Novel Thermophilic Anaerobes Phylogenetically Related to Genera Thermoanaerobacter and Clostridium Isolated from Automobile Radiators

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Abstract— This study was based on the isolation of thermophilic bacteria from automobile radiators. Water sample from three different automobile radiators were used for the screening of thermophilic bacteria at 70°C and pH 7.65 using nutrient broth medium and their turbidity were measured spectrophotometrically at 600nm. Results of this study suggest that optimal isolation rates of thermophiles from automobile radiator samples were achieved by culturing the sample on nutrient broth at high temperature and isolation on nutrient agar.

.**Keywords — Automobile radiators, thermophilic bacteria, *Thermoanaerobacter,* Clostridium**

1 INTRODUCTION

The realization that extreme environments harbor different kinds of prokaryote lineage has resulted in a complete reassessment of our concept of microbial evolution and has given considerable impetus to extremophile research [9], [3]. Extremophlic microorganisms especially thermophilic bacteria have developed a variety of molecular strategies in order to survive in harsh conditions. Thermophiles are microorganisms that thrive at relatively high temperatures, between 45 °C and 80°C. 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 thereby 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. 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

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the stability of thermophilic proteins [4]. Also, thermophilic proteins appear to be smaller and in some cases more basic, which may also result in increased stability [8].

A number of thermophiles, belonging to different taxonomic groups, produce thermostable enzymes. This class of enzyme is important not only for the study of biocatalysis and protein stability at extreme conditions but also for the many biotechnological opportunities they offer. This group of organism facilitate enzymatic degradation of polymeric substrates such as starch, cellulose, xylan, pectin and chitin

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

2.0 MATERIALS AND METHODS

2.1 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 respectively. The samples were immediately taken to the laboratory for subsequent sampling to reduce the proliferation of mesophilic organisms.

2.2 Sampling of Thermophilic Microorganisms from Automobile Radiators

0.2ml 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 McCantney bottles which were labeled accordingly. The samples were incubated in a water bath at 70°C for 7 days. A control medium consisted of sterilized nutrient broth only 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.

2.2 Isolation and biochemical identification 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 [7].

The thermophilic isolates were identified by the use of conventional methods for the presumptive identification by biochemical tests.

3.0 RESULT AND DISCUSSION

3.1 RESULT

Out of 3 samples cultured, all samples grew on nutrient broth and nutrient agar and they showed a clear growth 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 0.743nm, 0.427nm, 0.130nm and 0.050nm for sample A, B,C and Control

respectively as shown in Fig.1 below.

The result of the optical density (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 .

3.1.1 Description of *Thermoanaerobacter* and *Clostridium* spp.

The bacteria showed some morphological and biochemical characteristics. 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. *Clostridium* is a genus of Gram-positive bacteria, belonging to the *Firmicutes*. They are obligate anaerobes capable of producing endospores. Individual cells are rod-shaped with positive motility, negative lactose fermentation, lipase activity as opposed to positive indole production.

The genus *Thermoanaerobacter* falls into Clusters V in the phylogenetic interrelationship of *Clostridium* [2]. Cells are curved rods, 3–7 X 0.5μm, obligately anaerobe and gram positive. Growth substrates are glucose, fructose, lactose, rhamnose, arabinose, xylose, sorbitol, inositol, mannitol, sucrose, lactose, maltose, starch, pectin, pyruvate, and lactate. No growth on cellulose, succinate, citrate, formate, acetate. The major metabolic products are H_2, CO_2, and acetate; minor amounts of ethanol are produced along with trace amounts of lactate.

The thermophilic, anaerobic, endospore-forming bacteria are currently placed in the genera *Clostridium*, *Desulfotomaculum, Thermoanaerobacter, Thermoanaerobacterium, Caloramator, and Moorella* [2], [5], [10], [11]. Ecological studies have showed that both aerobic and anaerobic species and many morphological and physiological types of microorganisms can exist in thermophilic environments [1], [6]. This report is in accordance with the thermophilic organisms isolated during this experiment.

4.0 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.

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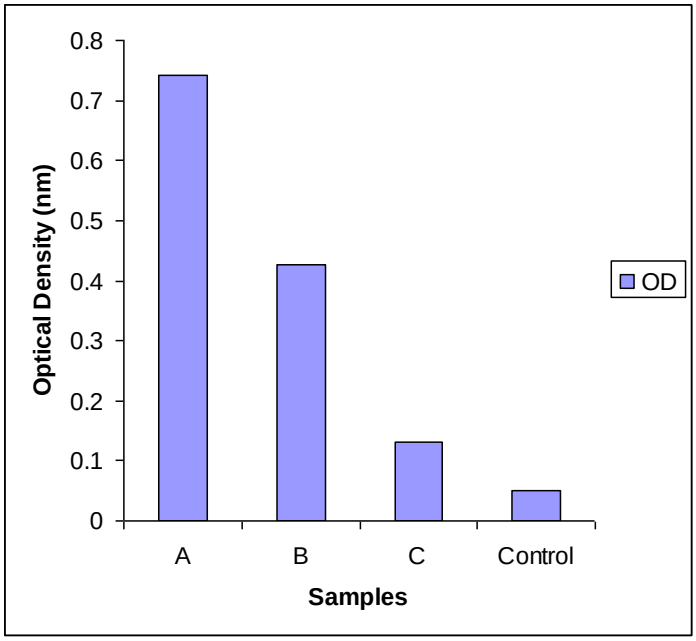

Fig 1. Result of the growth (turbidity) of the organisms in each sample and in comparison with the control.