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Full Length Research Paper

Identification of appropriate sample and culture method for isolation of new thermophilic bacteria from hot spring

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The purpose of this study was to identify appropriate samples and culture techniques on the isolation of thermophiles from local hot spring. Samples obtained from hot spring were used to assess the effects of sample type (water, biomat and sediment) and culture technique (on site or in laboratory after shipping in thermo flask) on thermopiles isolation rate. Overall, isolation rates were higher from sediment and biomat than from water sample. Isolation of thermophiles also occurred better when culture in laboratory compared with on site culture technique. Results of this study suggest that optimal isolation rates of thermophiles from sediment sample are achieved by culturing the sample on nutrient agar or broth at high temperature. For samples that cannot be processed immediately, acceptable handling techniques include storage at refrigeration for up to 5 days, shipping on thermo flask also recommended.

Key words: Thermophilic microorganisms, isolations, hot spring.

INTRODUCTION

Thermophilic microorganisms have received a great interest in recent years because they are not usually denatured by high temperature, even active at elevated temperature (Becker et al., 1997; Beg et al., 2000; Lee et al., 1999; Sonnleitner and Fiechter, 1983). Enzymes from these microorganisms also got special attention from the scientist from all over the world since this enzymes resistant to chemical reagents and extreme pH values in comparison to their mesophilic homologues. More recently, study of the molecular and physiological properties of extremophiles became of interest, therefore many studies devoted to the comprehension of molecular basis of the adaptation to high temperature. To date most of the scientist currently work to offer new hyperthermostable enzymes obtained from thermophilic microorganisms which catalyze the polymerization of DNA. Isolation and identification of the thermophilic microorganisms from hot spring is the initial task to achieve this goal. Hence the current study was carrying out with the objective to identify the appropriate spot in hot spring for isolation and identification of thermophilic microorganisms. In this study we compare tree sources of hot spring namely water source, sediment and microbial mat to identified the best sample for the isolation of thermophiles from our local hot spring in Kedah, Malaysia.

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MATERIALS AND METHODS

Sampling of thermophilic microorganisms from hot spring

The Ulu Legong hot spring was chosen as the site for thermophilic microorganism isolation. Water, biomats and sediments samples were collected from the main pool located in Ulu Legong, Baling, Kedah, Malaysia. There were two methods for sampling; first method, at each site, 1 ml of each sample recovered by sterile syringe was transferred to each of the bottles, consisting of 10 ml media in a 30 ml sterilizes universal bottles. The sampling was conducted in triplicates. In the second method, the samples were collected using 3 bottles of 500 ml sterile thermo flasks by each was

Figure 1. Location of Ulu Legong Hot spring and various sites of for sample collection (a) Ulu Legong hot spring, (b) water, (c) biomat and (d) sediment.

collected from the water surface, biomats and sediments, respectively. These three thermo flasks were sent to the laboratory within a period of three hours before inoculation was done. One milliliter of sample from each flask was transferred to each of the bottles, consisting of 10 ml media in a 30 ml sterilizes universal bottles and was conducted in triplicates. The temperature was 60°C and pH was 7.81.

Isolation and identification of microorganisms from waters, biomats, and sediments samples

Nutrient broth and nutrient agar were used for isolation of thermophilic microorganism from the hot spring. One milliliter sample from of each waters, biomats, and sediments sample will be added to nutrient broth (10 ml) and incubated in water bath at 60°C for 7 days. Growth was followed by measuring the increase in turbidity at 600 nm. Then, the culture was streaked onto a nutrient agar plate. Isolation of pure culture was done by using spread plate method and streak plate method recommended by Rath and Subramanyam (1998).

Characterization and identification of the isolates

Morphological studies

Morphological properties were investigated by using 18 h old bacterial cultures growing on thermus agar plates. These included the wet mount preparations using light microscope Gram staining to confirm Gram reaction. Light micrograph was also done.

Biochemical tests

The thermophilic isolate was identified by the use of conventional methods for the presumptive identification by biochemical. These tests were; Gram reaction, catalase production and oxidase production.

Statistical analysis

All values are mean±S.E.M. obtained from triplicates. For statistical analysis, one-way ANOVA with Duncan's variance (SPSS 15) was used to compare the groups. In all the cases a difference was considered significant when p was <0.05.

RESULTS

Location of Ulu Legong hot spring, Kedah

Figure 1 shows the location of Ulu Legong hot spring located in Kedah, Malaysia where the sample for this study was collected.

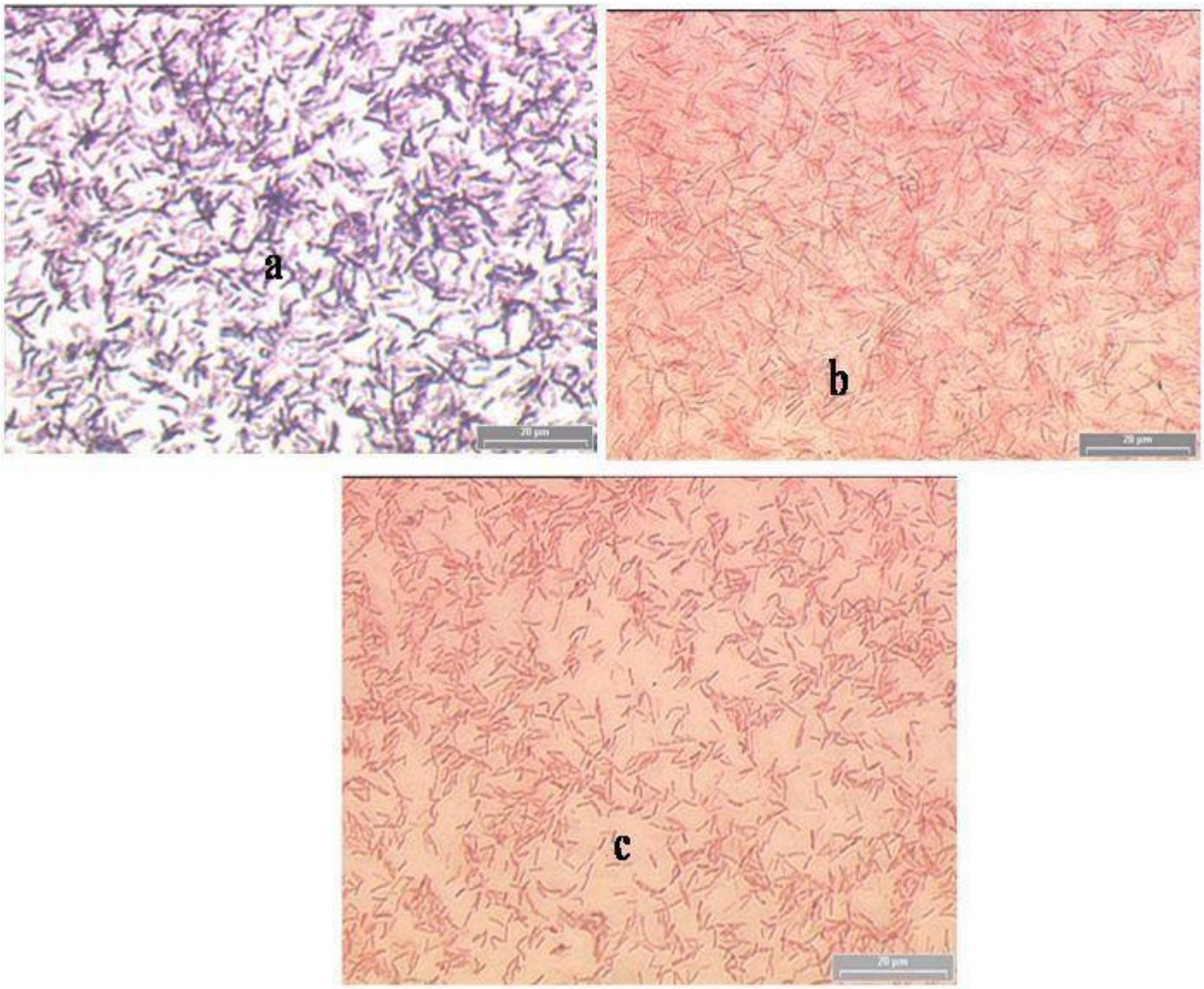

Figure 2. Gram reaction of the isolates (a) Gram positive and (b) Gram negative (short rod) and (c) Gram negative (long rod).

Isolation

Out of 9 samples cultured, all samples grew on nutrient broth and nutrient agar.

Characterization and identification of the isolate

Morphological studies

The Isolate is Gram-positive and Gram negative (Figure 2).

Biochemical tests

The fact that the Isolate is strictly aerobic microorganisms was further supported by the presence of catalase and oxidase activity in isolates.

Identification of appropriate site for the sample collection

The sample collected from sediment showed the higher growth rate compared to others sampling site such as water and biomat. Moreover, sample cultured in the laboratory after transported from sampling location were show higher growth rate compare to on site culture methods (Figure 3).

DISCUSSION

During the past few years, most research on the microbe

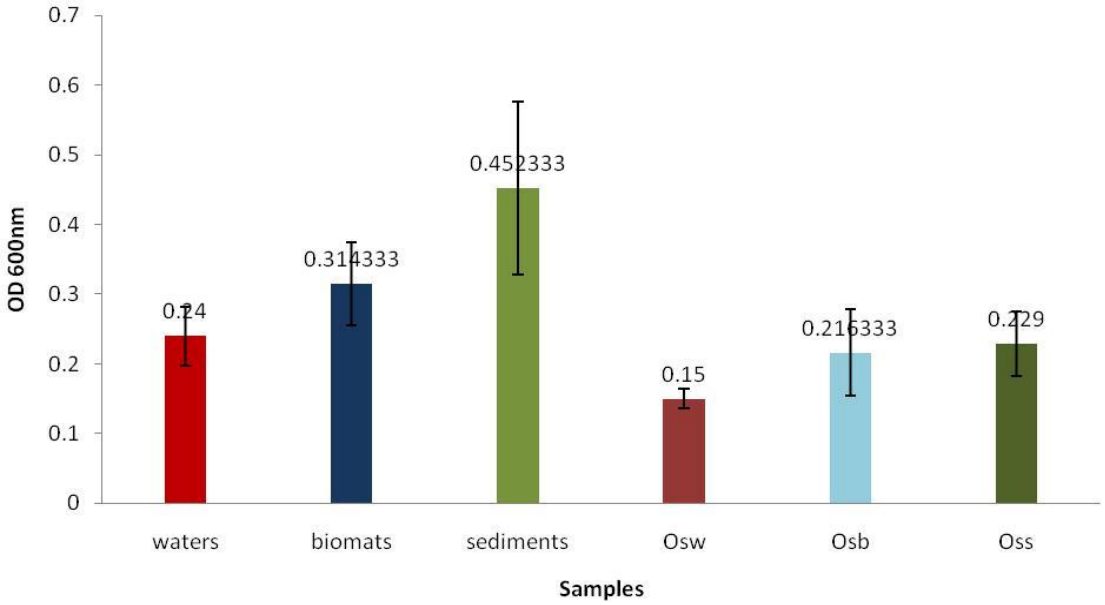

Figure 3. Growth rates of the bacteria from different sampling site and different culture method. Osw, on site culture of water sample, Osb, on site culture biomat sample, Oss, on site culture of sediment sample P< 0.05.

of hot springs has concentrated on cultivating and isolating extreme thermophilic and acidophilic strains (Belkova et al., 2007). Microbial enzymes already occupy a prominent position in modern biotechnology, optimizing or even replacing processes that already exist. The majority of the industrial enzymes known to date have been derived from bacteria and fungi. Therefore, isolation of the microbe of hot springs and its identification is an important task for modern scientist. Unfortunately, definitive sample for isolation and identification of the microbe of hot springs are uncommonly made. Because various sample available namely water, sediments and microbial mat associated with isolation and identification of the microbe of hot springs are easily confused, microbiologist may overlook the possibility of samples and fail to submit appropriate samples for isolation and identification of the microbe of hot springs. If appropriate samples are submitted for culture, sample handling techniques that are appropriate for isolation and identification of the microbe of hot springs may be resulted isolations of targeted microbes.

Recommendations for sampling and culturing samples obtained from hot springs with suspected thermophiles are common in the microbiology literature; however, data to support these recommendations is notably lacking. In order to investigate these problems more fully, a prospective evaluation of samples and culture techniques for the isolation of the microbe of hot springs from local geothermal water source samples was designed and completed.

Results of this study suggest that isolation of the microbe of hot springs from geothermal water source is not difficult when appropriate sample and culture techniques are employed. In our study, thermophilic bacteria were isolated from thermal springs water of Ulu Legong hot spring, Kedah, Malaysia. The isolate was as spore-forming bacilli with catalase and oxidase activity. Morphological studies conducted on the strain showed interesting observations that can be summarized as follows: The bacterial isolate are long and short rod shaped Gram positive and Gram negative.

For isolation of the thermophiles from water, sediment and biomat sample of geothermal water source, sediments sample appear to be far superior to water and biomat as a source of inoculums. This is not surprising given the fact that biomats are composed of metabolically diverse microorganisms, including cyanobacteria, diatoms, and bacteria, that compete for available nutrients (e.g. N, P, Fe and C) and environmental niches (Olson et al., 1999). Other researcher also reported that soil and aquatic sediments from temperate environments are known to possess thermophilic bacteria with optimal growth temperatures above 50°C (Madigan and Martinko, 2005; Takai et al., 2003; Miroshnichenko et al., 2003; Greene et al., 1997). The nutrient agar and broth used in this study appeared to adequately support the growth of microbe of hot springs when incubated at high temperature. For fresh samples, the use of on site culture method not significantly supports the culture of microbe of hot spring. This was likely due to decreased in optimum temperature for growth of thermophilic bacterial cells. In addition, for the collected samples, the highest rates of thermophiles isolation were achieved when the transported samples were cultured in the laboratory in a

water bath at high temperature were utilized.

Conclusion

In conclusion, based on the results of this study, isolation of thermophiles from hot spring is highly successful if sediment used as a sample at high temperature of 4°C, or in media within 24 h of collection. To minimize proliferation of bacterial contaminants (which may negatively affect thermophiles isolation), samples should be processed within 24 h of collection whenever possible. However, if necessary, sediments sample can be stored for up to 5 days either refrigerated solution without significantly compromising thermophiles isolation rates as long as they are subsequently plated on appropriate selective media.

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REFERENCES

Becker P, Abu-Reesh I, Markossian S (1997). Determination of the kinetic parameters during continuous cultivation of the lipase-producing thermopile Bacillus sp. IHI-91 on olive oil. App. Microbiol. Biotechnol., 48: 184-190.

Beg QK, Bhushan B, Kapoor M, Hoondal GS (2000). Production and characterization of thermostable xylanase and pectinase from Streptomyces sp. QG-11-3. J. Ind. Microbiol. Biotechnol., 24: 396-402.

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.

Greene AC, Patel BK, Sheehy AJ (1997). *Deferribacter thermophilus* gen. nov., sp. nov., a novel thermophilic manganese- and ironreducing bacterium isolated from a petroleum reservoir. Int. J. Syst. Bact., 47: 505–509.

Lee DW, Koh YS, Kim KJ, Kim BC, Choi HJ, Kim DS, Suhartono MT, Pyun YR (1999). Isolation and characterization of a thermophilic lipase from Bacillus thermoleovorans ID-1. FEMS Microbiol. Lett., 179: 393-400.

Madigan MT, Martinko JM (2005). Brock biology of microorganisms, 11st edn. Prentice-Hall, Upper Saddle River, NJ.

Miroshnichenko ML, Slobodkin AI, Kostrikina NA, L'Haridon S, Nercessian O, Spring S, Stackebrandt E, Bonch-Osmolovskaya EA, Jeanthon C (2003). Deferribacter abyssi sp. nov., an anaerobic thermophile from deep-sea hydrothermal vents of the Mid-Atlantic Ridge. Inter. J. Syst. Evol. Microbiol., 53: 1637–1641.

Olson JB, Litaker RW, Paerl HW (1999) Ubiquity of heterotrophic diazotrophs in marine microbial mats. Aquat Microb Ecol., 19: 29-36.

Rath CC, Subramanyam VR (1998). Isolation of thermophilic bacteria from hotsprings of Orissa, India. Geobios, 25(2-3): 113-119.

Sonnleitner B, Fiechter A (1983). Advantages of using thermophiles in biotechnological processes: expectations and reality. Trends Biotechnol., 1: 74-80.

Takai K, Kobayashi H, Nealson KH, Horikoshi K (2003). *Deferribacter desulfuricans* sp. nov., a novel sulfur-, nitrate- and arsenate-reducing thermophile isolated from a deep-sea hydrothermal vent. Int. J. Syst. Evol. Microbiol., 53: 839–846.