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Research article

Optimization and characterization of industrially used microflora, isolated from soil and water samples

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Abstract:

The aim of this study was to determine the importance of microflora in industry. The samples were collected from various places of Lucknow for "Optimization, production and characterization of industrial microflora". According to result basis total 10 cultures were isolated and out of 10 only 4 cultures were identified through Bergey's Manual. Further production, optimization were performed through various sources. The obtained cultures were *Pseudomonas aeruginosa, Staphylococcus aureus, Micrococcus luteus* and *Bacillus megaterium*. The culture conditions were checked at different parameters like-carbon sources, nitrogen sources, pH and also metal ions at the concentration of 1%, 1% and 0.2% respectively. Further to check the better growth in various sources the optimized production media was prepared, according to the best result of optimization the activity was checked to enhance the production of various proteins, enzymes and secondary metabolites.

Key Words: Optimization; Bergey's manual; enzymes and secondary metabolites.

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Introduction

Microorganisms are of major important in industrial wastewater treatment, agricultural and aquaculture. They reside in the sediment and other substrates, and in the water of aquaculture facilities, as well as in and on the cultured species. Microorganisms may have positive or negative effects on the outcome of aquaculture operations. Positive microbial activities include elimination of toxic materials such as ammonia, nitrite, and hydrogen sulphide, degradation of uneaten feed, and nutrition of aquatic animals such as shrimp, fish; production of aqua-farmer. These and other functions make microorganism's key players in the health and sustainability of aquaculture.

Beneficial microorganisms are those that can fix atmospheric nitrogen, decompose organic wastes and residues, detoxify pesticides, suppress plant diseases and soil-borne pathogens, enhance nutrient cycling, and produce bioactive compounds such as vitamins, hormones and enzymes that stimulate plant growth. The world of microorganisms is made of bacteria, fungi, algae, protozoa, and viruses. They are group together only because of their small size, and not by their function unlike larger organisms, the morphology of microorganisms is relatively poor and is confined to few shapes and colors. However, their poor morphology is compensated by great physiological versatility.

Biotechnology or industrial microbiology is defined as the application of organisms such as bacteria, fungi and algae to the manufacturing and services industries[1].

These include: Fermentation processes, such as brewing, baking, cheese and butter manufacturing, bacteria, often lactobacillus in combination with yeasts and moldshave been used for thousands of years in the preparation of fermented foods such as cheese, pickles, soy sauce, sauerkraut, vinegar, wine, and yogurt[2].

Chemical manufacturing such as ethanol, acetone, organic acid, enzymes, perfumes etc. In the chemical industry, bacteria are most important in the production of enantiomerically pure chemicals for use as pharmaceuticals or agrochemicals[3].

The present study is carried out by Optimization, Production and Characterization of importantly used microflora in industry, isolated from various sources. In this process the cultures were isolated and characterized and further used to enhance applications and for all the cultures optimization parameters involved pH, carbon sources, nitrogen sources and metal ions.

Methodology

Sample collection:

Samples were collected from various regions: Soil sample from Lucknow regions:-

- Amausi chemical industry
- Gomtinagar
- Water sample: Chinhat

Isolation of microbes through serial dilution method:

Microbes are very small in size, so counting the number of bacteria in a sample can be difficult at best, although direct counts are possible with a microscope, they require a lot of time & expertise. An easier method is to spread bacteria over a wide area & count the number of colonies that grow. If the bacteria are spread out enough, each bacterial cell in the original sample should produce a single colony. Usually, bacterial sample must be diluted considerably to obtain reasonable counts.

Dilution = volume of the sample /total volume of the sample & the diluents.

Procedure:

Serial dilution was performed to get reduced number of bacterial colonies in order to get pure colonies. The 6 test tubes were taken and in all 0.85% NaCl solution was added. The volume was taken 5ml in Ist test tube and remaining all should have 4.5 ml. After autoclaving 0.5 gm of soil was suspended in Ist test tube. Thus stock solution formed. From the stock solution, 0.5 ml solution was pipetted out and transferred in the second test tube containing 4.5 ml of NaCl. Now the second test tube was vortexed, from the above solution, a series of serial dilution were made. From each dilution, 50µl solution was pipetted out and spread on the surface of sterile petriplates of different prepared solidified agar medium by spread plate method. Now the plates were kept for incubation for overnight at 37°C.Observed result in the form of bacterial colonies. Sub culturing and pure culturing was done by streaking method.

Characterization of Bacterial cultures:

Characterization was done with the help of Bergey's manual[4]. According to Identification chart several tests were performed like -Gram staining of bacteria and endospore staining.

Confirmatory test for *Pseudomonas aeruginosa* using Brain Heart Infusion Agar:

Oxidase test:

This test is used for the confirmation of desired bacteria. Cytochrome oxidase is an enzyme found in some bacteria that transfer electrons to oxygen. Presence of cytochrome oxidase can be detected through the use of an oxidase reagent (Gordn Mcleod reagent) which acts as an electron donor to cytochrome oxidase. If bacteria oxidize the reagent it turns dark purple due to formation of indophenol blue indicating positive reaction[4].

Confirmatory test for Bacillus culture using starch hydrolysis test:

Starch is a complex carbohydrate (Polysaccharide) composed of two constituents amylose, a straight chain polymer of 200-300 glucose units and amylopectin a larger branched polymer with phosphate groups.

Growth kinetics

Growth is the orderly increases in all major constituents of an organism involving several structures, nucleic acid, protein & all components. From nutrient obtained from outside the cell. Growth kinetics process was used to determine the time period at which the culture showed optimum activity.

Optimization parameters:

Carbon sources: The effect of carbon sources such as glucose, dextrose, sucrose, lactose, beef extract at a concentration of 1% was examined by replacing in the production media[5].

Nitrogen sources: Various nitrogen sources like Urea, Peptone, NH_4Cl, Na_2HPO_4, NaH_2PO_4 at a concentration 1% replacing in the production media.

Metal ions: Various metal ions like- $FeSO_4$, $CaCl_2$,Pb(NO3)2, $ZnCl_2$, $CuSO_4.5H_2O$ at 0.2% concentration.
pH: The effect of pH such as- 5,7,9,11 were checked by adjusting pH of Nutrient broth.

Antibiotic sensitivity test (*Pseudomonas aeruginosa*):

This method is used for the detection of antibacterial activity by Agar well diffusion method. If zone of inhibition observed then antibiotic will be sensitive & if zone of inhibition will not be observed then cultures will show full growth in the presence of antibiotics[5,10].

Biosurfactant activity:

Biosurfactant are amphiphilic compounds produced by some bacteria & fungi which reduces the surfaces & interfacial tension. Biosurfactant producing micro-organism were naturally present in the oil contaminated soil[6].

Methicillin-resistant Staphylococcus aureus:

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a bacterium responsible for several difficult-to-treat infections in humans. It is also called multidrug-resistant *Staphylococcus aureus* and oxacillin-resistant *Staphylococcus aureus* (ORSA). MRSA is any strain of *Staphylococcus aureus* that has developed resistantto beta-lactam antibiotics, which include the Penicillins (methicillin, dicloxacillin and oxacillin, etc.) and the cephalosporins. Strains unable to resist these antibiotics are classified as methicillin-sensitive *Staphylococcus aureus* or MRSA[7].

Hydrocarbon & wax degradation (Micrococcus luteus):

Hydrocarbon utilizing microorganisms are ubiquitously distributed in the marine environment following oils spills, these microorganisms are naturally degrade numerous contaminating petroleum hydrocarbons and cleansing the oceans of oil pollutants. Aromatics with one, two or three aromatic rings are also efficiently biodegraded those with four or more aromatic ring are quite resistant to biodegradation. The application of fertilizer increased rates of biodegradation 3-5 times[8,9].

Production of enzyme by (Bacillus megatarium):

Enzyme assay was performed by DNS method for preparation of standard graph. DNS is 3,5 di nitro salicylic acid which reacts with reducing sugar and itself converted in to 3 amino 5 nitro salicylic acid. DNS act as a stopping agent as well as colouring agent. To stop the reaction DNS will give orange colour solution at λmax 540 nm. In this method maltose was used as a reducing sugar.

Crude enzyme assay:

In this method enzyme reacts with substrate and make enzyme substrate complex after that they will make product.

$$[E] + [S] \longrightarrow [ES] \longrightarrow [P]$$

$$\text{Starch} \longrightarrow \text{Amylase} + \text{Amylopectin}$$

This process is known as saccherification.

Results:

A mixed culture of bacteria were isolated from different areas of Lucknow & out of different isolates there were 4 cultures detected for their industrial uses & that cultures were maintained for optimization of carbon sources, nitrogen sources, metal ions and pH. The antibiotic sensitivity, MSAR, hydrocarbon & wax degradation were also performed.

Serial dilution:

The serial dilution method was performed in order to get pure & reduced number of bacterial colonies & there were different isolates formed & 4 cultures were used for further work.

Figure 1. Showed the mixed culture plate for all samples.

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Colony morphology:

Three samples were used for further analysis:
C_2**-Chemical industry sample(Amausi)** S_4**-soil sample(Chinhat).**
G_3**-Gomtinagar sample.**

Table 1. Colony morphology of isolated cultures

Characteristics	C2	S4	G3
Shape	Circular	Circular	Circular
Colour	White	White	White
Texture	Smooth	Rough	Smooth
Margin	Entire	Lobate	Entire
Opacity	Flat	Flat	Flat
Elevation	Opaque	Opaque	Opaque

Sub culturing:

The procedure of transfer on micro-organisms from their parent growth source to a fresh one or from one medium to another is sub culturing.

Figure 2. Showed sub culturing results for all isolates.

Table 3 showed that for all isolates there were different biochemical tests were performed and some cultures were showing positive as well as negative result according to Bergey's manual.

Table 3. Biochemical analysis of isolated bacterial culture

Biochemical Test	C2-A	C2-B	S4	G4
Gram Staining	-ve rods	+ve cocci	+ve rods	+ve cocci
Catalase Test	+ve	+ve	+ve	+ve
Endospore Test	-ve	-ve	+ve	-ve
Carbohydrate Test	+ve	-ve	-ve	-ve
Mannitol Test	-ve	+ve	-ve	-ve
Glucose Test	+ve,	-ve	-ve	+ve

Confirmatory Test:

- **BHI Oxidase test (Gram -ve rods):**

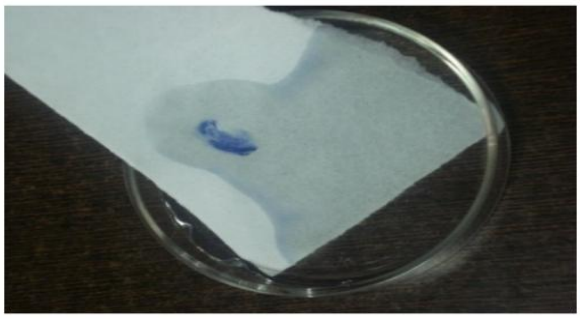

Figure 3. Colour changes showed confirmation

Figure 4. BHI Culture *Pseudomonas* in C_2 culture

Starch hydrolysis test (Gram +ve rods)

To determine the presence or absence of starch in the medium by using iodine solution

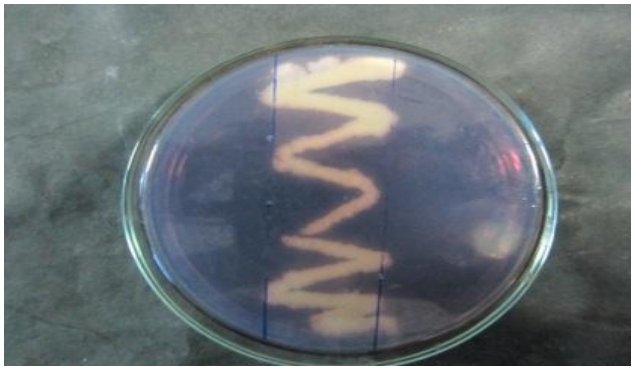

Figure 5. Confirmation of *bacillus* in G_3 culture.

The zone (yellow) showed that this culture was hydrolyzing starch and also blue colour was obtained.

Optimization:

Optimization was performed by using suitable carbon sources, nitrogen sources, pH and metal ions.

Table 4. Optimization of bacterial culture in carbon sources

Cultures	Glucose	Sucrose	Beef extract	Dextrose	Lactose
C2-A	0.163	0.690	0.844	0.143	0.235
C2-B	0.044	0.097	0.446	0.447	0.067

Table 4 showed that best carbon sources were obtained for culture C2A and C2B- Beef extract and Dextrose respectively.

Table 5. Optimization of bacterial culture in nitrogen sources:

Culture	NH_4cl	Urea	Peptone	NaH_2PO_4	Na_2HPO_4
C2-A	0.042	0.006	0.185	0.018	0.022
C2-B	0.407	0.112	0.190	0.112	0.085

Table 5 showed that best nitrogen sources were obtained for culture C2A and C2B- Peptone and Ammonium chloride respectively.

Table 6: Optimization of bacterial culture in metal ions

Culture	$CuSO_4.5H_2O$	$Pb(NO_3)_2$	$FeSO_4$	$CaCl_2$	$ZnCl_2$
C_2 A	0.532	0.690	0.332	0.530	0.332
C_2 B	0.170	0.486	1.270	0.175	0.294

Table 6 showed that best metal ions were obtained for culture C2A and C2B Lead nitrate and Ferrous sulphate respectively.

Table 7. Optimization of bacterial culture at different pH

Culture	pH5	pH7	pH9	pH11
C2A	0.415	0.610	0.098	0.075
C2B	0.005	0.114	0.150	0.015

Table 7.Sshowed that best pH was obtained for culture C2A and C2B-pH 7 and pH 9 respectively.

Applications: Antibiotic sensitivity test for Pseudomonas for culture S. aureus.

Antibiotic sensitivity test against various pathogens by using production media.

S. aureus *E. coli* *B. subtilis*

Figure 6. Showed Antibacterial activity against various pathogens

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Biosurfactant activity:

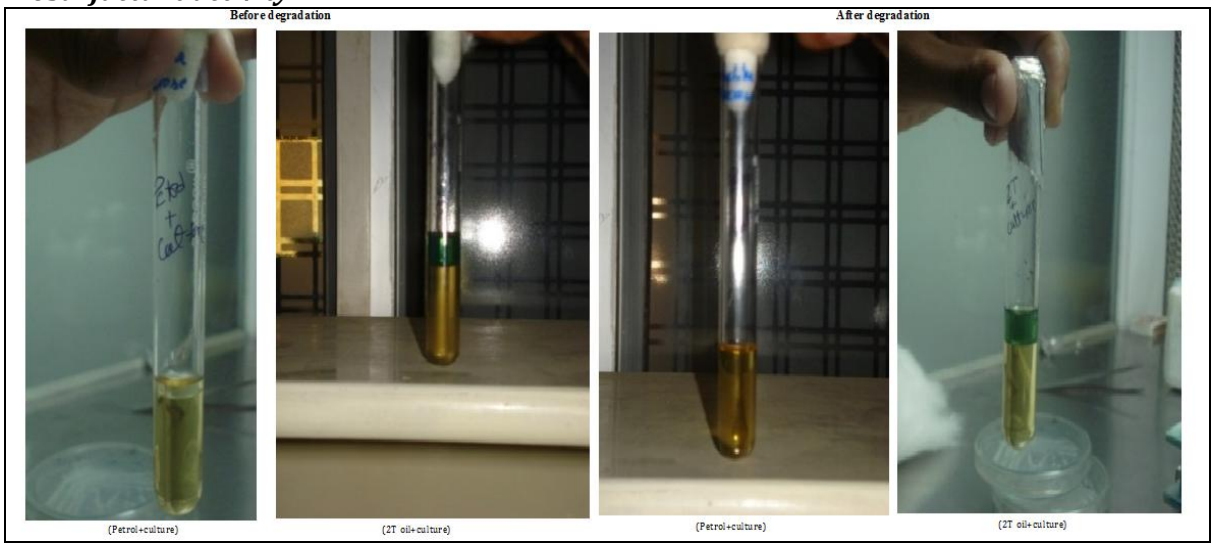

Figure 7. Biosurfactant activity against petrol & 2-T oil and best result is obtained in petrol.

Methicillin-resistant *Staphylococcus aureus:*

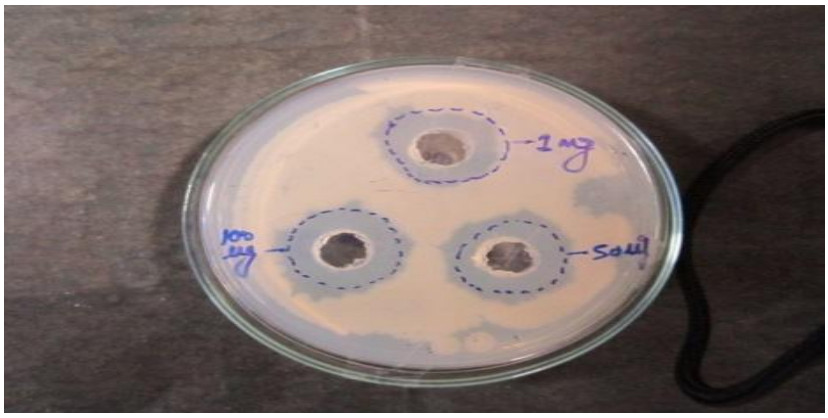

Figure 8. Shows zone of inhibition against antibiotic(amoxycillin)Conc.(1mg,100µg,50µg).

Hydrocarbon & wax degradation (*Micrococcus luteus*):

Figure 9. Wax degradation

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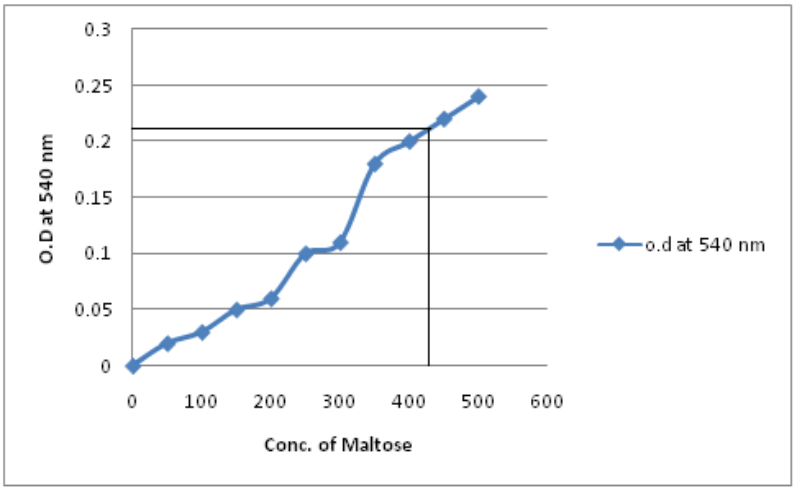
Table 7. Enzyme Assay by DNS Method

S.No.	Vol of Maltose (µl)	Vol of D/W (µl)	Concentration of Maltose	Vol. of DNS	Incubation Time	Vol. of D/w	O.D at 540 nm.
1	0	1000	0	1ml	10 min in	5ml	0
2	100	900	50	1ml	Boiling	5ml	0.02
3	200	800	100	1ml	Water	5ml	0.03
4	300	700	150	1ml	Bath	5ml	0.05
5	400	600	200	1ml		5ml	0.06
6	500	500	250	1ml		5ml	0.10
7	600	400	300	1ml		5ml	0.11
8	700	300	350	1ml		5ml	0.18
9	800	200	400	1ml		5ml	0.20
10	900	100	450	1ml		5ml	0.22
11	1000	0	500	1ml		5ml	0.24

Table 8. Enzyme Activity

S.No.	Vol. of [E]	Vol. of [s]	Incubation time in RT	Vol. of DNS	Incubation in B.W.B.	Vol. of D/w	O.D .
1	0.5ml	0.5ml	15 min	1ml	10 min	5ml	0.00
2	-	-	-	1ml	10 min	5ml	1.05



Graph 1. Measurement of Enzyme activity

Enzyme activity of test tube = mg maltose released*0.36/vol. of enzyme used /incubation time.

1.05*0.36/0.5/15= 0.0504mg/ml/min

Enzyme activity of test sample in production media= mg maltose released*0.36/vol. of enzyme used/incubation time.

2.15*0.36/0.5/15= 0.103mg/ml/min.

Discussion

The microbes are the most successful group of all living species occupying each habitat in water, soil, plants and animals including humans with enormous success. This leads to a fundamental impact on all research areas in modern biology and medicine.

Biotechnologically designed and employed microorganisms for applications in food industry, chemistry and pharmacy significantly increase the importance. Because of their small size sophisticated technology is required for detection and characterization.

As importantly use of microflora in industrial area is beneficial for humans as they are used in pharma industry for producing antibiotics which can be obtained through useful microbes. Likeway, some more microbes are used for enzyme production, wax degradation, hydrocarbon degradation etc. The present study is carried out for Optimization, production and characterization of importantly microflora used in industry which is isolated from various soil and water sample.

The optimization involved providing suitable condition for growth and the parameters used for optimization were pH, carbon, nitrogen sources and metal ions by replacing in production media as the suitable culture conditions of 3 isolated were obtained. According to result basis the suitable for *Pseudomonas*, *Staphylococcus*-Carbon sources (1%): Dextrose, sucrose, glucose, lactose & beef extract. Nitrogen sources(1%)-Na_2HPO_4, urea, peptone, NH_4Cl, NaH_2PO_4.Metal ions (0.2%)-$FeSO_4$,$CaCl_2$,$Pb(NO_3)_2$,$ZnCl_2$ & $CuSO_4.5H_20$. pH-5,7,9 &11 and same as for *Bacillus &Micrococcus*[11].

According to result basis total 10 cultures were isolated and out of 10 only 4 cultures were identified through Bergey's manual. Further production and optimization was performed and obtained cultures were *Pseudomonas aeruginosa, Staphylococcus aureus, Micrococcus luteus* and

Bacillus megaterium and their applications in industries[12].

Conclusion

Finally based on the above study it can be concluded that at the end of all the experiment it was identified that out of all the bacterial cultures isolated from various types of area, for were industrial used microflora. Isolate C_2 (a) are gram –ve rods C2 (b) are gram +ve cocci, G_3 are gram +ve cocci & S_4 are Gram +ve rods. All these four isolated bacterial cultures are catalyse +ve. Isolates are identified as, C_2 (a) was identified as *Pseudomonas aeruginosa,* C_2(b) was *Staphylococcus aureus,* G3 was *Micrococcus luteus* & S_4 was *Bacillus megaterium.* All these four isolates are importantly used in industries for commercial purpose because of their application. At present time many industries are using antibiotics (Pharma industry) that antibiotic can be obtained through Microbes, only like this way some of the microbes are used for MRSA, Enzyme Production, Hydrocarbon & Wax degradation etc.
After optimization and characterization of microbes, its application was observed that the isolated cultures are bacteriostatic in nature.

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