PERSPECTIVES IN MICROBIOLOGY

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Published For

Association of Microbiologists of India

by

NATIONAL AGRICULTURAL TECHNOLOGY INFORMATION CENTRE

Perspectives in Microbiology
Proceedings of the 34th Annual Conference of the
Association of the Microbiologists of India
held at PunjabAgricultural University, Ludhiana
February 9 - 11, 1994

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Published by:

National Agricultural Technology Information Centre
89, I Block, Sarabha Nagar,
P.O. Box No. 340
Ludhiana - 141 001, India

Printed at:

Swami Printers, Ludhiana

STUDIES ON AROMATIC COMPOUNDS DEGRADING MICROORGANISMS FROM INDUSTRIAL EFFLUENT

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Samples from the effluent treatment plant of the Pharmaceutical unit were collected and analysed for total bacterial counts with nutrient agar and mineral medium on 0.2 % benzoate. Fifteen predominant isola-tes on benzoate agar were purified, amongst these eleven were gram positive and four were gram negative. These isolates showed ortho cleavage pathway except isolate NIX which followed meta cleavage pathway and also showed extracellular production of intermediate compound. This isolate, tentatively identified as *Pseudomonas mendocina* was also capable of utilizing cresol, adipic acid, p-hydroxybenzoic acid and 3-hydroxybenzaldehyde substrates. The UV analysis of the yellow product indicated that it is Hydroxy Muconic Semialdehyde (HMS). Specific activity of the enzyme catechol 2.3 dioxygenase was found to be 6.318. After treatment with Mitomycin -C culture NIX did not show accumulation of HMS but it grew on sodium benzoate.

Aromatic and heterocyclic compounds are the pollutants that are discharged in the industrial effluents. Pollution by aromatic and heterocyclic compounds has been controlled to considerable extent in recent years by using metabolic capabilities of micro-organisms (1) The degradation of aromatic compounds by micro-organisms constitute an essential step in carbon cycle, with regard to oxidation of both natural products and aromatic compounds.

Industrial effluent of a Pharmaceutical company at Kundai Industrial Estate, Ponda, Goa, was found to contain different types of aromatic compounds as Sodium benzoate was one of the raw material and was used for isolation of aromatic compound degrading micro-organisms.

EXPERIMENTAL RESULTS AND DISCUSSION

The samples collected from the tanks were found to be highly turbid. The maximum turbidity was observed from tank 3. The pH of this sample was tested and found to vary between 6.4 to 7.6 as seen from table 1. The maximum pH was observed in the first tank and the minimum in the soil sample. This could be due to the growth of organisms which are known to produce acidic components during metabolism resulting in decline in pH. It was interesting to note that the viable count of sample 4 was maximum on sodium benzoate medium whereas sample 1 showed maximum number of colonies on nutrient agar as seen in Table 1.

Amongst the cultures isolated, the culture NIX showed the presence of metapathway and excess production of yellow coloured compound in both solid and liquid mineral media. So, this culture was selected for further studies. The culture was identified as *Pseudomonas*

Table 1 pH and total viable counts in the samples from effluent treatment tanks

Sample	pH	Media	
		Nutrient Agar cfu x 10/ml	Sodium Benzoate cuu x 10/ml
1	7.6	2.66	1.38
2	6.5	1.75	1.14
3	6.8	1.23	0.84
4	6.4	Matgrowth	168800

Table 2 Utilisation of organic substrates as carbon source by SBI

Substrate	Growth
Naphthalene	-
Anthracene	-
Phenanthrene	-
Toluene	-
p- cresol	++
m- cresol	++
Adepic acid	+++
Phenol	-
p-Hydroxy benzoic acid	+++
m-Hydroxy benzoic acid	-
Protocatechuic acid	-
Catechol	+++
Chlorobenzoate	-
3-Hydroxybenzaldehyde	++
Pthalic acid	-

Key ++ Growth after 48 hrs, +++ Growth after 24 hrs

mendocina (2,3). Besides benzoate, the culture also utilised some of the aromatic compounds as shown in Table 2. The organism utilised p- and m- cresol, p- hydroxybenzoic acid, adipic acid, 3-hydroxy benzaldehyde, however it failed to utilise any poly aromatic compounds like naphthalene. Anthracene and compounds like chlorobenzoate, toluene, 3-hydroxyquinoline etc. Earlier reports have indicated that benzoate is normally broken down by ortho pathway, while ring cleavage in this culture occurs via meta pathway. The culture under study *Pseudomonas mendocina* therefore appears to have a unique enzymatic system as compared to other organisms(4).

Studies were hence further undertaken to find optimum concentration of benzoate by the culture. Optimum concentration of sodium benzoate for growth of culture was found to be

0.7% indicating resistant nature of the culture. As seen from the growth curve of *Pseudomonas mendocina* in presence of 0.2% and 0.7% benzoate, this organism reaches the stationary phase after 12 hours and 14 hours respectively. It was observed that the culture broth becomes yellow to orange in colour as it enters stationary phase. This is due to accumulation of one of the intermediate in culture broth as well as on solid media.

The UV scan (Fig. 1) of the supernatent of culture showed highest peak at 376 nm and a small peak at 286 nm indicating that HMS is accumulated in excess along with little of catechol. Such accumulation of catechol is reported with *Micrococus* culture grown on substituted benzoic acid (8). The optimum concentration of benzoate for maximum production of HMS was found to be 0.9% (Table 3).

The UV analysis indicated that semialdehyde (HMS) is produced in high concentration with absorbance 2.399 at 376 nm in the culture broth after 24 hours. These studies indicated that the initial reaction from Benzoate to Catechol and HMS are occurring at a faster rate as compared to the later part of the conversion i.e. HMS to pyruvic acid.

The specific activity of the enzyme 2,3 dioxygenase in the crude extract was found to be 6.318 units/mg/min (Table 4) further established this fact. In *Pseudomonas arvilla* (ATCC 23973) specific activity of 2.3 dioxygenase in crude extract has been reported to be 4.0 units/mg/min (5). The protein profile of crude extract on SDS - PAGE showed two bands indicating two sub units of meta pyrocatechase.

Table 3 HMS production at different concentrations of benzoate

Concentration of Benzoate	Absorbance at 376 nm
0.1 %	2.297
0.3 %	2.353
0.5 %	2.377
0.7 %	2.390
0.9 %	2.399
1.00 %	2.372

Table 4 Enzyme assay of Catechol 2,3 dioxygenase

Substrate for growth		Activity abs/min	Specific activity abs/min/mg.
Glucose	- Wholecellls	0.235	N.D
Benzoate	- a) Whole cells	0.322	N.D
	b) Crude extract	0.276	6.318

N.D = not done.

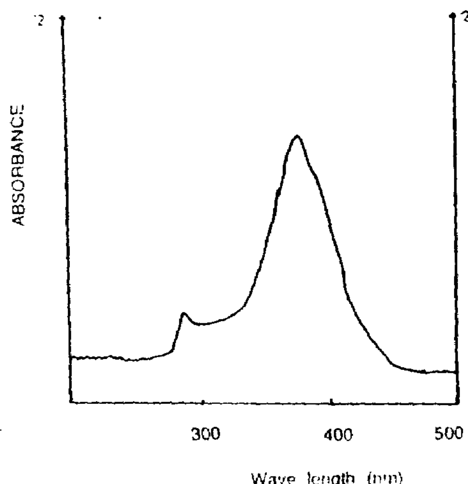

Fig. 1 UV scan of yelow coloured product accumulated in culture broth.

The culture NIX showed unique characteristics of excess accumulation of HMS extracellularly. When this culture NIX was treated with Mitomycin-C (50 μg/ml), the culture lost the characteristics of producing yellow coloured compound (HMS) on benzoate plate but showed ability to grow on benzoate. It was interesting to note that this cured culture showed the cleavage by ortho pathway indicating the presence of both the pathways in the wild type. Such organisms are reported to have the genes encoding for meta cleavage located in plasmid (3). This culture may also have a plasmid which codes for a meta cleavage enzyme system. Plasmids are known to be important components of genetic makeup of *Pseudomonas*. Some of them give the cells, capacity for degradation of unusual carbon sources, thus contributing nutritional versatility (3).

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