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## COMPARATIVE STUDY OF FAME AND SEQUENCE ANALYSIS FOR IDENTIFICATION OF BACTERIA FROM INDUSTRIAL WATER OF KRIBHCO

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### ABSTRACT

There have been several systems developed in the past few years for rapid microbial identification. In the present study, two methods (GC FAME analysis and 16s rDNA sequencing) were compared for identification of bacteria. The results when compared, we found that the FAME results are equally similar to that of genotypic sequencing. five bacterial samples obtained from KRIBHCO plant and were analyzed, wherein FAME analysis, the automated system identified sample 1 to be *Bacillus atrophaeus* with 0.804 SI. The genotypic analysis also showed similar results, after BLAST the organism was identified to be *Bacillus atrophaeus* with 97% identity match. In case of sample 2, FAME analysis identified the organism to be *Pseudomonas aeruginosa* with 0.765 SI and sequencing also identified the organism to be *Pseudomonas aeruginosa* with BLAST showing 98% identity.

### KEYWORDS

Bacteria, FAME, Sequencing, BLAST, Identification.

### INTRODUCTION

Accurate identification of bacterial isolates is an essential task of the clinical microbiology laboratory. For many slow-growing and fastidious organisms, traditional phenotypic identification is difficult and time-consuming. Several commercial systems offer computer-assisted identification of a wide variety of bacterial organisms. The Biolog system (Biolog, Inc., Hayward, Calif.) was evaluated for the identification of strains likely to be encountered commonly in clinical laboratories [1,2], and the gas-liquid chromatography system developed by MIDI, Inc. (Newark, Del.), based on the cellular fatty acid profile. An automated cellular fatty acid (CFA) bacterial identification system, Microbial Identification System was compared with a conventional system for the species level identification of microorganisms. MIS identifications were based exclusively on the CFA composition. The MIS, as the first automated CFA identification system, is an accurate, efficient, and relatively rapid method for the identification of microorganisms [3,4].

Genotypic identification is emerging as an alternative or complement to established phenotypic methods. During the last decade, methods based on the analysis of DNA polymorphisms were added to this list of methodologies, for instance Random Amplified Polymorphic DNA (RAPD) [5], Arbitrarily Primed PCR (AP-PCR) [6] and micro satellite-primed PCR [7]. Polymerase chain reaction-mediated direct sequence determination can also be used as a rapid and reliable method for the identification of isolates in the clinical laboratory [8, 9]. The sequence of small-subunit rRNA varies in an orderly manner across phylogenetic lines and contains segments that are conserved at the species, genus or kingdom level [10, 11]. This method should be useful for increasing the amounts of bacterial 16S ribosomal DNA sequences for the purposes of sequencing and probing. This approach may make it possible to identify new, non-culturable organisms [12, 13]. The amplified segment is sequenced and compared with known databases to identify a close relative. The method is evaluated with respect to accuracy, sensitivity to modified nucleotides in the template RNA, and phylogenetic usefulness, by examination of several 16S rRNAs whose gene sequences are known [8,14]. In the present study, two methods, such as GC-FAME analysis and 16s rDNA sequencing were compared for identification of bacteria.

### MATERIALS AND METHODS

The two cultures were obtained from soil sample and pure cultured onto Trypticase soya broth agar (TSBA) media at 28°C for 24 hours. The fatty acids were extracted and methylated to form fatty acid methyl esters (FAME). These FAME's were analyzed using Gas Chromatography (Agilent 6850 Series II) with the help of MIDI Sherlock software for FAME. Aerobic library was used. For DNA sequencing, the genomic DNA was extracted from the bacterial culture. The bioinformatics analysis was performed using NCBI BLAST (<http://ncbi.nlm.nih.gov/blast>) identifying the microorganism using online databases.

### RESULTS AND DISCUSSION

We have analyzed two microbial cultures obtained from soil using FAME and sequencing approaches. On comparing FAME results with that of sequencing, it was found that both are similar to each other. Figure 1 shows peaks corresponding to the fatty acids identified through FAME analysis of bacterial sample one. The MIDI Sherlock microbial identification system using RTSBA 6 method identified the organism to be *Bacillus-thuringiensis-kurstakii* with 0.74 SI. The sequence analysis also showed similar results (Table 1).

**TABLE 1: BACILLUS-THURINGIENSIS-KURSTAKII SEQUENCE ANALYSIS BY BLAST**

	Max score	Total score	Query cover	E value	Ident	Accession
<a href="#">Bacillus thuringiensis serovar konk</a>	3.524e+05	8.702e+06	78%	0.0	99%	<a href="#">AE017355.1</a>
<a href="#">Bacillus cereus F837/76, complete</a>	3.049e+05	8.440e+06	77%	0.0	99%	<a href="#">CP003187.1</a>
<a href="#">Bacillus cereus 03BB102, complete</a>	3.045e+05	8.527e+06	78%	0.0	99%	<a href="#">CP001407.1</a>
<a href="#">Bacillus anthracis str. H9401, comp</a>	2.403e+05	8.912e+06	80%	0.0	99%	<a href="#">CP002091.1</a>
<a href="#">Bacillus anthracis str. SVA11, compl</a>	2.403e+05	8.887e+06	80%	0.0	99%	<a href="#">CP006742.1</a>
<a href="#">Bacillus anthracis str. Sterne, comp</a>	2.403e+05	8.925e+06	80%	0.0	99%	<a href="#">AE017225.1</a>
<a href="#">Bacillus anthracis str. A0248, compl</a>	2.403e+05	8.924e+06	80%	0.0	99%	<a href="#">CP001598.1</a>
<a href="#">Bacillus anthracis str. Ames Ancest</a>	2.403e+05	8.924e+06	80%	0.0	99%	<a href="#">AE017334.2</a>
<a href="#">Bacillus anthracis str. Ames, compl</a>	2.403e+05	8.924e+06	80%	0.0	99%	<a href="#">AE016879.1</a>
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<a href="#">Bacillus anthracis str. CDC 684, cor</a>	2.402e+05	8.933e+06	80%	0.0	99%	<a href="#">CP001215.1</a>
<a href="#">Bacillus anthracis str. A16R, compl</a>	2.402e+05	8.921e+06	80%	0.0	99%	<a href="#">CP001974.1</a>
<a href="#">Bacillus cereus AH820, complete q</a>	2.313e+05	9.346e+06	82%	0.0	99%	<a href="#">CP001283.1</a>



After the completion of BLAST analysis, the organism was identified to be *Bacillus-thuringiensis-kurstakii* with 97% identity match. Figure 2 depicts the chromatogram with fatty acid peaks identified through the FAME analysis of the second bacterial sample. The automated system identified the organism to be *Pseudomonas aeruginosa* with 0.765 SI. The sequence analysis also identified the organism to be *Ps. aeruginosa* with 98% identity match after BLAST (Table 2).

TABLE 2: *PSEUDOMONAS AERUGINOSA* SEQUENCE ANALYSIS BY BLAST

Description	Max score	Total score	Query cover	E value	Ident	Accession
<a href="#">Pseudomonas aeruginosa strain C gene island PAGI-4(C) sequence</a>	63522	63522	100%	0.0	100%	<a href="#">AY258138.1</a>
<a href="#">Pseudomonas aeruginosa plasmid Rms148 transposon Tn4661 DNA_comple</a>	23462	23462	36%	0.0	100%	<a href="#">AB375440.1</a>
<a href="#">Pseudomonas aeruginosa strain PACS88 clone fa1398, complete sequence</a>	23255	23255	36%	0.0	99%	<a href="#">EU595754.1</a>
<a href="#">Pseudomonas aeruginosa PA7, complete genome</a>	23255	54692	93%	0.0	99%	<a href="#">CP000744.1</a>
<a href="#">Pseudomonas aeruginosa strain PACS171b clone fa1386, complete sequence</a>	23254	23254	36%	0.0	99%	<a href="#">EU595748.1</a>
<a href="#">Pseudomonas aeruginosa PA38182, complete genome</a>	22676	59779	96%	0.0	99%	<a href="#">HG530068.1</a>
<a href="#">Pseudomonas aeruginosa UCBPP-PA14, complete genome</a>	20332	60022	97%	0.0	97%	<a href="#">CP000438.1</a>
<sup>1</sup> <a href="#">Pseudomonas aeruginosa SCV20265, complete genome</a>	20325	61291	96%	0.0	97%	<a href="#">CP006931.1</a>
<a href="#">Pseudomonas aeruginosa DK2, complete genome</a>	20194	64969	98%	0.0	99%	<a href="#">CP003149.1</a>
<a href="#">Pseudomonas aeruginosa M18, complete genome</a>	19752	20449	32%	0.0	99%	<a href="#">CP002496.1</a>
<a href="#">Pseudomonas aeruginosa RP73, complete genome</a>	19664	34631	58%	0.0	99%	<a href="#">CP006245.1</a>
<a href="#">Pseudomonas aeruginosa c7447m genome</a>	19642	22656	36%	0.0	99%	<a href="#">CP006728.1</a>
<a href="#">Pseudomonas aeruginosa LES431, complete genome</a>	19640	20336	32%	0.0	99%	<a href="#">CP006937.1</a>
<a href="#">Pseudomonas aeruginosa LESB58 complete genome sequence</a>	19640	20336	32%	0.0	99%	<a href="#">FM209186.1</a>
<a href="#">Pseudomonas aeruginosa PAO1-VE13 genome</a>	19631	22734	36%	0.0	99%	<a href="#">CP006832.1</a>

Species level identification of microbes is possible through MIDI Sherlock microbial identification. Abel and colleagues first suggested that microorganisms could be classified by gas chromatographic analysis [16]. These concepts found application in the form of the Sherlock system. The Sherlock microbial identification system is solely based on computer comparison of the unknown organism's fatty acid methyl ester profile with the profiles of a predetermined library of known isolates with pattern recognition software. Identification of bacteria by conventional methods usually requires 48 h after a discrete colony has been isolated. Two weeks are required for the identification of many slow-growing and fastidious organisms by conventional methods. In some circumstances, no identification can be made after weeks of analysis, even by an experienced technologist [17].

ECL Deviation: 0.006 Reference ECL Shift: 0.016 Number Reference Peaks: 10

Total Response: 442595 Total Named: 414136

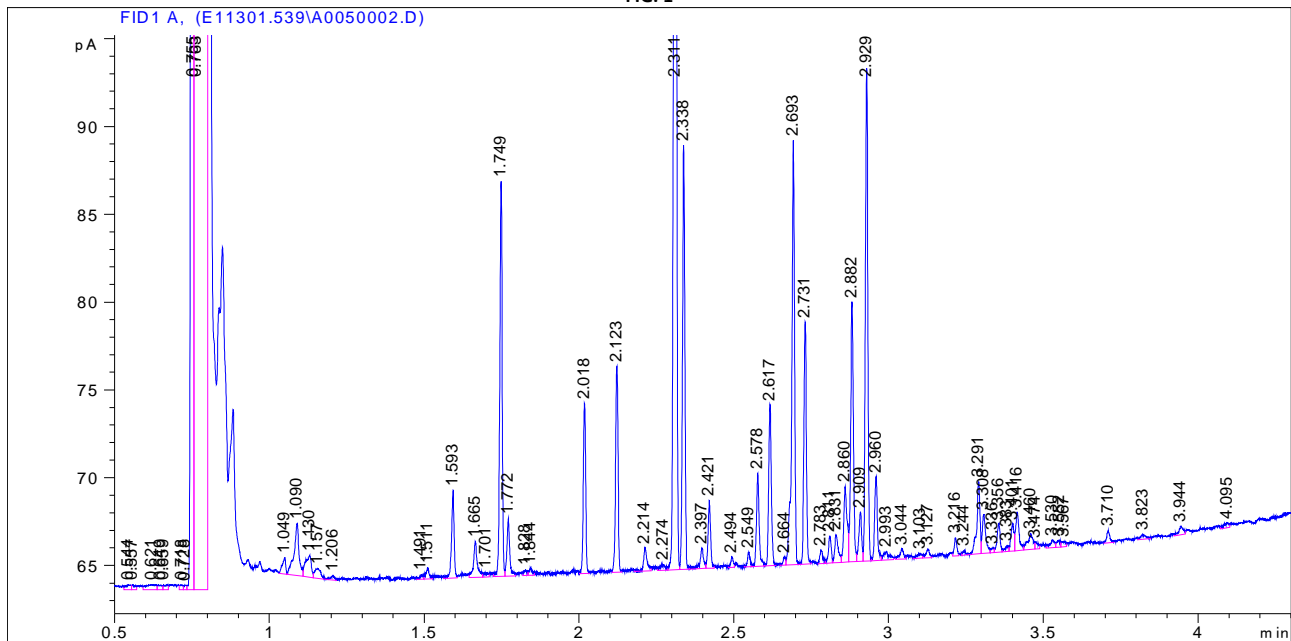
Percent Named: 93.57% Total Amount: 394254

Matches

TABLE 3

Library	Sim Index	Entry Name
RTSBA6 6.00	0.605	Bacillus-cereus-GC subgroup A
	0.370	Bacillus-thuringiensis-kurstakii

FIG. 1

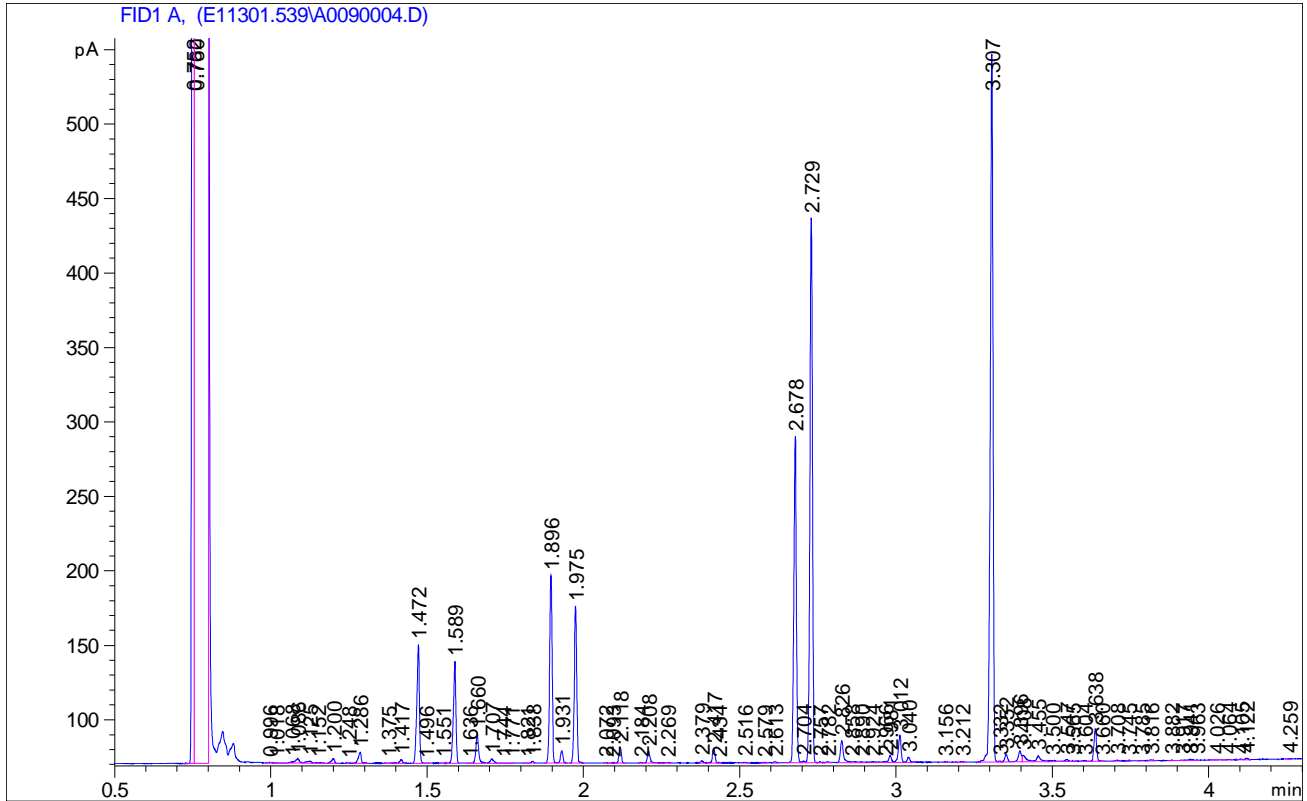


ECL Deviation: 0.006 Reference ECL Shift: 0.007 Number Reference Peaks: 15  
 Total Response: 1904747 Total Named: 1791748  
 Percent Named: 94.07% Total Amount: 1719475  
 Matches

TABLE 4

Library	Sim Index	Entry Name
RTSBA6 6.00	0.765	<i>Pseudomonas-aeruginosa</i>

FIG. 2

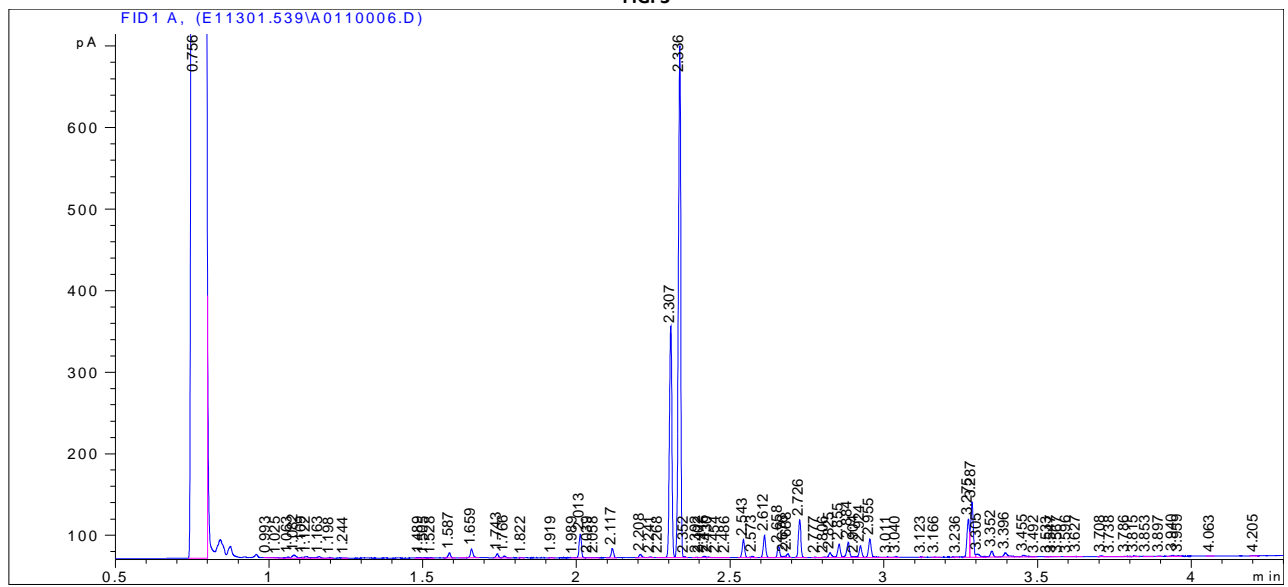


ECL Deviation: 0.004 Reference ECL Shift: 0.009 Number Reference Peaks: 18  
 Total Response: 1483894 Total Named: 1414122  
 Percent Named: 95.30% Total Amount: 1351343  
 Matches:

TABLE 5

Library	Sim Index	Entry Name
RTSBA6 6.00	0.716	<i>Bacillus-megaterium-GC subgroup A</i>

FIG. 3

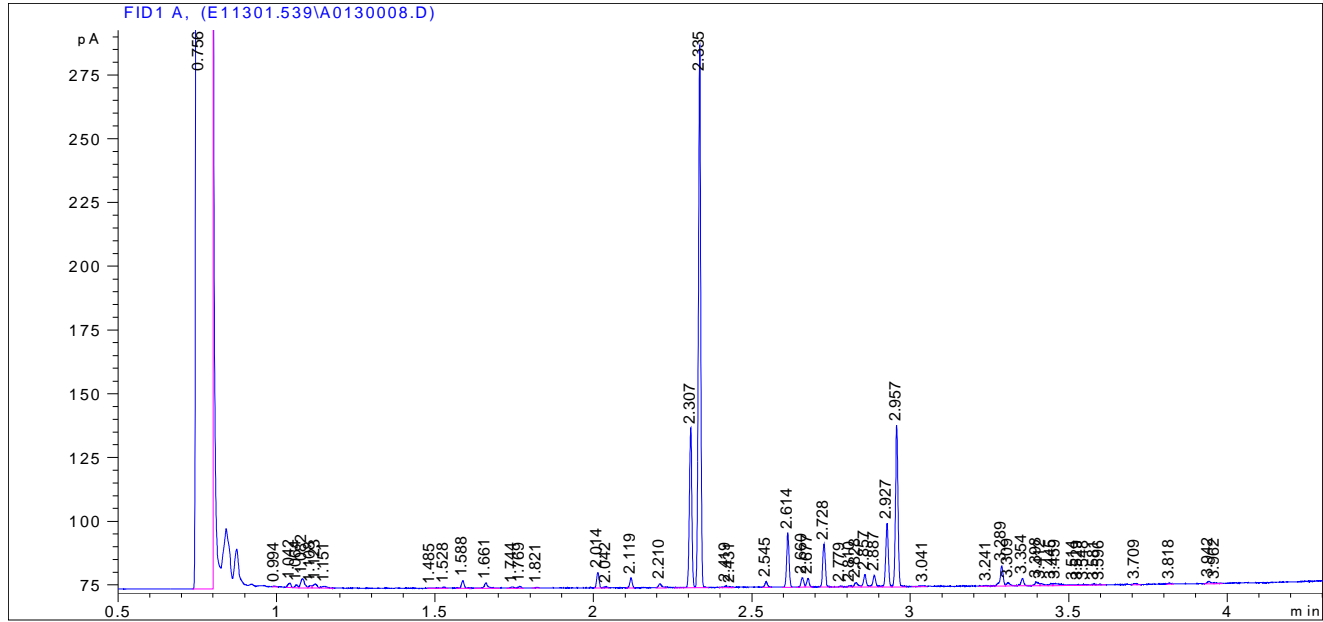


ECL Deviation: 0.004 Reference ECL Shift: 0.005 Number Reference Peaks: 20  
 Total Response: 532865 Total Named: 505577  
 Percent Named: 94.88% Total Amount: 482369  
 Matches:

TABLE 6

Library	Sim Index	Entry Name
RTSBA6 6.00	0.804	<i>Bacillus-atrophaeus</i>

FIG. 4

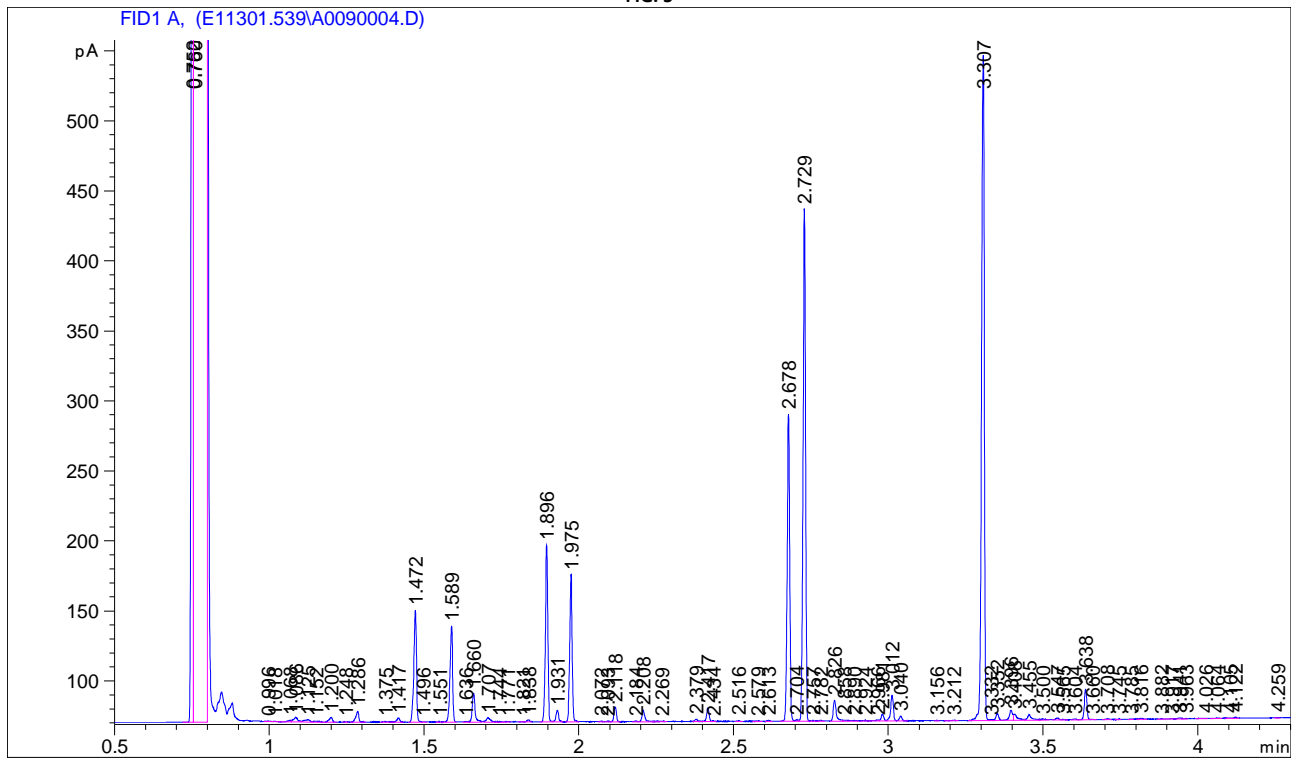


ECL Deviation: 0.006 Reference ECL Shift: 0.007 Number Reference Peaks: 15  
 Total Response: 1904747 Total Named: 1791748  
 Percent Named: 94.07% Total Amount: 1719475  
 Matches:

TABLE 7

Library	Sim Index	Entry Name
RTSBA6 6.00	0.765	<i>Pseudomonas-aeruginosa</i>

FIG. 5



Direct sequence determination of 16S rRNA gene fragments represents a versatile method for identification of bacteria to the species level. But one of the potential limitations of the 16S rDNA sequencing application is the inability to assign a species for recently diverged species [18,19]. Cost is a critical issue in the evaluation of 16S rDNA sequence analysis as a diagnostic tool. Driven in part by the technology underlying the human and microbial genome projects, sequencing costs will probably continue their rapid trend upward.

### CONCLUSION

Through the above study we can conclude that FAME analysis is equally reliable and cost effective as compared to sequencing. Identification of bacteria through FAME is rapid, accurate and less expensive, thus this technology should be brought within the reach of microbiologists and into routine practice.

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