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ISOLATION AND IDENTIFICATION OF PSEUDOMONDS FOR DEGRADATION OF 4-CHLOROBENZOIC ACID

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ABSTRACT

A total eight *Pseudomonas* species were isolated from fifty samples of water and soil taken from different places. Nearly all isolates were produces proteinase, catalase, esterase, lipase and hemolysin. The several isolates were able to grow at 42^{0}C and reduces nitrogen. The four isolates *P. putida, P. flurescens P. chichori and P. aeruginosa* were most common and able to grow on 5 mM concentration of 4-chlorobenzoic acid.

KEYWORDS: *Pseudomonas* species, 4-chlorobenzoic Acid, Degradation

Man made chemicals used a pesticides, are cause of considerable environmental pollution and human health problems as a result of their persistence, toxicity and transformation into hazardous metabolites (Alexander, 1981). 4-Chlorobenzoic acid is an intermeditary metabolites of a number of chlorinated compounds such as chlorinated biphenyles and herbicides.

Microorganisms by their rapid growth and indensible enzyme system are capable of degradation and their by elimination of a wide range of chemical from the environment, the enzyme involved in the sequential steps leading to bioremediation of toxic compound are incoded in plasmids and chromosomal genes. The species *of Pseudomonas* are widely distributed in the environment including those normally in habiting water and soil. Soil micro organisms play major role in the bioremediation of toxic compound.

We were interested to know what *Pseudomonads* might be present in the soil and whether they passed of the morphological and physiological properties, normally associated with virulence.

MATERIALS AND METHODS

Isolation of Pseudomonads

The sample of water and soil were taken from different places and serially diluted in to distilled water and each dilution were plated individually on to restrictive medium, viz., minimal salts agar with 0.5% (v/v) glycerol as a carbon source medium salt agar was based on that of Hageman 1966 and contained (per/litre) KH_2PO4, 4g/l; $(NH_4)_2$ SO_4,, 1 g/l; $CaCl_2$ H_2O, 66/mg/1, $(NH_4)6$ Mo7O24. H_2O, 185mg/I, $FeSO_4$, $7H_2O$, 200 mg; MgSO4, 400 mg/1; carbon source, 5g/l and agar 15 g/l. The pH was adjusted to 6.5 and all the plates were incubated aerobically for 48 hours at 28^{0}C.

Morphological Identification

Colonies showing *Pseudomanads* like morphology plus representative colonies of differing morphologies were picked and replicated on the some type of medium. After over night of incubation at 20 °C on the fresh medium colonies were transferred to nutrient broth supplemented with 15% (v/v) glyceral. The microbial cells, appeared on the nutrient agar pates were characterize on the basis of morphology of the colonies including parameters of diameter, colour, opacity, form, elevation, margin smoothness, texture and spreading nature. The different colonies that appeared on nutrient agar plates were streamed a new nutrient agar plates. The process was repeated three time to ensure the purity of each isolates.

Biochemical Identification

Colonies appeared on the nutrient broth were using for the test of oxidation, fermentation by the method of Cowon 1974. Phycocyanin and fluoresceine production were detected on king medium A and B respectively. Catalase production and growth at 42 °C was tested in nutrient broth, nitrate reduction were tested in nutrient broth supplemented with 0.05% $NaNO_3$. Arginine hydrolysis was detected by the methods of Thornley 1960. Tests for carbohydrates metabolism were performed by spotting colonies on Difca ammonium salts agar (Smith *et. al.*, 1952). All tests were carried out in duplicate and results were read after the recommended time, the keys of King and Philips 1977 where confirmation were used for preliminary identification of isolates to the species level.

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Virulance Factor

The ability of isolates to produce certain enzyme associated with virulence was tested. Esterase production was detected by growth on mineral salt agar supplemented with 0.5% (v/v) glyceral monocitrate. Protease production was screaned by using 10 ml of minimal salt broth containing 10 μg of Azocoll (acollegen - based substrate) per ml. Lypase production was screaned with 0.005% victoria blue and hemolysin were screaned with 7% horse blood agar. Protease test were incubated aerobically for 72 hours at 28°C on orbital incubator shaker at rpm 250. Lipase and esterase tests were incubated for 48 hours at 28°C and hemolysin was incubated at 28°C for 24 hours (Holt 1971).

4-Chlorobenzoic Acid

The utilization of 4-chlorebenzoic acid was determined by using the minimal salt agar supplemented with 5 mM 4-chlorobenzoic acid as a sole source of carbon. Each *Pseudomanads* isolates were plated individually on the MSA medium. The plates were incubated at 28°C and 42°C serially. The colony forming units (cfu) were determined after 24 hours.

RESULTS AND DISCUSSION

The fifty samples of soil and water of different places yields eight *Pseudomonas species*. These isolates producing large, flat, oval, rough and circular colonies after incubation at 28^0C for 30 hours on minimal salt medium containing of chlorobonzoic acid as a sale source of carbon and energy. Few isolates producing green, brown and yellow pigments on king medium. The colonies can be varying size and diameter. The isolates produces transparent translucent and opaque opacity. All the isolates are spreading nature. The margin are either circular, wavy and rhizoidal type. The elevation are flats raised and converse. The eight different isolates are identified by morphological character are *P. aeruginosa P. putida, P. alcaligens, P. fluorescens P. mendocina P. pseudomalli. P. cichori* as shown in Table 1. The similar morpholgocial characters described by Wilson *et. al.*, 1984.

The biochemical characterization using the keys of king and Phillips-1977. Majority of the isolats producing oxidase and esterase reaction. The Table 2 show that all isolates produces proteinase with exception of *P. fluourescens* and *P. putida* failed to grow at 42^0C. The catalase activity were strongly produces by maximum isolates. Gilardi 1966 characterize Pseudomonads which are capable of growth at 42^0C. These isolates were screaned for their ability to grow at high temperature, produces catalase and reduces nitrates.

The colony forming unit reported here indicated the maximum. *Pseudomonas* species have ability to grow on 4- chlorobenzoic acid containing medium at 28^0C except. *P. Pseudomalli*. The Figure 1 show that the *P. putida* have maximum utilization capability of 4-chlorobenzoic acid then the other. Dorn *et. al.*, 1974 reported that large incubation period were required for growth of *Pseudomonas* species in media supplemented with higher concentration of 4-chlorobenzoic acid. The five species of *Pseudomonas* could degrades 4- chlorobenzoic acid with one percent glucose medium. The above report describe *Pseudomonas* species which utilize of 4-chlorobenzoic acid as sole source of carbon and energy.

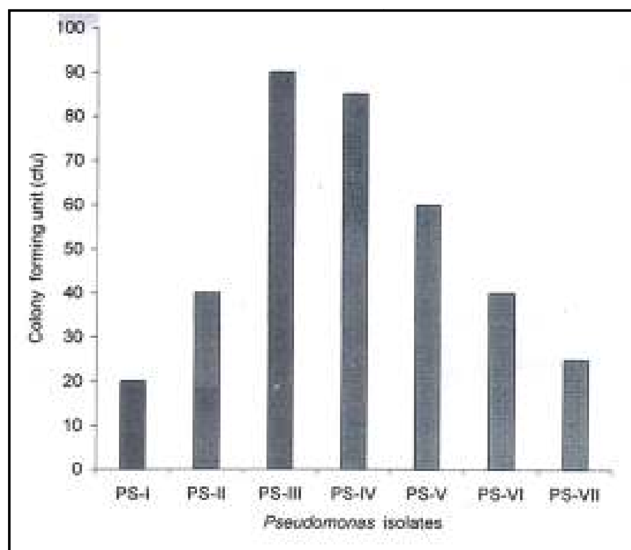

Figure 1: Colony forming unit of Pseudomonads recorded after 72 hrs in minimal salt agar medium supplemented with 4-chlorobenzoic acid

(Abbreviations: PS-I = *P. psendomallei,* PS-II = *P. fluorescens,* PS-III = *P. aeruginosa,* PS-IV = *P. putida,* PS-V = *P. cichorii,* PS-VI = *P. alcaligenes,* PS-VII = *P. mendocina*)

Table 1: Morphological characteristics of the isolates of *Pseudomonas* spp. on nutrient agar

Pseudomonads isolates	Diameter (mm)	Colour	Opacity	Form	Elevation	Margin	Smoothness	Texture	Spreading nature
P. pseudomallei	1	Yellow	0	C	C	E	S	D	Yes
P. fluorescens	2	Light gray	s	C	R	E	S	U	Yes
P.cichorii	2	Yellow	s	c	R	S	D	U	Yes
P. aeruginosa	1	Yellow	T	c	U	S	S	D	Yes
P. putida	2	Light yellow	0	c	F	U	S	U	Yes
P. mendocina	1	Creamish	0	c	C	U	D	U	Yes
P. alcaligenes	1	Yellow	s	c	C	S	S	V	Yes

(Abbreviations: Opacity, T- Transparent, S-Translucent, O-Opaque Elevation, C - Convex, F-Flat, R-Raised, U-Umbonate Margin, E-Entire, S-Serrate, U-Undulate Smoothness, D-Dully, S-Shiny Taxture, D-Dry, V-Viscous Form, C - Circular.)

Table 2: Result of biochemical tests performed for the *Pseudomonas* spp

Biochemical test	*P. pseudomallei*	*P. cichorii*	*P. aeruginosa*	*P. putida*	*P. mendocina*	*P. alcaligenes*	*P. fluorescens*
Catalase test	+	+	+	+	+	+	-
Oxidase test	-	+	+	+	-	+	+
Citrate test	+	+	+	+	-	-	+
Starch hydrolysis	+	-	-	-	-	-	-
Indol production	-	-	-	-	+	-	+
Methyl red test	+	-	-	-	+	+	-

(Abbreviation: **+** is positive test, **-** is negative test)

REFERENCES

Alexander M., 1984. Biodegradation of chemicals of environmental concern. Science, **211**:132-138.

Cowon S.T., 1974. Cowon and Steel's manual for the identification of medical bacterial 2nd ed. Cambridge. University Press, Great Britain.

Dorn E., Nellwing M., Renakek W. and Nackmass K., 1974. Isolation and chractionzation degrading Pseudomonad. Biocham. J., **174**: 73-89.

Gilardi G.L., 1996. *Pseudomonas* species in clinical microbiology Mt. Sinai J. Med., **43**:710-726.

Hegeman G.D., 1966. Synthesis of the enzymes of the mendelate pathway of *Pseudomonas putida*, Synthesis of enzymes by the wild type. J. Bacteriol., **91**:1140-1154.

Holt R.J., 1971. The detection of bacterial esterase and lipases. Med. Lab. Technol., **28**: 208-210.

King A. and Phillips J., 1977. The identification of Pseudomonads and related bacteria in a clinical laboratory. J. Med. Microbiol., **11**: 165-176.

Smith N.R., Gordon R.E. and Clark F.E., 1952. Aerobic spare forming. Monograph no.16. U.S. Department of Agriculture, Washington D.C.

Thornley M.J., 1960. The differentiation of Pseudomonas from other gram-negative bacteria on the basis of arginine metabolism. Applied Bacteriol., **23**: 37-52.

Wilson J.M., Miles A.A. and Parker K., 1984. Principle of Bacteriology Virology and Iimunity. 7th edn. London; Edward, Arnold.