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Fame Analysis for Identification of Clinical Isolate Siderophore Producing Pseudomonas Species

KEYWORDS: FAME analysis, Clinical, Siderophore, Pseudomonas

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ABSTRACT *The genus Pseudomonas contains more than 140 species most of which known to cause disease in humans. Identification of Pseudomonas by conventional methods usually requires 48 h and it's time consuming process. In this study 13 Pseudomonas spp were isolated from patients and hospital environment, they were identified as Pseudomonas spp on the basis of morphology and biochemical test. All 13 isolates were shown siderophore production on Chromo azurol S agar plate. Further their identities were confirmed by FAME analysis which revealed out of 13, four strains exactly similar to Pseudomonas aeruginosa.*

INTRODUCTION

Pseudomonas spp have been known for their siderophore production for many years (Decheng et al., 2005; Wilhelmina et al., 2004). It is a ubiquitous free-living bacterium found in natural habitats like soil, fresh water, marine environments and also isolated from clinical instruments, and medical products (Franzetti and Scarpellini, 2007).The genus *Pseudomonas* contains more than 140 species most of which known to cause disease in humans (Lyczak et al., 2000). While certain members of the genus are considered to be important phyto-pathogens, carriers of human infections, and exhibit activities of bioremediation and biocontrol (Tripathy et al. 2006). The species of *Pseudomonas* include *P. aeruginosa*, *P. fluorescens*, *P. putida*, *P. cepacia*, *P. stutzeri*, *P. maltophilia*, and *P. putrefaciens*. Identification of *Pseudomonas* spp based on biochemical and physiological test were difficult and time-consuming. Recent advances in the biochemistry of microorganisms revealed that analysis of cell components, such as proteins and fatty acids, can be effectively applied to bacterial identification, providing the basis for chemotaxonomy (Komagate and Suzuki, 1987). The microbial identification system produced by MIDI (Newark, DE, USA) is widely used for identification of microorganisms by fatty acid analysis (Olson, 1996).

In present study thirteen *Pseudomonas* spp was isolated from patients and hospital environment. Siderophore production by different strain was studied on chromo azural S (CAS) agar plate and isolate were identified by FAME analysis by MIDI Sherlock.

MATERIALS AND METHOD

Isolation of *Pseudomonas* spp

Thirteen *Pseudomonas* spp were isolated from patients and hospital environment (Table-1) out of which 04 from urinary tract infection, 03 from burn skin, and 03 from wound, and 03 from hospital environment. The sample collected were serially diluted with distilled water and plated on citramide agar which is selective media for *Pseudomonas* spp.

Biochemical and physiological characterization

The study of biochemical and physiological characterization of isolate was carried out using a combination of colonial morphology, Gram stain characteristics, motility tests, pigmentation, oxidation-fermentation tests, Catalase and oxidizer activity tests and pyocyanin production (Cheesbrough, 1993).

Siderophore Production

Siderophore production by different strain of *Pseudomonas aeruginosa* was tested by chromo azural S (CAS) assay (Schwyn and Neilands, 1987). The strains were spread over citramide agar plate and incubated for 48h at 30°C.After incubation a thin layer of CAS reagent in 0.7% agar was spread on the bacterial growth and plates were again incubated for 24h at 30°C formation of yellow orange zone around the colonies indicates siderophore production.

FAME analysis

FAME analysis for identification of *Pseudomonas* spp was carried out as per methods described by MIDI (Newark, DE, USA) on Agilent 6890N Network GC system. All strains were grown on Trypticase Soy Broth Agar and their fatty acid was extracted by flowing sampling process. The reagents 1, 2, 3 and 4used for FAME analysis were prepared as per MIDI.

Sampling process

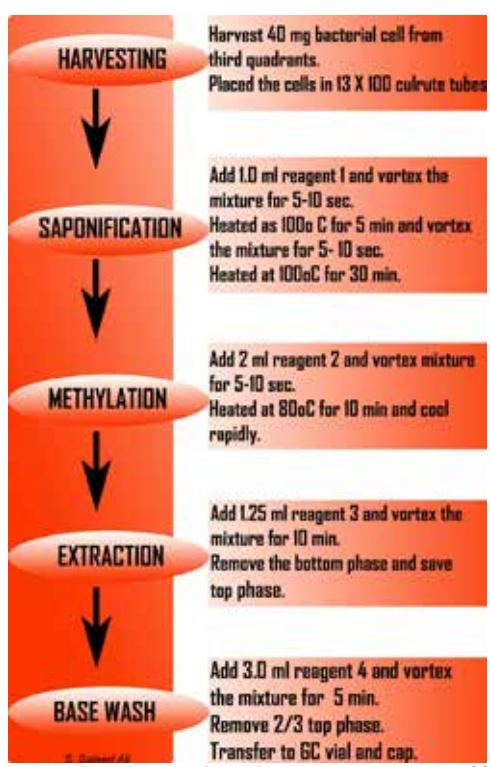

Fig. 1: Flow chart explains the extraction process of fatty acids.

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RESULTS AND DISCUSSION

All the thirteen bacterial strain were isolated from urinary tract infection, wound, burn skin of patients and hospital environment (Fig. 2) was identified as a *Pseudomonas* spp on the basis of morphology and biochemical tests (Table. 1).

Fig. 2: Colonies of Pseudomonas spp on citrimide agar plate.

Table-2 Biochemical and physiological characterization of Pseudomonas spp

Sr. No	Biochemical Test	Results
1	Gram staining	Gram Negative, Single rods
2	Motility	Motile
3	Colony Morphology on Nutrient Agar	Bluish green colours colonies
	MacConkey agar	Non lactose fermenting colonies
	Cetrimide agar	Bluish green colour colonies
4	Oxidase	Positive
5	Catalase	Positive
6	Growth at different temperature	
	5^0C	Negative
	15^0C	Positive
	37^0C	Positive
	42^0C	Positive
7	Growth at different pH	
	5.7	Positive
	6.8	Positive
	8.0	Positive
8	Growth on Nacl(25%)	Positive
9	Simmon's citrate medium	Positive
10	Urease	Negative
11	Indole	Negative
12	Methyl red	Negative
13	Vogues Prosker	Negative
14	Nitrate Reductase	Positive
15	Gelatin Hydrolysis	Positive
16	Glucose	Positive
17	Lactose	Negative
18	Manitol	Positive
19	Arginine dihydrolase	Negative
20	Starch hydrolysis	Negative

Siderophore production by different *Pseudomonas spp*. were confirmed by growing them individually on citramide agar, after spreading layer of CAS reagent and incubation each colony has developed yellow to orange colored zone on CAS agar plate indicating siderophore production (Fig. 3). The color change from blue to orange resulting from siderophoral removal of Fe from the dye. (Wilhelmina M. Huston *et al.*, 2004. Further the *Pseudomonas* spp were identified by gas chromatographic (GC) analysis.

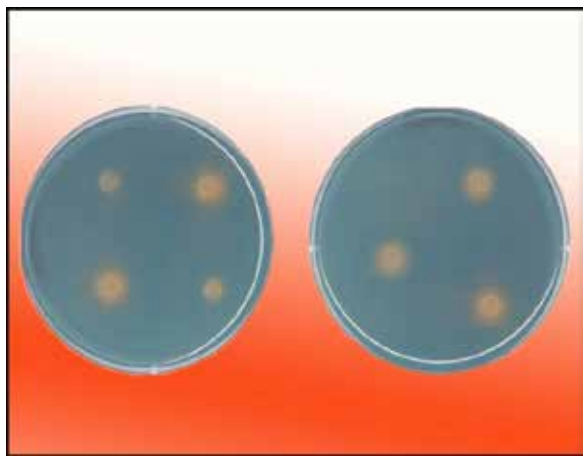

Fig. 3: Siderophore production by Pseudomonas spp.

The chromatogram obtained in this experimental analysis (Fig. 4) is more descriptive and elaborative. It confirms and correlates the presence of saturated and unsaturated forms of fatty acids in the bacterium. The result of Sherlock MIDI software revealed that out of thirteen *Pseudomonas* spp four strains were similar to RTSBA6 6.00 strain i.e. *Pseudomonas aeruginosa.*

Conclusion

In above study thirteen *Pseudomonas* spp were isolated and identified as *Pseudomonas* spp based on biochemical and physiological characterization. All strain were shown siderophore production on CAS agar plate. Further their identity confirmed by FAME analysis which revealed that four strains were exactly matching with *Pseudomonas aeruginosa*. 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.

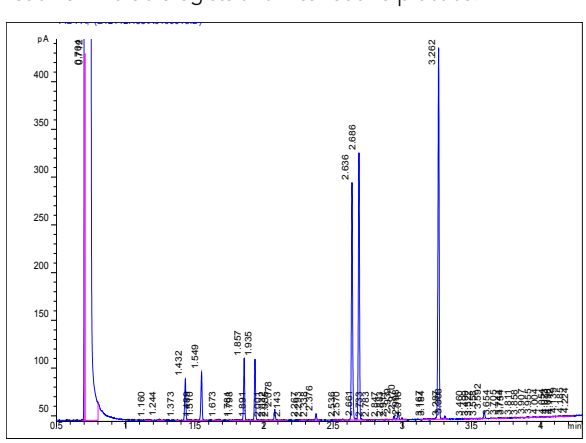

Fig. 4: Chromatogram of fatty acids.

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