp (Adiguzel *et al.*, 2009). FA profiles of T14 strain was the same as that of first group including FA 12:0, 15:0 iso, 16:0 iso and 16:0, but their concentrations were different (5.08 and 39.16%). This strain was identified as the member of the *Geobacillus*. First group strains consisted of three sources from steamed white rice (SWR), aerated fish pond water (AFPW) and kitchen waste (KW). The second group of the strains including T3, T4 and T5 had similar fatty acid profiles containing 15:0 iso, 15:0 anteiso, 16:0 iso and 17:0 anteiso at the concentration ranging between 3.98 and 42.24%.

Beneficial strains

Five beneficial strains were isolated from three different IMO sources and these are; *B. cereus*, *B. sphaericus*, *B. megaterium*, *Acinetobacter calcoaceticus* and *Microbacterium barkeri*. *B. cereus* are reported to inhibit the mycelia growth of *Fusarium oxysprorum* by 57.7%, *Scleroticum rolfsii* (68.8%), *Pythium aphanidermatum* (72.2%), *Helminthosporium maydis* (57.7%), *Maccrophomina phaseolina* (64.4%), *Rhizoctonia solani* 45.5% zones of growth inhibition (Muhammad and Amusa, 2003). Soils inoculate with *B. cereus* reduced seedling infection and that the efficiency of antagonists increased with increase in dose Lytic enzymes is known to be produced by *B. cereus* (Muhammad and Amusa, 2003). These enzymes and other antibiotics produced by *B. cereus* have been reported to have antagonistic effects on some microorganisms (Bankole and Adebanjo, 1998). Both substances, produced by *B. cereus*, may have been responsible for inhibiting the growth of the pathogens and probably play an important role in the prevention of seedling blight diseases of crops which have remained very serious constraints to maize production (Muhammad and Amusa, 2003).

Table 1. Cellular fatty acid composition (%w/w) of strains from three IMO sources

Fatty acid concentration (%)

Fatty acids	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14	T15	T16	T17	T18	T19	T20	T21
12:0	3.84	11.66	3.42	3.07	2.67	5.22	4.34	4.34	6.07	8.44	10.10	2.63	2.76	4.80	3.28	7.54	4.82	5.32	3.45	3.54	9.39
12:0 2OH	n.d.	4.43	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
12:0 3OH	n.d.	6.10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
13:0 iso	n.d.	n.d.	n.d.	n.d.	n.d.	8.39	n.d.	n.d.	n.d.	n.d.	n.d.	5.95	6.50	n.d.	n.d.	n.d.	n.d.	n.d.	11.19	n.d.	n.d.
14:0 3OH	9.44	7.49	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	8.06	n.d.	n.d.	2.77	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	12.01
14:0 iso	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	7.66	n.d.	4.04	3.08	n.d.	5.07	n.d.	n.d.	5.44	3.82	n.d.	8.43
14:0	9.66	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	6.77	8.15	n.d.	2.87	3.90	n.d.	n.d.	n.d.	4.59	5.00	4.89	n.d.	n.d.
15:0 iso	n.d.	n.d.	3.98	12.03	12.74	31.44	22.80	22.44	n.d.	16.43	n.d.	23.64	37.45	39.16	43.13	54.11	33.37	14.86	29.20	12.81	n.d.
15:0 anteiso	n.d.	n.d.	38.00	31.55	35.61	4.85	51.51	51.45	n.d.	41.95	n.d.	4.41	4.50	20.81	30.83	8.73	28.60	44.39	3.02	43.33	n.d.
16:0 3OH	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	6.18	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
16:0 w6c	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	6.75	6.67	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
16:0 w7c	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	28.26	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	38.52
16:0 w11c	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.77	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
16:0 iso	n.d.	n.d.	12.36	22.18	19.82	9.86	4.67	4.30	n.d.	9.53	n.d.	9.56	6.10	8.08	3.34	11.84	4.92	10.93	7.34	n.d.	n.d.
16:0	30.38	12.17	n.d.	3.33	2.04	7.50	3.93	4.35	19.69	7.84	23.84	8.32	7.30	5.04	5.67	n.d.	12.05	6.74	8.19	7.53	15.96
16:1 w6c	n.d.	n.d.	n.d.	n.d.	n.d.	8.00	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	7.90	n.d.	n.d.
16:1 w7c	4.66	19.02	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	9.61	n.d.	n.d.	n.d.	n.d.	n.d.
17:0 cyclo	16.86	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
17:0 iso	n.d.	n.d.	n.d.	5.38	5.39	14.23	5.36	5.54	n.d.	n.d.	n.d.	16.49	9.95	5.08	3.11	8.16	n.d.	n.d.	9.80	6.12	n.d.
17:0 anteiso	n.d.	n.d.	42.24	22.47	21.73	n.d.	7.39	7.58	n.d.	n.d.	n.d.	2.46	n.d.	17.02	2.80	n.d.	n.d.	7.31	n.d.	11.35	n.d.
17:0	2.72	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
17:1 anteiso A	n.d.	2.68	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
17:1 w8c	n.d.	6.21	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3.48
17:1 iso w5c	n.d.	n.d.	n.d.	n.d.	n.d.	5.37	n.d.	n.d.	n.d.	n.d.	n.d.	4.80	4.12	n.d.	n.d.	n.d.	n.d.	n.d.	5.95	n.d.	n.d.
17:1 iso w10c	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.46	2.46	n.d.	n.d.	n.d.	n.d.	n.d.	2.70	n.d.	n.d.
18:0	n.d.	n.d.	n.d.	n.d.	n.d.	5.13	n.d.	n.d.	n.d.	n.d.	11.35	2.86	5.21	n.d.	n.d.	n.d.	11.65	n.d.	2.55	8.14	2.80
18:1 w7c	13.00	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	24.97	n.d.	7.15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	9.42
18:1 w9c	n.d.	30.23	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	47.56	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3.50	n.d.
19:0 cyclo w8c	9.44	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
20:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3.68	n.d.

n.d. not detected.

B. sphaericus has demonstrated the only biological larvicide capable of providing residual control in highly organic environments, including sewage, waste lagoons, animal waste ponds and septic ditches. Surendran and Vennison (2011) demonstrated that *B. sphaericus* is a soil bacterium that can effectively kill mosquito larvae present in water. Application of compost that contains this strain is a useful involvement on monoculture (aquaculture and agriculture) sectors that have practices in some countries such Philippines and Indonesia. Rahul *et al.* (2011) reported that this spore-forming bacterium also has the ability to efficiently degrade oxidation of benzene, toluene, ethylbenzene and xylene isomers (BTEX), has a high capability for entirely degrading BTEX at concentration lower that 200 mg L-1. But for xlene at a concentration of 200 mg L-1, degradation is incomplete with around 90% of the xylene degraded after 72 hrs. This intimates that *B. sphaericus* has the potential to be used in biofilters for the anticipation of BTEX contaminated environments and it might be advantageous when applied these strains in compost.

Erdoğan *et al.* (2012) and Van Gestel *et al.* (2003) reported that *B. megaterium* and *Acinetobacter calcoaceticus* been used as degrading bacteria in biological method in oil-contaminated soil. Crude oil contains complex mixture of hydrocarbons and other organic compounds, including some heavy metals and metallic compounds that would be more of a concern for acute toxicity to organisms. Physical and chemical methods to reduce hydrocarbon pollution are expensive and time consuming than biological method. These bacterial also can degrade a wide range of target constituents present in oily sludge as reported by Kishore and Ashis (2007). Bioremediation activities make use of these indigenous oil-degrading microorganisms by enhancing and fertilizing them in their natural habitats as in compost. Barje *et al.* (2008) reported *Acinotobacter calcoaceticus* were abundantly at 20 to 40°C in compost pile indicating a fall in unsaturated fatty acids with temperature increase while the enxymatic unsaturated fatty acids deactivated when the temperature rose from 20 to 30°C for *B. megaterium*.

Microbacterium spp. have frequently been isolated from the soil and used as biocontrol agents (Santori *et al.*, 2012). Rau *et al.* (2009) reported *M. barkeri* is one of the species which belongs to the high GC-content and some reports also have demonstrated that *M. barkeri* strains are strong in plant colonization and play important role in biocontrol. Cottyn *et al.* (2009) isolated these bacterium strains because the potential biocontrol ability in rice seeds. It has been stressed that this bacterium genome contains a repertoire of antimicrobial, antibiotic and metabolism associated pathways and epiphytic fitness genes (Liu *et al.*, 2012).

4. CONCLUSIONS

FAMEs are good biomarkers for bacterial genus identification at species level. The most critical point of FAME analysis of bacterial strains is the amount of cell mass used. Therefore, this cell mass has to be exactly weighed out between 30 to 45 mg. In case of identification difficulties with unknown isolates, a correlation curve of cell mass with the resulting SI value might be useful in figuring out the best amount of cell mass correlated with the highest probability of good library matches. In addition, a further subculture might stabilize fatty acid formation and thus facilitate library comparison.

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