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Research Article

Protein profiling of *Pseudomonas associated with* fish pathogens

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ABSTRACT:
Bacteria were isolated from the fishes collected from different regions of Nanded district. Total ten species i.e. two species from each region were selected for the further studies. Species were identified as fluorescent pseudomonas based on morphological and biochemical activities such as grams nature, oxidase, fluorescent pigment, arginine hydrolysis. The study of whole cell protein profile was done for showing relationship among the isolated strains by UPGMA cluster analysis. Similarly index suggested the isolates were distinguished into four clusters representing different area or region.

Keyword: Protein profiling of Pseudomonas associated with fish pathogens

INTRODUCTION

Pseudomonas sp. is one of significant fish pathogens among the etiological agent of bacterial fish diseases. The pathogen causes ulcer formation diseases together with ulcerative syndrome, tail and fin rot, gill rot and dropsy[1]. The bacterial pathogen has several modes to cause disease in their host. Adhering to the host and joining the host tissues are the most essential steps in commencing infections. Virulent bacteria secretes tissue degrading enzymes and toxins to evade the immune defense response of the host. Cell surface structure like pili works as adhesion factors for infection process as well as extracellular products have been studied widely in bacterial fish pathogens[2]. Several virulence factors have also been described for bacterial fish pathogens like EPS present in the capsule or lipopolysaccharides in cell wall are related to virulence in pathogens[3].

Proteins comprise 55% of the dry mass in bacterial cells and can be separated by electrophoretic techniques such as polyacrylamide gel electrophoresis (PAGE) of whole-cell soluble proteins to achieve a protein electrophoregram[4]. The electrophoretic protein patterns can be used to assess similarity among strains at species and subspecies levels. Also, protein profiles combined. with computer-aided analysis have potential in phylogenetic and taxonomic studies. Separation of cellular proteins by electrophoresis is a sensitive technique that can provide information of strains at the sub species level. Total cell protein separation by SDS-PAGE was used in bacterial identification[5]. The cell envelope protein fraction had been used to characterize *Pseudomonas* spp. and to screen the bacteria from different source and locations[6]. This study was aimed to examine the degree of phenotypic and genotypic diversity

within a selection of closely related pseudomonad isolates sampled from fish.

MATERIAL AND METHODS

Isolation of bacteria

Bacteria were isolated from the fishes, water samples were collected and shaken gently to remove the adhering material. The suspension from all samples were vortexed and serial dilutions were made and dilutions of 10^{-6} with three replications for each sample. One mL of this dilution was pour plated on Pseudomonas Isolation Agar for fluorescein and pyocyanin production. 0.1ml of each dilution was spread on Luria Bertani plates and plates were incubated at 27±2°C until colony development was observed.

Pseudomonas was identified on the basis of cell morphology, colony morphology, and gram staining.

A total of fifteen bacterial strains were isolated on the basis of colony morphology from the diseased fishes collected from different sites of Maharashtra, India.

Biochemical tests and Characterization

Bacteriological characteristics of the isolates were examined by using the methods described by Palleroni [7]. Gram stain, colony color, LOPAT test (Levan, Oxidase, Pectate liquefaction, Arginine hydrolysis, Tobacco hypersensitivity) Fluorescent pigments, Aesculin hydrolysis, Nitrate reduction, Gelatin hydrolysis, Sucrose Utilization, Inositol utilization, L-Arabinose utilization, Tyrosinase, fluorescent pigment on King's medium B.

The results of all these tests were recorded as either positive or negative for distinguishing into different biovars [8].

Isolation of protein from the Bacteria and SDS-PAGE of whole cell protein

Whole cell protein isolation and SDS-PAGE of whole cell protein was performed by using modified method described [9].

Determination of phylogenetic relationships

The strains were compared on the basis of presence or absence of protein bands in the gel. The presence or absence of band was used as 1 or 0 for preparing binary matrix and used for analysis using NTSYS.

The cluster analysis was performed by unweighted pair group method using arithmetic averages (UPGMA) and the dendrogram was generated with the NTSYS-PC to show the similarity coefficient between the genotypes.

RESULTS AND DISCUSSION

A total of 187 strains from 12 different banana growing field were isolated. These stains were subjected to screening of their disease inducing characteristic in fishes. The result thus obtained were recorded and of these 187 strain 12 strains with relatively high virulence were selected. The selection was done such that one strain from each location is represented. *Pseudomonas fluorescens* is a physiologically diverse species of opportunistic bacteria (gamma-proteobacteria) found throughout terrestrial habitats. The 12 strains were highly virulent to the experimental fish in which they caused mortality ranging from 70% to 95% within 48 h. Table 1.

The biochemical and physiological tests identified different strains within the genus *Pseudomonas* based on the different biochemical and physiological tests. The identification of a small group of strains could not be confirmed using the biochemical and physiological analysis. In the present study, most of the species were identified as *P. fluorescens*. The results of the biochemical and physiological tests were considered individually for each strain to compare the phenotypic diversity present within them. All the strains were positive for oxidase and arginine hydrolysis. Three of the strains were negative for sucrose utilization, levan production and inositol utilization. The biochemical and physiological tests identified different strains within the genus *Pseudomonas*. The substrate utilization pattern of the strains within each site is given in Table 2. The strains fell into five groups based on analysis

of the patterns of total proteins following SDS-PAGE. These groups were identical to those indicated by biochemical tests. Protein profiles comprised 23 to 26 reproducible bands, ranging from about 4 kDa to more than 97 kDa. The protein profile of all the strains appeared to be similar; there was variation in the protein profile. Although strains in each group appeared to be homogeneous in protein profiles but differed with respect to low molecular weight proteins, about 3.5 - 20 kDa, in that three bands were apparent whereas three different bands were apparent. Furthermore, an intense band was present at more than 20 kDa in all lane (Fig. 1). UPGMA analysis performed on SDS–PAGE profiles of protein extracts revealed the existence of five different groups of strains. Strain membership to the grouping identified by wholecell protein analysis showed a high degree of similarity.

The isolates were mainly distinguished into five major clusters representing five different biovars. The cluster I representing biovars IV, cluster II representing biovar III, cluster III representing biovar I, cluster IV representing biovars II and cluster V representing biovars V. Thus, the isolates belonging to rhizosphere of same crop from different geographic location were distributed into different phenotypic clusters (Fig. 2). *P. fluorescens* is an opportunistic species long recognized for its genetic, physiological and functional diversity19. The previously sequenced genome of isolate Pf-5 offered a glimpse of genome content and organization, but in the absence of comparative data sheds little insight into the extent of genomic diversity[10].

The genus *Pseudomonas* comprises of a diverse group bacteria with high levels of physiological and genetic diversity within species. This is evident from the facts that they can colonize a wide range of habitats. Diversity arises and is maintained through the interaction between ecological and genetic factors[11].

Genetic factors are the ultimate determinants of patterns of diversity but the products of these genetic factors – the protein are the ultimate expression of these factor.

Physiological state of the cell is reflected from the proteins present in the cell, this is expressed in the form of variation in banding pattern of protein bands.

REFERENCES:

1. Paniagua C., Octavio R., Juan A., and German N., Pathogenicity Factors and Virulence for Rainbow Trout (Salmo gairdneri) of Motile Aeromonas spp. Isolated from a River. J. Clin. Microbiol.,1990, Vol. 28, No. 2, pp. 350-355
2. El-Sayyad, H. I., Zaki, V. H., El-Shebly, A. M. and El-Badry, D. A. 2010. Studies on the effects of bacterial diseases on skin and gill structure of *Clarias gariepinus* in Dakahlia Province, Egypt. Annals of Biological Research, 1 (4): 106-118.
3. Arias, C.R., Shoemaker, C.A., Evans, J.J. and Klesius, P.H. (2003) A comparative study of Edwardsiella ictaluri parent (EILO) and E. ictaluri rifampicin-mutant (RE-33) isolates using lipopolysaccharides, outer membrane proteins, fatty acids, Biolog, API 20E and genomic analyses. J Fish Dis 26, 415–421.
4. Kersters, K., Numerical methods in the classification of a bacteria by protein electrophoresis. In: Jones, D., Priest, F.G. (Eds.), Computer-Assisted Bacterial Systematics. *Academic Press*, London, 1985; 337-368.
5. Law, M. 2001. Differential diagnosis of ulcerative lesions in fish. Environmental Health Perspectives, 109 (5): 681–686.
6. Noga, E. J. 2000. Skin ulcers in fish: Pfiesteria and other etiologies. Toxicologic Pathology, 28: 807–823.
7. Palleroni, N. J. Genus I. Pseudomonas Migula. In Bergey's Manual of Systematic Bacteriology, 1; 141-199. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins. 1984.
8. Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T., Williams, S.T., Bergey's Manual of

Determinative Bacteriology. Williams and Wilkins Press, London. 1994.

9. Khan F.G. 1996. A preliminary study of finger printing of Pseudomonas aeroginosa of whole cell protein analysis by SDS-PAGE. Indian Journal of medical research.104: 342- 348.
10. Hafeez A. and A. Sultana. 1992. Study of the whole cell and outer membrane protein profile of E. coli by SDSPAGE. Pakistan Journal of Zoology.1:66-68.
11. Kim S., Reuhs B.L. and Mauer L.J. 2005. Use of Fourier transform Infrared spectra of crude bacterial lipopolysaccharides and chemometrics for differentiation of Salmonella enterica serotypes. J. Appl. Microbiol. 99: 411-417.

Table 1. Virulence results for Pseudomonas species after intramuscular injections

Isolate No.	Number of fish/ isolate	Fish mortality / isolate/ hrs.	% Virulence
Pb1	20	19/24	95
Pb2	20	18/24	90
Pb3	20	15/48	75
Pb4	20	16/48	80
Pb5	20	17/48	85
Pb6	20	14/96	70
Pb7	20	13/120	65
Pb8	20	15/48	75
Pb9	20	17/48	85
Pb10	20	14/96	70
Pb11	20	16/48	80
Pb12	20	14/96	70
Control I	10	0/10	0

Table 1. Biochemical characters of fluorescent Pseudomonas species used for differentiating various strains

Biochemical Characteristic	Pb1	Pb2	Pb3	Pb4	Pb5	Pb6	Pb7	Pb8	Pb9	Pb10	Pb11	Pb12
Levan	-	+	+	-	+	+	+	+	-	-	+	+
Oxidase	+	+	+	+	+	+	+	+	+	+	+	+
Pectate hydrolysis	+	+	+	+	+	+	+	-	+	-	+	+
Arginine hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+
Tobacco hypersensitivity	-	+	-	+	+	+	-	-	+	-	+	+
Fluorescent pigments	-	+	+	+	+	+	+	+	+	-	+	+
Nitrate reduction	+	-	+	-	+	+	+	-	-	+	+	+
Gelatin	-	+	+	+	+	+	+	-	+	-	+	+

hydrolysis												
Sucrose Utilization	-	-	+	-	+	+	+	+	+	-	+	+
Inositol utilization	-	-	+	-	+	+	+	+	+	-	+	+
L-Arabinose utilization	+	-	+	-	+	+	+	+	+	+	+	+
Tyrosinase	+	-	-	-	+	+	-	+	+	+	+	+
Lecithinase	+	-	+	-	+	+	+	+	+	-	+	+
D-Xylose utilization	+	+	+	+	-	-	+	+	+	+	-	-
D-Galactose	-	+	+	+	+	+	+	+	+	+	-	-
D-Mannose	-	+	+	+	+	+	+	+	+	+	+	+
Sorbitol	-	+	-	-	+	+	-	+	+	-	+	+
L-Tartarate utilization	-	+	-	+	-	-	-	-	-	-	-	-
BIOVAR	III	V	I	BV5	II	II	I	IV	II	III	II	II

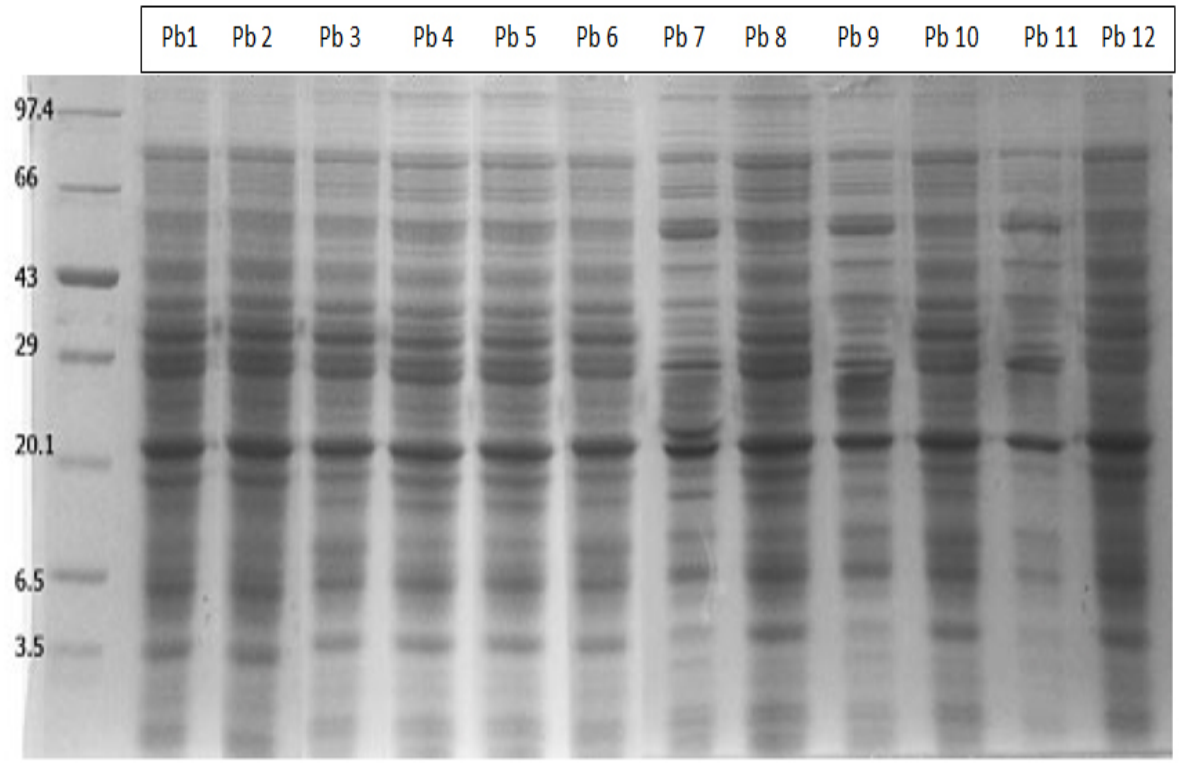

Fig. 1. SDS-PAGE analysis of 12 representative *Fluorescent pseudomonads*

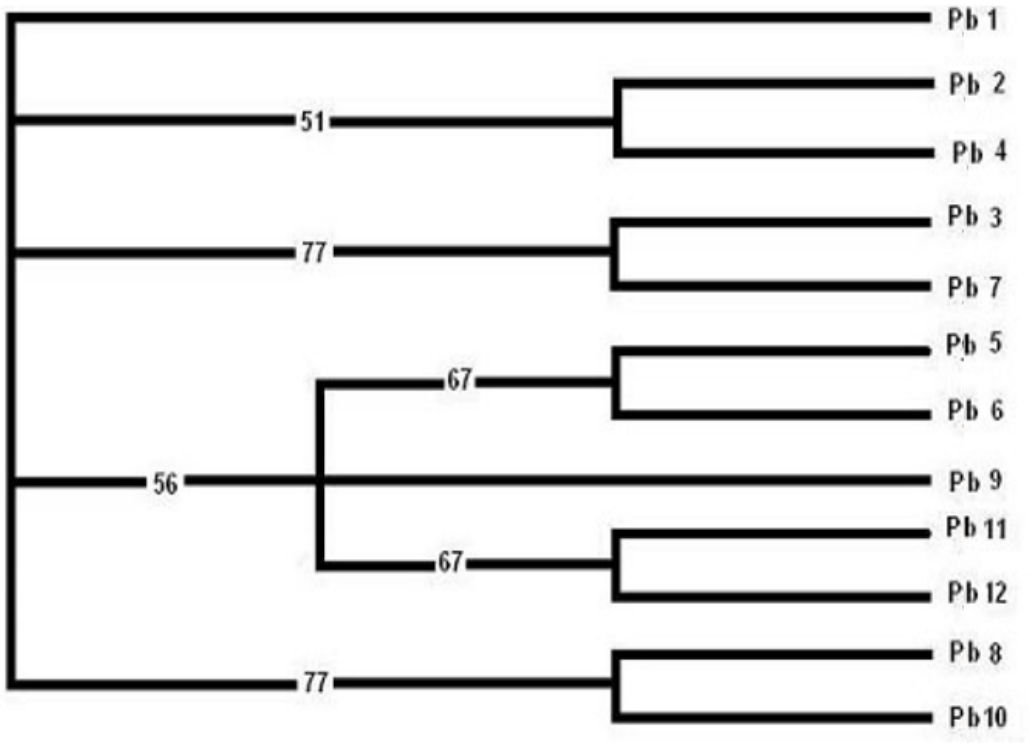

Fig. 2. Dendrogram representing the relationship between 12 representative *Fluorescent pseudomonas* isolates based on total cellular proteins resolved by SDS-PAGE