Assay of population density of amylase producing bacteria from different soil samples contaminated with flowing effluents

Bahadure R.B., Agnihotri U.S. and Akarte S.R.
Department of Zoology, Vidya Bharati Mahavidyalaya Amravati.444 602, rgene1@yahoo.co.in & srakarte@rediffmail.com

Abstract- Industrial microbiology deals with soil terms with life, from the distinct viruses to larger bacteria, fungi, algae and protozoa, up to macroscopic worms and insects. Many microorganisms that live in the soil play indispensable role in maintaining life of this planet degrading or chemically modifying molecules. Considerable human interest in soil organisms stems from their ability to synthesize a variety of useful chemicals. Environmental conditions affect the density and composition of the flora of the soil.
Academic observation can yield practical possibilities and industrial fermentation industries often keep close watch on theoretical studies being carried out in laboratories. Industrial microbiology concern itself with the isolation and description of microorganism from natural environments, such as soil or water. The population density of microorganisms based on their survival and their enzyme producing activity can be primarily screened along with its propagation activity. Considering this soil sample from different stations were collected to observe the amylase producing activity of bacterial population and thereby further identifying different types of such bacteria in effluent contaminated soil and water.

Introduction

The early detection of small percentage of useful microorganisms that are presence in the soil and water can be screened primarily for detection and isolation of only those microorganisms which are of interest from among the large microbial population. Many successful industrial fermentation processes heavily depend on enzymes from microorganisms. Amylases are produced by variety of living organisms, ranging from bacteria to plants and humans. Bacteria and fungi secret amylases to the outside for their cells to carry out extra cellular digestion. These amylases are employed commercially for preparation of sizing agents and removal of starch sizing from woven cloth, preparation of starch sizing plate's liquefaction of heavy starch pastes formed during heating steps in the manufacture of corn and chocolate, syrups, production of breads, in brewing industry. These Bacteria are screened from natural resources, for its ability to grow on cheap substrates, producing enzymes at high stable rate, no toxic substances. So for study of the population density of amylase producing bacteria, a survey of soil samples near by flowing effluents was done.

Materials and Method

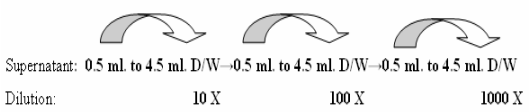
Collection of Samples:
Soil samples were collected from different spots along the bank of flowing effluent through local stream of water for the study of enzyme producing microorganisms. The locations selected were from and around the Amravati city and other places away from the Amravati city where the flowing water is discharged outside the city.
Preparation of culture media for Microorganisms:
A culture media of Agar solution with Carbon sources and Salt solution-prepared kept in Autoclave and then inoculated on pouring plates.

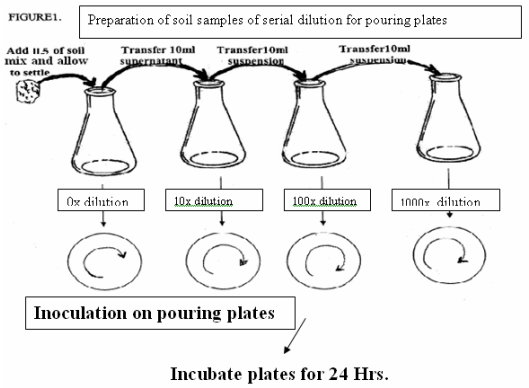
Preparation of Soil extract:
After collecting the soil samples from various localities in the Amravati City.

→ 0.1 gm. of soil sample + 10 ml. of doubled D/W.

→ Mix thoroughly by vortexer and incubate it for 30 min. at 37^0 C in incubator.

→ Allow it to settle, and use 0.2 ml. of the supernatant for plating directly for serial dilution use sterile doubled D/W, and dilute as shown bellow.





For Inoculation 0.2 ml of each dilution on Carbon source of plates. Incubate at 37^0 C for 1 to 2 days. Count the number of colonies in each plate of dilutions.
Staining of plates for Amylase producing Bacteria:
Iodine / Potassium Iodide:-
0.2 % I_2 in 2 % KI in doubled D/W (leave it on a shaker to dissolve iodine). After 24 hrs. of growth

the starch plates are flooded with the above iodine solution cleared zones are seen around amylase producing colonies under blue background.

Discussion and Conclusion

So it is far necessary to screening the soil and water bacteria for their amylase, cellulase production which could be a major source of enzyme after pure culture on large scale. The productions of enzymes for cost effectiveness can be processed by chemicals and antibiotics adopt three different strategies are as fallows.

i) Proper selection of strain.
ii) Improvement of strain for mutates the existing strain.
iii) Modification of strain for molecular cloning of genes for overproduction of enzymes.

The most attention is paid forwards an enzyme producing bacteria in recent years because of their great potential for microbiological and Biotechnological exploitation on the basis of following criteria

Screening for microorganisms in soil for starch degrading enzymes.
Screening for organic acids.
Screening for antibiotics.
Production of antibiotics against specific microorganisms.
Screening for lipase producing microorganisms.
Production of cellulase degrading enzymes.

The preliminary screening of environmental bacterial has prime importance as the soil and water are the major sources for varieties of bacteria as well as their enzyme producing activity. The first starch-degrading enzyme alkaline amylase was produced in Horikoshi-II medium by cultivating alkaliphilic *Bacillus species.* Strain A-40-2(Horikoshi, 1971). The second report of alkaline amylase producing three alkaliphilic *Bacillus strain* reported by McTigue *et al.* 1994. Kimura and Horikoshi *et al.* 1990, isolated at number of starch-degrading psychrotrophic microorganisms from the environment. Two highly alkaliphilic pullulanase producing bacteria were isolated from Korean soil (Kim et al. 1953). Matsuzawa *et al.* 1975, estiblished the industrial production of cyclodextrin (CD), by using crude of Cyclomaltodextrin Glycosyltransferase (CGTase) which is bacterial Origin produce cyclodextrin. Georganta *et al.* 1993 isolated CGTase producing Psychrophilic alkaliphilic bacteria from samples of deep-sea bottom mud.

References

[1] Georganta G., Kaneko T., Kudo T. and Horikoshi K. (1993) *Starch* 45:95-99.
[2] Horikoshi K.(1971) *Agric.Biol.Chem.* 35:1783-1791.
[3] MatsuzawaM.,.Kawano M., Nakmura N. and Horikoshi K. (1975) *Starch* 27:410-413.
[4] McTigue M. A., Kelly C.T., Fogarty W.M. and Doyle E.M. (1994) *Biotecnol. Lett.* 16:569-574.

Observations and Results

Sample No.	Selection Plate	Volume (ml)	Dilution Number of colony counted			
			0x	10x	100x	1000x
1. Chattri Lake	Soil plate	200 (ml)	187	56	50	30
2. Pedhi River	Soil plate	200 (ml)	221	152	98	44
3. Chudaman River	Soil plate	200 (ml)	155	133	102	91
4. College (Botanical Garden).	Soil plate	200 (ml)	110	55	33	25
5. Ambanala Origin (Wadli Lake).	Soil plate	200 (ml)	202	192	180	167
6. Ambanala Ending.	Soil plate	200 (ml)	314	283	262	187
7. Industrial Effluent Origin.	Soil plate	200 (ml)	290	269	241	224
8. Industrial Effluent Ending.	Soil plate	200 (ml)	357	320	246	170

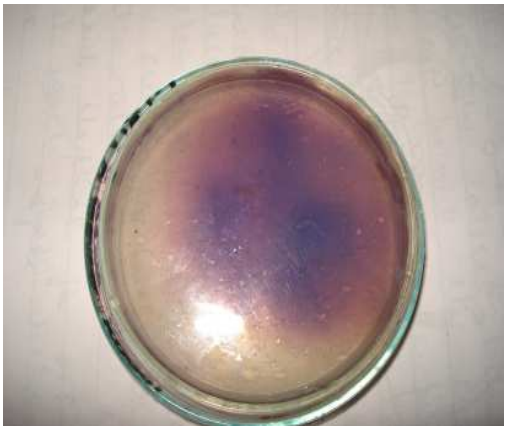
Staining the plates for Amylase producing Bacteria:(Soil Samples)

Chatri Lake (1000 XDilution)

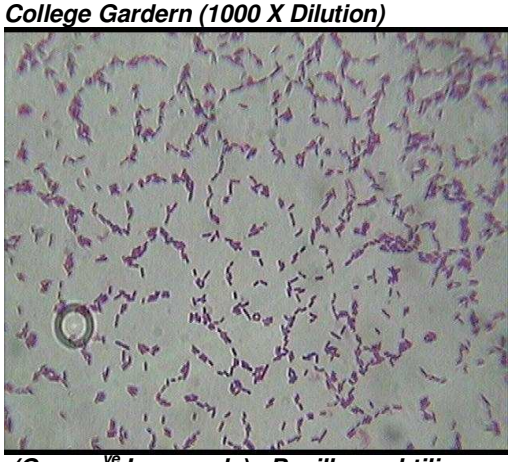
Pedhi River (1000 X Dilution)

Chudaman River (1000 X Dilution)

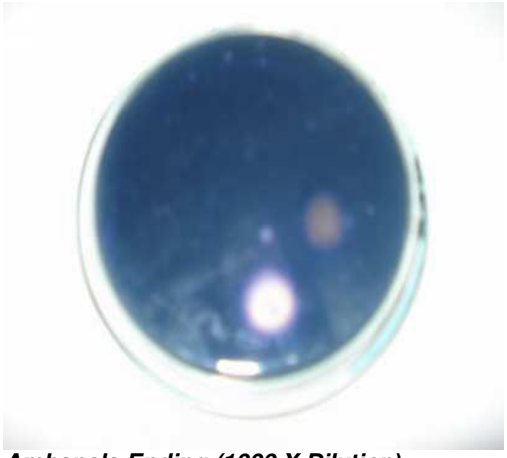
College Gardern (1000 X Dilution)



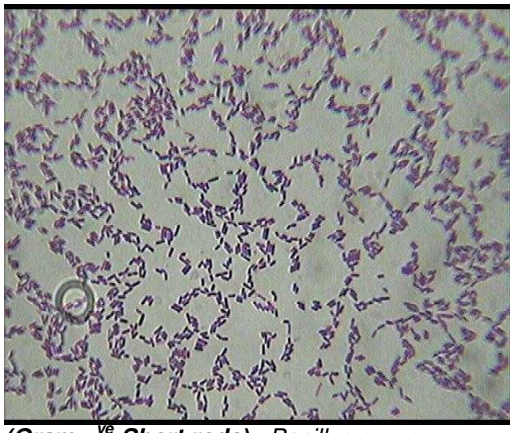
Ambanala Origin (1000 X Dilution)



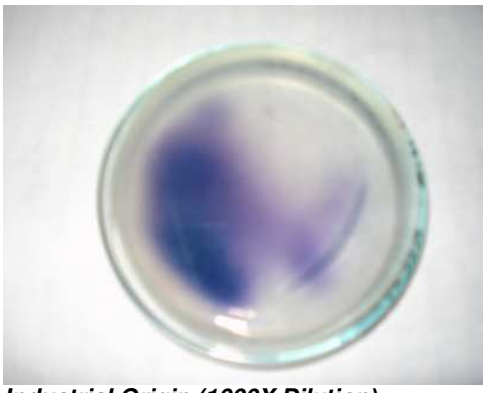
(Gram +^{ve} Long rods) –Bacillus subtilis



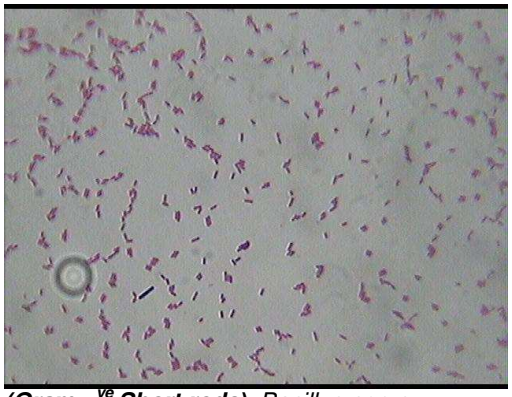
Ambanala Ending (1000 X Dilution)



(Gram +ve Short rods) *–Bacillus cerus*

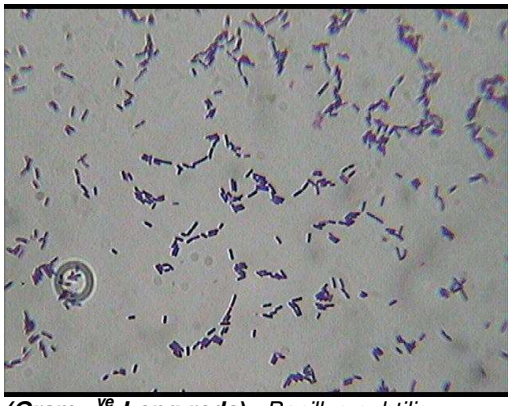


Industrial Origin (1000X Dilution)



(Gram +ve Short rods)*–Bacillus cerus*

Industrial Ending (1000 X Dilution)



(Gram +ve Long rods) *–Bacillus subtilis*