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Isolation, Purification and Characterization of Oil Degrading Bacteria from Different Oil Cake Samples

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ABSTRACT

Oil cakes of mustard and linseed were collected from Rambagh, Allahabad and Mahewa Gate, Allahabad. Two samples of mustard and one sample of linseed cake were taken as a sample for project. Bacteria were grown on these oil cake samples. From it different cultures were isolated and each was separately grown. The pure cultures grown were tested for oil degrading property. Those cultures, which showed oil degrading property, were selected and isolated. The cultures were then identified using Gram staining technique. A series of biochemical tests were done for the cultures consuming oil. The tests include starch hydrolysis test, urease production test and catalase production test. DNA was then isolated from these oil degrading cultures.

Keywords: oil cakes, bacterial growth, pure culture, oil degradation, gram staining.

Oil Cake: Oil cake is the solids remaining after pressing something to extract the liquids. Their most common use is in animal feed. Some foods whose processing creates press cakes are olives for olive oil (*pomace*), peanuts for peanut oil, coconut flesh for coconut cream and milk (*sapal*), grapes for wine (*pomace*), apples for cider (*pomace*), and soybeans for soy milk (used to make tofu) (this is called *okara*) or oil. Other common press cakes come from flax seed (linseed), cottonseed, and sunflower seeds. However, some specific kinds may be toxic, and are rather used as fertilizer, for example cottonseed contains a toxic pigment, gossypol, that must be removed before processing. Oil cake**,** coarse residue obtained after oil is removed from various oilseeds, rich in protein and minerals and valuable as poultry and other animal feed. Oil cakes from certain seeds such as castor beans and tung nuts are toxic and are used as fertilizers rather than feed. Oilseeds from which oil cake used as feed is produced include soybeans, peanuts, flaxseed (linseed), rapeseed, cottonseed, coconuts (copra), oil palm, and sunflower seeds.

Edible Oil Cakes have a high nutritional value especially have protein content ranging from 15% to 50%. Due to their rich protein content, they are used as animal feed, especially for ruminants and fish. Non-edible oil cakes such as castor cake, karanja cake, neem cake are used as organic nitrogenous fertilizers, due to their N P K content. Some of these oil cakes are found to increase the nitrogen uptake of the plant, as they retard the nitrification of soil. They also protect the plants from soil nematodes, insects, and parasites; thereby offer great resistance to infection.

Oil cakes have been widely used for the production of industrial enzymes, antibiotics, biopesticides, vitamins and other biochemicals. There are several reports describing production of various enzymes using oil cakes as substrate in solid-state fermentation (SSF), or as supplement to the production medium. The supplementation of oil cakes with rice straw substrate colonized by the mushroom, Pleurotus sajor-caju increased the mushroom yields between 50% and 100%, compared to the unsupplemented substrate.

Oil cakes have also been reported for use in production of antibiotics and antimicrobials. Arun and Dharmalingam (1999) reported evaluation of alternative media constituents like carbon sources and buffers for the large-scale production of daunorubicin. Streptomyces peucetius cultivated on the media containing oil cakes as carbon source with HEPES or phosphate buffer showed good yield of the antibiotic, and the intermediates were also converted into the final product more efficiently.

The media containing linseed oil-cake agar, mustard oil cake agar, neem oil-cake agar, beef extract agar, Emerson agar and YPSS agar were used for growing an endoparasite of nematodes. In general, maximum radial growth of most of the isolates was recorded on linseed oil-cake agar medium.

Microbial Biodegradation is the chemical dissolution of materials by bacteria or other biological means. The term is often used in relation to ecology, waste management, biomedicine, and the natural environment (bioremediation) and is now commonly associated with environmentally friendly products that are capable of decomposing back into natural elements. Some microorganisms have a naturally occurring, microbial catabolic diversity to degrade, transform or accumulate a huge range of compounds including hydrocarbons (e.g. oil), polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), pharmaceutical substances, radio nuclides, pesticide, and metals. Decomposition of biodegradable substances may include both biological and abiotic steps.

Interest in the microbial biodegradation of pollutants has intensified in recent years as humanity strives to find sustainable ways to clean up contaminated environments.Petroleum oil contains aromatic compounds that are toxic for

most life forms. Episodic and chronic pollution of the environment by oil causes major ecological perturbations. Marine environments are especially vulnerable since oil spills of coastal regions and the open sea are poorly containable and mitigation is difficult. In addition to pollution through human activities, about 250 million litres of petroleum enter the marine environment every year from natural seepages. Despite its toxicity, a considerable fraction of petroleum oil entering marine systems is eliminated by the hydrocarbon-degrading activities of microbial communities, in particular by a remarkable recently discovered group of specialists, the so-called hydrocarbonoclastic bacteria (HCB). *Alcanivorax borkumensis* was the first HCB to have its genome sequenced. In addition to hydrocarbons, crude oil often contains various heterocyclic compounds, such as pyridine, which appear to be degraded by similar, though separate mechanisms than hydrocarbons.

Materials and Methods

Sampling

Three samples were collected for the entire project. Two samples were of mustard and one of flax seed or linseed was taken. The samples were oil cakes collected from different places. Sample 1 (mustard cake) was collected from an oil mill near Mahewa Gate, Naini, Allahabad. Sample 2 (mustard cake) was collected from an oil mill in Kotha Parcha, Rambagh, Allahabad. Sample 3 (linseed cake) was collected from an oil mill infront of Gau Ghat petrol pump, Rambagh, Allahabad. All the three samples were grinded using mortar and pestle. Then the samples were air dried for 24 hrs.

Isolation of Oil Degrading Bacteria

Oil degrading bacteria were isolated by observing the oil degrading property. 100 ml of nutrient agar was prepared by adding 0.5 gm peptone, beef extract 0.3 gm, 0.5 gm NaCl in 50 ml distilled water. The pH of solution was maintained at 6.8 the volume was then made upto 100 ml. The solution was then autoclaved and poured in autoclaved petri plates and left for overnight incubation at room temperature. Next day the samples were serially diluted and the concentration of 10-6 and 10-7 was used for inoculation. Inoculation was done in laminar air flow and the plates were then left in incubator at room temperature for growth. After the growth was observed, a pure culture was prepared for each culture in previously prepared agar slants. After observing growth in the slant, calculated amount (1 ml) of mustard oil was poured in each slant. The slants were then kept in incubator for few days to observe oil degradation. LB broth was prepared and the cultures which degraded oil, were inoculated in different flasks containing LB.

Identification of Isolates

Gram staining

A smear of culture was prepared on a slide. The slide was then heat dried. The slide was then kept in a container containing crystal violet for one minute. After one minute the slide was taken out and washed off in tap water. Then the slide was kept in a container containing Gram's iodine for one minute. After one minute, washing was done using tap water. Decolourization was done with alcohol (95% for 10 to 20 sec) till no more colour washes off. Slide was washed off tap water. Slide was dipped in safranin for one minute. Slide was then washed off with tap water. Then slide was air dried and observed under microscope. The above straining was performed for all the samples an d cultures.

Starch hydrolysis test

100 ml of water was taken in a flask. 0.3 gm of beaf extract, 0.5 gm of peptone, starch 0.2 gm and 1.5 gram of agar were added in it and pH was maintained at7.2. The solution was mixed thoroughly by stirring. It was then boiled and then poured into plates. The bacterial culture was inoculated in the plates and left for 48 hrs in incubator. Plates were flooded with iodine solution and observed results. The process was repeated with all the cultures.

Catalase test

A few drops of bacterial broth culture were placed on cavity slide. Same amount of hydrogen peroxide were dropped on plate. The plate was observed for bubble formation. The process was repeated for all the cultures.

Urease production Test

0.1 gm of peptone, 0.1 gm dextrose, 0.5 gm NaCl, 0.2 gm potassium phosphate, 2 gm urea and 0.0012 gm phenol red in 50 ml of distilled water. Suspended the 1.5 gm agar in 50 ml of distilled water, boiled to dissolve completely, and autoclaved at 121oC and 15 psi for 15 minutes. Agar was cooled to 50 to 55oC. Aseptically add 100 ml of filter-sterilized urea base to the cooled agar solution and mixed thoroughly. Mixed well and distributed into culture tubes and allowed the medium to solidify in a slanting position to form slopes. The tubes of the media with bacterial culture were labelled. Incubate inoculated broth for 24-48 hrs at 37°C. The process was repeated for all the cultures.

Measurement of oil degradation

The slant in which oil was degraded, were separated. Using the pipette, all the oil was sucked out from each slant. The amount of oil sucked from each slant was measured and recorded.

Results and Discussion

When the petriplates were inoculated with serially diluted sample and left for incubation, colonies of bacteria developed. From it different cultures were separated and grown in different agar slants. From each sample two cultures were prepared, such that there was a total of six slants. In these six slants, after 24 hours, growth was observed. Figure 1 and figure 2 represents the two cultures obtained from sample 1.

Figure 1

Figure 2

Figure 3 and figure 4 represents the initial level of oil in the two cultures from sample 1.

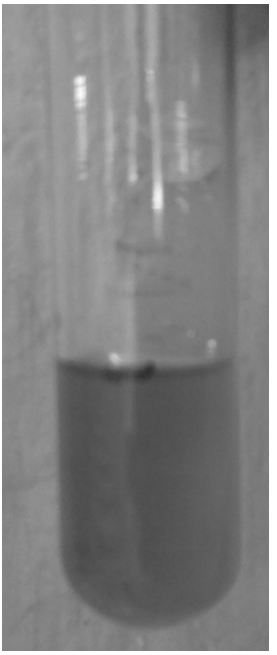

Figure 3

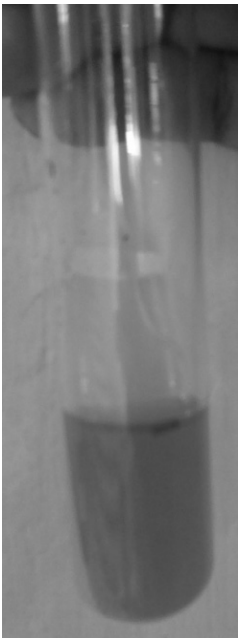

Figure 4

Figure 5 and figure 6 represents the oil degradation observed in both the cultures from sample 1.

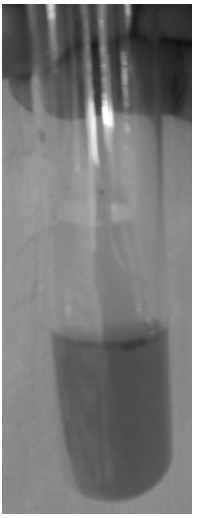

Figure 5

Figure 6

Figure 7 and figure 8 shows the initial oil content in both cultures from sample 2.

Figure 7

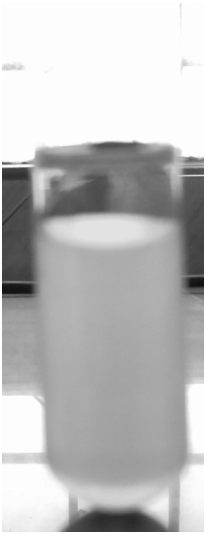

Figure 8

Figure 9 and figure 10 shows the oil degradation observed in both the cultures from sample 2.

Figure 11 and figure 12 shows the growth of cultures from sample 3. Figure 13 and figure 14 shows the oil degraded by both the cultures of sample 3.

Figure 9

Figure 10

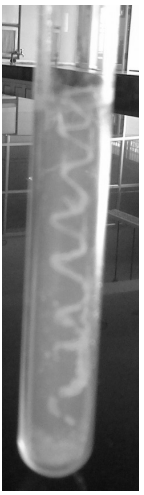

Figure 11

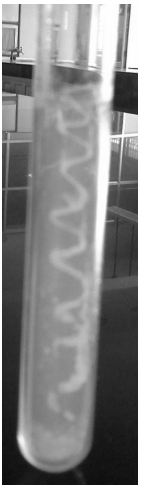

Figure 12

Figure 13

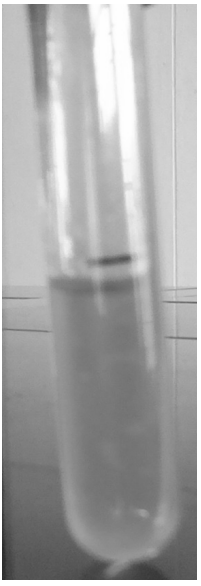

Figure 14

The following table 1.0 shows the amount of oil degraded by each culture from all the three samples. The oil degraded was measured by pippetting out the oil from all the slants using pipette. All the cultures which degraded oil, were inoculated in a LB broth. Growth was observed in every beaker containing broth.

Table 1 : Oil consumed by different bacterial cultures from all the three samples.

Samples	Culture	Observations
Sample 1	Culture 1-1	0.4 ml
Sample 1	Culture 1-2	0.5 ml
Sample 2	Culture 2-1	0.2 ml
Sample 2	Culture 2-2	0.1 ml
Sample 3	Culture 3-1	0.3 ml
Sample 3	Culture 3-2	0.4 ml

Various biochemical tests, namely starch hydrolysis test, catalase test and urease production test were done on all the cultures. The following table shows the results obtained from the Starch hydrolysis was the first test for all the samples. It was done to check if the culture can use starch or not as a carbon and energy source for growth. Different samples gave different results for the above test. The next test was catalase production. The catalase test facilitates the detection of the enzyme catalase in bacteria. It is essential for differentiating catalase-positive *Micrococcaceae* from catalase-negative *Streptococcaceae. All the samples gave positive result. The next and the last test was urease production test.* The urease test identifies those organisms that are capable of hydrolyzing urea to produce ammonia and carbon dioxide. It is primarily used to distinguish urease-positive Proteeae from other Enterobacteriaceae.

Table 3.2 Biochemical Characterization of bacterial cultures

S. No.	Biochemical Test	Sample 1	Sample 2	Sample 3
1.	Starch hydrolysis	+	-	-
2.	Catalase production	+	+	+
3.	Urease production	+	-	-

Conclusion

Environment pollution caused by released of a wide range of compound as a consequence of industrial progress has assumed serious proportions. To prevent development of hazardous waste the process of bioremediation has been followed. My present study follows the isolation of oil degrading bacteria from different oil cakes. Different oil cakes were grinded and dried. 0.1 gm of dried sample was added to 100 ml of DW and then serially diluted. Then bacteria was grown using

this serially diluted liquid. Different cultures grew on the media. All the cultures were separated and grown separately in agar slants using streaking technique. In each test tube oil was poured to check bacteria's oil degrading property. Those cultures which possessed the property, were isolated. DNA was isolated and purified from these cultures. Also DNA was run through gel electrophoresis. A number of biochemical tests like starch hydrolysis test, catalase test and urease production was done.

Bacteria, which degrade oil, could also be degrading the oil content of the seed, if it infects the plants. So, necessary steps need to be taken to find out methods to arrest its growth. Also these bacteria also might help in cleaning oil spills.

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