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Microbiology

ENRICHMENT AND ISOLATION OF N-HEXANE TOLERANT MICROORGANISMS FROM PHARMACEUTICAL INDUSTRIAL EFFLUENT.

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ABSTRACT India is developing country. To fulfill the requirements of each individual from India, lot of attention had paid on the increased population of India. So there is need of increased modern industrialization to fulfill the demands of people. Pharmaceutical industries are the prime industries in India, which produce tones of pharmaceutical products in each year. During the production of pharmaceutical products in industry huge amount of organic solvent such as n-hexane, methanol, butanol etc. were used to make drug concentrate. Residual concentration of these solvents are mixed in wastewater and discharged away from industry. These organic solvent are bioaggumented and slowly turns in to biomagnified compound in nature. So there is immediate need to find out any solution for this problem. Bacterial isolates *B.safensis*, *A.denitrificance* and *B.lichniformis* tolerate **30 % (v/v) n-hexane** concentration. Use of these potential of microorganisms to remediate such heavily loaded effluent is efficient and eco-friendly method.

KEYWORDS :

Introduction:

Organic-solvent-tolerant bacteria are a relatively novel group of extremophilic microorganisms. They overcome the toxic and destructive effects of organic solvents due to the presence of various adaptive mechanisms. Extensive studies done on the toluene tolerance of certain *Pseudomonas* strains have led to an understanding of the mechanisms of organic solvent tolerance involving novel adaptations such as the toluene efflux pumps, cis-trans isomerisation of membrane fatty acids, rapid membrane repair mechanisms, etc. However, there is practically no information available on the tolerance mechanisms of the reported Gram-positive organic-solvent-tolerant bacterial strains like *Bacillus*, *Rhodococcus* and *Arthrobacter*. This research article discusses the general aspects of organic-solvent-tolerant bacteria, their history, biodiversity, mechanisms of tolerance and proposes certain probable adaptations of Gram-positive bacteria in tolerance to organic solvents.

Materials and methods:

Collection and characterization of Pharmaceutical Industrial effluents:

Pharmaceutical effluent samples from waluj MIDC Aurangabad was collected in polythene bottles half filled. Pharmaceutical effluents with different colours were brought to working laboratory.

Pharmaceutical effluents with five number were collected in polythene bottles. Their physic-chemical property were analysed such as color, odour, consistency and pH. These effluent samples were named as A to E and a boar well ash sample named as F. Their physical characterization is represented in table no. 1.0.

Table no. 1.0

Characteristics	Effluents					
	A	B	C	D	E	F
Colour	Dark red	Pale yellow	Dark green	Light brown	Dark brown	Ash
Turbidity	Turbid	Turbid	Turbid	Turbid	Not found	Turbid
Odour	-	-	-	-	Aromatic	-
pH	9.57	3.88	12.04	8.76	Un-readable	8.5

Gas-Chromatography analysis pharmaceutical industrial effluent

Amongst the five pharmaceutical effluent sample, two effluent sample designated as A and B were selected on the basis of their pH; 9.57 and 3.88 respectively. Gas chromatography was performed in the Shraddha Analytical laboratories Ghatkopar (W), Mumbai. The presence of n-hexane in effluent sample B with 28.6 % (v/v) and methanol in effluent sample A with 13.08 % (v/v). The results were showed in Fig. no.1.0 and 2.0.

Fig. no. 1.0. and 2.0. Gaschromatography analysis of Pharmaceutical effluent (A and B)

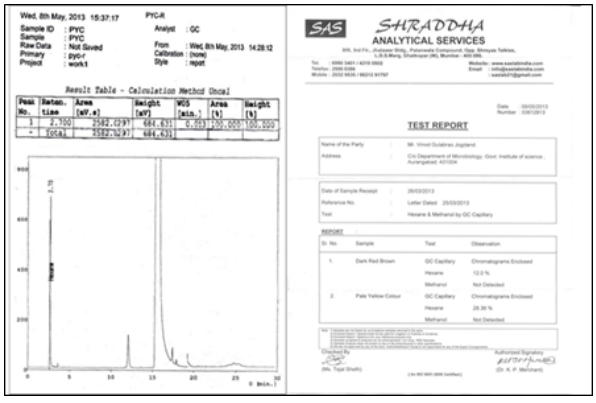
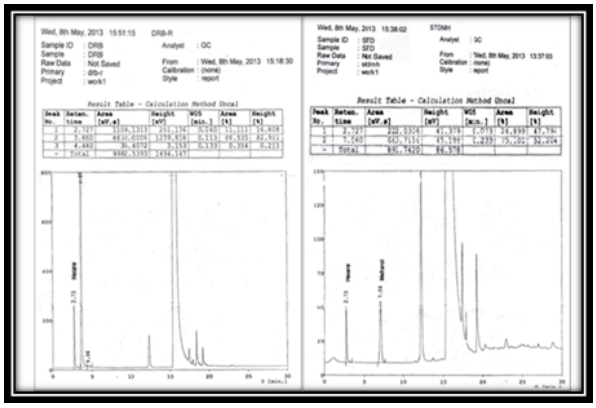


Fig. no. 2.0.



Enrichment and isolation of indigenous microorganisms

Effluents were collected from the MIDC area Waluj, Aurangabad. These effluent samples were collected in a empty plastic bottles, half of the bottle filled and tightly packed its cap. Physico-chemical properties of each individual effluent sample were noted. They were having a diverse pH ranging from 3.88 to 12.04.

These samples were enriched for the isolation of bacteria, actinomycetes and fungi in Nutrient broth, Starch-casein broth and Potato Dextrose Broth respectively. Ten ml of each individual effluent sample (A to E) and a ash sample was directly inoculated in sterile

broth medium as mentioned above and flasks were incubated at 30°C for three days with 150 rpm on a rotary shaker incubator.

Simultaneously the same procedure was repeated and 10ml of effluent sample was taken in a beaker and its pH was adjusted with help of 0.1N NaOH / HCl. Then they were inoculated in respective broth and incubated for three days on a rotary shaker with 150 rpm at 30°C temperature. Spread plate and pour plate method was performed for the isolation of microorganisms. Bacterial isolates (26) were isolated. No growth on Potato dextrose agar and casein starch agar was found. Result are depicted in Table no. 2.0

Table No. 2.0. Bacterial isolates with respect to pharmaceutical effluents.

Bacterial isolates with respect to pharmaceutical effluent			
Sr. No.	Effluent sample	No. of isolate	Colony appearance
1	A	9	Serrate margin redish coloured big in size. Another one was white with smooth margin, pin point and small in size
2	B	2	pinpoint yellow colour colonies
3	C	2	white and red colour colonies
4	D	1	white colour colony
5	E	10	white and transparent pinpoint colonies
6	F	2	white colour colony

n- hexane tolerance studies

To check their potential to tolerate/ degrade n-hexane, mineral salt medium was used. This medium is suggested by Fritche and Hofrichter (2000). For this experiment screw caped tubes with 30ml volume capacity were used to avoid evaporation of solvent. In these screw caped tubes 20ml mineral salt medium with 0.5 to 10% (v/v) concentrations of n-hexane were prepared and loopful of 26 bacterial isolates were inoculated and incubated at 30°C. Amongst the 26 bacterial isolates only four bacterial isolates named as Y, C2, B and D showed the tolerance to n-hexane (10% v/v). These four bacterial isolates were used for further studies.

Biochemical and 16s r-DNA sequencing identification of bacterial isolates.

Table no. 3.0. Biochemical identification of bacterial isolates

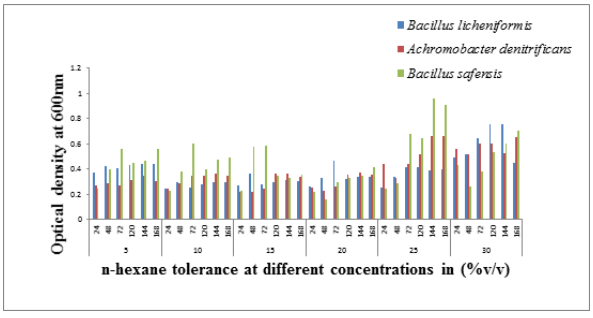
Biochemical identification of bacterial isolates				
Bacterial Isolate	***B. lichniformis***	***B.safensis***	***A. denitrificans***	***Lysinibacill usodyssey***
Code	Y	C2	B	D
Grams nature	Gram positive rods	Gram positive rods	Gram negative short rods	Gram positive cocci.
Major pigmentation	Yellow	-	-	Red
water soluble pigment	-	-	-	+
Growth on simmons citrate	-	+	+	-
growth on citrimide	-	+	+	-
growth on inorganic nitrogen	-	-	-	-
Acetoin	-	-	-	-
nitrate reduction	-	+	+	-
Oxidase	-	+	+	+
Glucose	-	+	+	-
Mannose	-	-	-	-
Motility	-	+	+	-
Catalalase	+	+	+	+
Fluroscence	-	+	+	-
Gelatinase	-	+	+	-
Indol	-	-	-	-
Methylred	-	-	-	-
Vogusprocuros	-	-	-	-
Citrate utilization	-	-	-	-

The selected four bacterial isolates were identified on the basis of different biochemical tests performed in my laboratory and 16s r-DNA analysis was performed by National Centre Cell Science, Pune. The automized sequencer process helps to have 1200 bp stretch of 16s r DNA. Obtained sequence were further evaluated by Bioinformatics with the help of BLAST tool to know the closet relative and name of bacterial isolate. Following were the 16s-r-DNA sequences of bacterial isolates (Tancsics, *et al*., 2014). The four bacterial isolates were identified as *B. lichniformis* (Y), *B.safensis*(C2), *A.denitrificans* (B) and *L.odyssei* (D) and deposited with accession number for sequence **KY174334 to KY174337**at NCBI. Results are represented in Table no. 3.0

n-Hexane tolerance at 30 % (v/v) concentration.

Screened and identified four bacterial isolates were separately analysed to determine their potential to tolerate n-hexane concentrations. N-hexane tolerance potential was analysed by growing these 4 bacterial isolates in presence of successively high concentration of n-Hexane (Segura, *et al*., 2007; Ramos *et al*., 1996; Nielsen *et al*., 2005; Stancu, 2011). Kunz and Chapman *et al*. in (1981) by using M9 medium for this purpose (Basal salt medium) and the same medium was used for n- hexane tolerance studies. Results are shown in Fig.4.0.

Fig.4.0 n-Hexane tolerance by selected bacterial isolates.



Result and discussion:

The Pharmaceutical industrial effluent is inhabit with microorganisms. These microorganisms were enriched, isolated, characterized and identified from NCCS Pune. As these three organisms can possibly showed growth at 30 % (v/v) n- hexane concentrations so they termed as extremophiles. These organisms can be used for the bioremediation of pharmaceutical effluents. Amongst the three bacterial culture *B.safensis* showed highest tolerance as compared *A.denitrificans* and *B.lichniformis.* B. safensis was the gram positive microorganisms but still showed highest degree of n-hexane tolerance. All the three bacterial species are newly reported as solvent tolerant strains.

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