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Identification of Appropriate Sample and Culture Method for the Isolation of Thermophilic Bacteria from Automobile Radiators

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I. Introduction

Microorganisms can be grouped into broad categories according to their temperature ranges for growth; psychrophiles, mesophiles, thermophiles and hyperthermophiles. Thermophiles are microorganisms that thrive at relatively high temperatures, between 45 °C and 80 °C. During the past few years, most research on the microbes of hot springs has concentrated on the cultivating and isolating of extreme thermophilic and acidophilic strains (Belkova *et al*., 2007). It is generally believed that at high temperature, biomolecules such as enzymes denature thereby losing their function and hence, stopping the metabolism. Also, the fluidity of membranes increases significantly, disrupting the cell.

The molecular basis for adaptations of thermophilic organisms to extreme environments is to prevent denaturation and degradation. Their membrane lipids contain more saturated and straight chain fatty acids than do mesophiles, which grow typically between 15 °C and 40 °C (Ulrih *et al*, 2009). This allows thermophiles to grow at higher temperatures by providing the right degree of fluidity needed for membrane function. The presence of chaperones which refold denatured proteins increase the stability of thermophilic proteins (Jaenicke *et al*, 1996). Also, thermophilic proteins appear to be smaller and in some cases more basic, which may also result in increased stability (Kumar *et al*, 2001).

The study of extreme environments has considerable biotechnological potential. For example, the two thermophilic species *Thermus aquaticus* and *Thermococcus litoralis* are used as sources of the enzyme DNA polymerase, for the polymerase chain reaction (PCR) in DNA fingerprinting, etc. The enzymes from these organisms are stable at relatively high temperatures, which is necessary for the PCR process which involves cycles of heating to break the hydrogen bonds in DNA and leave single strands that can be copied repeatedly. Another thermophile, *Bacillus stearothermophilus* (temperature maximum 75°C) has been grown commercially to obtain the enzymes used in 'biological' washing powders. Extremophilic microorganisms, especially thermophilic bacteria, can facilitate the enzymatic degradation of polymeric substrates such as starch, cellulose, xylan, pectin and chitin (Stetter *KO*, 1996; Kristjansson *JK* and Hreggvidsson *GO*, 1995; Bertoldo *C* and Antranikian *G*, 2001).

Hence, the current study was carrying out with the objective to isolate and identify thermophilic organisms from automobile radiator.

II. Materials and Methods

a) Collection of Sample

Water sample from three different automobile radiators; Honda, Jetta and Passat were collected using sterile syringe, one for each and labeled as sample A, B and C accordingly. The samples were immediately taken to the laboratory for subsequent sampling to reduce the proliferation of mesophilic organisms.

b) Sampling of Thermophilic Microorganisms from Automobile Radiators

0.5ml of each sample recovered by sterile syringe was transferred to each of the bottles, consisting of 10 ml sterilized nutrient broth (pH 7.65) in a

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McCartney bottles which were labeled accordingly. The bottles were incubated in a water bath at 60°C for 7 days. A control medium consisted of sterilized nutrient broth only and was incubated in the water bath alongside with the samples. Growth was followed by measuring the turbidity at 600nm at the 7th day of incubation.

c) Isolation of Thermophilic Organisms

After the 7th day of incubation in water bath, the cultures were introduced into a prepared plate containing nutrient agar using streak method and incubated for 2 days. Isolation of pure culture was done using spread plate method and streak plate method recommended by Rath and Subramanyam (1998).

d) Characterization and identification of microorganisms

Colonial characteristics of the bacterial isolates were determined using parameters such as size, elevation, pigment, surface, opacity, edge and shape. Cellular characteristics of the isolates were determined through the following experiments:

e) Gram's staining

Smear of each bacterial isolate was prepared on a clean slide. In preparing the smear a drop of sterile distilled water was placed in the middle of the slide. A sterilized inoculating needle was used to pick from the bacterial colony and rubbed on the slide containing a drop of sterile distilled water. The bacterial cells were spread into a thin smear, air dried and heat fixed (Fawole and Oso, 2001).

The heat fix smear was stained with crystal violet for 1 to 2 minutes after which the stain was poured off. The smear was rinsed off with Gram's iodine and the iodine was allowed to react for 1 minute with the smear. The slide was then washed with 95% alcohol until the violet was seen to stop running from the slide. The slide was rinsed with gentle running tap water and counterstained with safranin for 1 to 2 minutes.

The slide was rinsed with water, blotted dry and examined under microscope with oil immersion (Olutiola *et al.,* 1991). Gram positive cells appeared purple while gram negative cells appeared pink.

f) Motility Test

The hanging-drop method was used to determine the motility of the bacterial isolates. A little Vaseline was placed around the edge of the hallow of a clean cavity slide. A loopful of each isolate was transferred to the center of a clean coverslip laid on the bench. The cavity slide was carefully inverted over the coverslip and the slide was pressed down gently in order to seal the coverslip with the slide.

The unit was then inverted in such a way that the loopful of the bacterial colony was in hanging position. The preparation was examined immediately under the X40 objective lens. The microscopy was done quickly in order to avoid excessive illumination, which could quickly cause the organism under study to lose motility (Olutiola *et al.,* 1991).

Motile cells came in view and were seen moving rapidly in the field while non-motile were not moving.

g) Spore Staining

Heat-fixed smear of each isolate was prepared in a slide. Malachite green solution was added to the smear and steamed for 10 minutes. The stain was not allowed to dry out. The stain was then washed off with cold water.

The smear was counterstained with safranin solution for 15 seconds. It was wash with water, blotted dry and examined under the microscope with the oil-immersion objective (Olutiola *el al.,* 1991). Spores appeared green and bacterial cells appeared red.

h) Capsule Staining

Air-dried smear of each isolate was prepared and fixed on a slide. Crystal violet was applied on the slide for 2 minutes and the slide was then steamed for 40 minutes. The crystal violet was rinsed off with copper sulphate solution. Each slide was blotted carefully, dried in the air and examined under the microscope using oil immersion lens (Olutiola *el al.,* 1991). Bacterial cells appeared deep violet while the capsules appeared pale violet.

i) Catalase Test

A thick emulsion of each test organism was prepared on a clean slide. Several drops of 3% hydrogen peroxide were added on each of the slides. A positive result was indicated by effervescence which was caused by the liberation of oxygen gas as a result of catalase production by the bacterium. There were no gas bubbles in the bacteria that do not produce catalase (Fawole and Oso, 2001).

j) Oxidase Test

A filter paper was soaked in 1% sodium oxalate solution. A portion of each bacterial colony was picked and rubbed on the filter paper. A blue colour change within 10 seconds indicated the production of the enzyme oxidase.

k) Methyl Red Test

10 ml of glucose phosphate broth was prepared into different test tubes. The test tubes were then inoculated with different bacterial isolates. The test tubes were incubated for 3 days at 37^{0}C. After 3 days, 5 drops of methyl red indicator was added to 5 ml of each cultured broth. Acid production was indicated by a yellow colouration.

l) Indole Test

One of the end products of the amino acid tryptophan hydrolysis is indole. Some microorganisms are capable of hydrolyzing tryptophan to give indole. Production of indole revealed the possession of the enzyme tryptophalase by the test organism.

1% tryptone broth was prepared in different test tubes. The test tubes were inoculated with each

bacterial isolate. The tubes were then incubated for 48 hours at 35^{0}C. After incubation, 2 ml of chloroform was added to each broth culture and was shook gently.2ml of Kovac's reagent was added to the broth culture and shook gently. The tubes were allowed to stand for 20 minutes in order to permit the reagent to rise to the top. A red colour at the reagent layer indicated indole production (Fawole and Oso, 2001).

m) Starch Hydrolysis

Starch agar was prepared following manufacturer's instructions. The agar was poured in sterile Petri dishes and allowed to set. The plates were inoculated with each test organism using streaking method. The plates were incubated for 48 hours at 37^{0}C. After incubation, each of the plates was flooded with iodine solution. There was no bluish black colour in the plates of the bacteria that utilized starch while bluish black colour was observed in the plates of the bacteria that did not utilized starch. Starch utilization indicated the possession of the enzyme amylase by the test organism (Fawole and Oso, 2001).

n) Citrate Utilization

Some bacteria are capable of utilizing citrate as the sole carbon source. In the test, citrate agar plates were inoculated with the bacterial isolates using streaking method. The plates were incubated at 37^{0}C for 24 hours. Colour change from green to blue on the plates indicated citrate utilization by the test organisms.

o) Sugar Fermentation

The sugar tested for fermentation included fructose, maltose, lactose, sucrose and glucose. Nutrient broth containing 0.5% of each of the sugar was prepared. Two drops of 0.01% phenol red indicator was added to each of the broth media. 10ml of each of the broth media was dispensed into test tubes containing inverted Durham tubes. The media setup was sterilized by steaming for 30minutes on three successive days.

Each indicator-sugar-broth was inoculated with a loopful of each of the bacterial isolates. One test tube of each medium was left uninoculated as a control. The test tubes were incubated at 35^{0}C for 4 days.

After incubated it was seen that growth occurred in the inoculated tubes while growth did not occur in the uninoculated tubes. The change in colour from red to yellow indicated acid production and the presence of air space in the Durham tubes indicated gas production (Fawole and Oso, 2001).

p) Oxygen Relationship

MacCarney bottles containing sterile nutrient agar were used. The bottles were inoculated while at semi-solid state with each of the bacterial isolate using stabling technique. The agar in the MacCartney bottles were allowed to solidify and incubated at 37^{0}C for 48 hours. Anaerobes grew at the bottom of the bottles, aerobes grew on the surface and facultative anaerobes grew from the bottom through the bottles to the top.

III. Result and Discussion

Out of 3 samples cultured, all samples grew on nutrient broth and nutrient agar and they showed a clear distinction compared to the control. The growth rate (Turbidity) of the thermophilic organisms in the nutrient broth at the 7th day of culturing were determined spectrophotometrically and the results of the optical density (OD) were 0.937, 0.897, 0.140 and 0.050 for sample A, B,C and Control respectively as shown in Fig.1.

The result of the OD showed that sample A has the highest growth rate follow by sample B and C. The increasing order of the growth rate is; Control < Sample C < Sample B < Sample A .

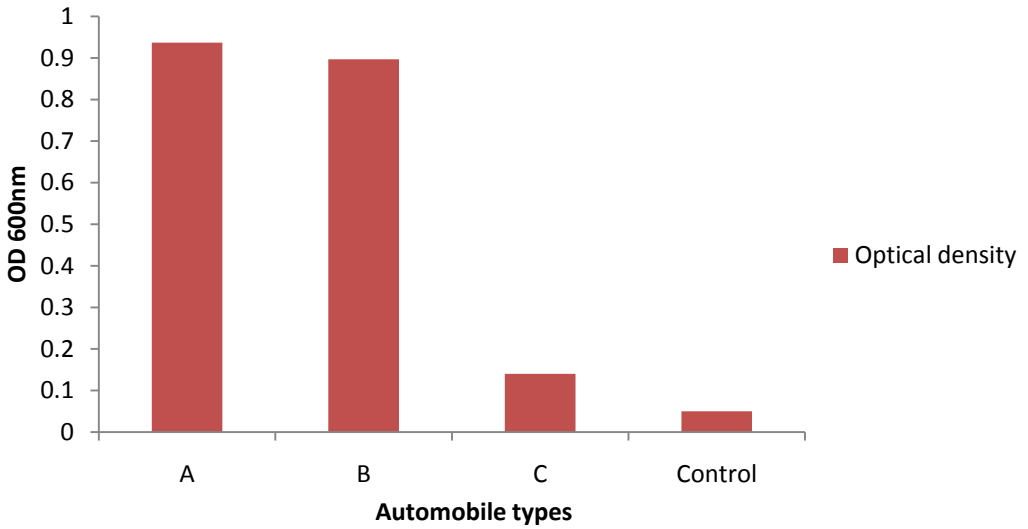

Figure 1 : Growth rate of bacteria from water samples of automobile radiators after 7days.

The bacteria showed some morphological and biochemical characteristics which were summarized in the Tab.1 below. Pure culture isolates were observed and identified from each of the sample cultured on nutrient agar while no growth was observed in the control. *Thermoanaerobacter* spp was isolated from sample A while *Clostridium* spp was isolated from sample B and C.

Table 1 : Colonial morphology, cellular morphology and biochemical characteristics of the bacterial isolates.

Isolate	Cellular shape	Colonial elevation	Colonial edge	Colonial opacity	Colonial surface	Colonial pigmentation	Cellular arrangement	Gram's staining	Motility test	Spore staining	Capsule staining	Catalase test	Methyl red test	Starch hydrolysis	Citrate utilization	Oxygen reaction	Action on simple carbohydrates					probable microorganism
																	Lactose	Glucose	Sucrose	Maltose	Fructose	
A	Rod	Raised	Lobate	Opaque	Smooth	Creamy White	Clusters	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	FAN	A	A	A	A	AG	*Thermoanaerobacter* species
B	Rod	Raised	Entire	Opaque	Rough	Cream	Chain	-ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve	AN	A	AG	A	AG	A	*Clostridium* species
C	Rod	Raised	Entire	Opaque	Rough	Cream	Chain	-ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve	AN	A	AG	A	AG	A	*Clostridium* species

IV. Conclusion

From our experiment, we were able to isolate rod shaped, obligately anaerobic and facultatively thermophilic bacteria belonging to the genera *Thermoanaerobacter* and *Clostridium* from automobile radiators. These organisms can be used in the production of various enzymes for industrial and biotechnological importance.

References Références Referencias

1. Bertoldo C, Antranikian G: Amylolytic enzymes from hyperthermophiles. *Methods Enzymol* 2001, 330:269-289.
2. Belkova NL, Tazaki K, Zakharova JR, Parfenova VV (2007). Activity of bacteria in water of hot springs from Southern and Central Kamchatskaya geothermal provinces, Kamchatka Peninsula. Russ. *Microbiol. Res.*, 162(2): 99-107.
3. Collins, M. D., P. A. Lawson, A. Willems, J. J. Cordoba, J. Fernandez- Garayzabal, P.
4. Fawole, M.O. and Oso, B.A. (2001) 'Laboratory manual of microbiology' Spectrum Books Limited, Ibadan, Nigeria.
5. Garcia, J. Cai, H. Hippe, and J. A. E. Farrow. 1994. The phylogeny of the genus *Clostridium*: proposal of five new genera and eleven new species combinations. *Int. J. Syst. Bacteriol.* 44:812–826.
6. Jaenicke, R. et al. (1996) Structure and stability of hyperstable proteins. *Adv. Protein Chem.* 48, 181–269.
7. Kristjansson, J.K. and Hreggvidsson, G.O. (1995). Ecology and habitats of Extremophiles. *World J Microbiol Biotechnol.* 11 : 17-25.
8. Lee, Y. E., M. K. Jain, C. Lee, S. E. Lowe, and J. G. Zeikus. 1993. Taxonomic distinction of saccharolytic thermophilic anaerobes: description of *Thermoanaerobacterium xylanolyticum* gen. nov., sp.nov., and *Thermoanaerobacterium accharolyticum* gen. nov., sp. nov.; reclassification of *Thermoanaerobium brockii, Clostridium thermosulfurogenes, and Clostridium thermohydrosulfuricum* E100-69 as *Thermoanaerobacter brockii* comb. nov., *Thermoanaerobacterium thermosulfurigenes* comb. nov., and *Thermoanaerobacter thermo-hydrosulfuricus* comb. nov., respectively; and transfer of *Clostridium thermohydrosulfuricum* 39E to *Thermoanaerobacter ethanolicus. Int. J. Syst. Bacteriol.* 43:41–51.
9. Olutiola,P.O., Famurewa, O. and Sonntag, H.G. (2000). An Introduction to microbiology, a practical Approach. Tertiary Text Book series.
10. Rath CC, Subramanyam VR (1998). Isolation of thermophilic bacteria from hotsprings of Orissa, India. Geobios, 25(2-3): 113-119.
11. S. Kumar, R. Nussinov, How do thermophilic proteins deal with heat? Cell. Mol. *Life Sci.* 58 (2001) 1216–1233.
12. Stetter KO (1996) Hyperthermophilic procaryotes. *FEMS Microbiol Rev* 18:149-158.

13. Wiegel, J. 1986. Genus *Thermoanaerobacter*, p. 1379–1383. In P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.), *Bergey's manual of systematic bacteriology*, vol. 2. The Williams and Wilkins Co., Baltimore, Md.
14. Wiegel, J. 1992. The obligate anaerobic thermophilic bacteria, p. 105–184. In J. K. Kristjannson (ed.), Thermophilic bacteria. *CRC Press, Boca Raton, Fla*.