Biosciences, Biotechnology Research Asia Vol. **7**(1), 449-451 (2010)

Isolation and characterization of thermophilic bacteria from hot spring in Orissa, India

HEMANT KUMAR KHUNTIA, SURYAKANTA SAMAL, RAJESH KUMAR SAHOO, SANTANU KUMAR KAR, BIBHUTI BHUSAN PAL* and KANCHAN MALA BIHARI

Microbiology Division, Regional Medical Research Centre,
Chandrasekharpur, Bhubaneswar- 751 023 (India).

(Received: November 01, 2009; Accepted: December 20, 2009)

ABSTRACT

The present study was conducted to isolate thermophilic bacteria from hot spring at Tarabalu Orissa, India and to study its physiological and biochemical properties The strain from hot springs at Tarabola was isolated by plating and screening on Nutrient Agar medium. It was characterized by the physiological, biochemical and antimicrobial analysis. The cells were Gram-negative, motile rods, non-spore forming and generally occurred singly or in pairs. The growth temperature ranges from 57^0 C to 100^0 C, optimum at 75^0 C; growth pH ranges from 5.5 to 9.0, optimum at 7.0. The strains used glucose and lactose as carbon and energy sources. The hot spring isolate was active for enzyme Amylase at 57^0 C. Further molecular study is required for its identification and taxonomic characterization.

Key words: Thermophillic, hot spring, bacteria, enzyme, temperature.

INTRODUCTION

Microbes are believed to survive and interact with different ecosystem through various ways for their survival in many environment. Thermophilic bacteria in hot springs survive at moderate to high temperature adopting different characteristics than the normal bacteria. Thermophilic bacilli grow best at temperature between 45^0 to 75^0 C and there fore they can survive in hot spring at higher temperature (1). For several decades thermophilic bacteria have been focused for biotechnological and academic interest. In particular phenotypic and genotypic characterization of thermophilic bacteria from hot spring has done from different region in world including India.(2,3,4).Although there are several hot springs present in Orissa India, few have been studied (3, 4) and many are left about the knowledge of microbial communities. Here we report the isolation and characterization of thermo stable bacteria isolated from a hot spring Tarabalu which has not explored earlier in Orissa, India.

MATERIAL AND METHODS

Water samples of volume 500ml were collected from 5-6 hot springs at Tarabalu present at one location scattered over 100 meter radius in sterile glass bottle and transported to the Microbiology Department, Regional Medical Research centre, Bhubaneswar, India and analyzed within 24hr.Temperature and P^H of the water of the hot spring was measured at the time of collection with thermo-meter and P^H indicator paper respectively. Immediately after reaching the laboratory, 100µl of water sample was streaked on to the Nutrient Agar (Himedia, Mumbai, India) plate which was then sealed in plastic bags to prevent from drying and incubated at 57^0 C for 7 days. Aliquots of 1ml, 2ml and 3ml of water were added to the Nutrient broth and incubated at 57^0 C in orbital shaking water bath for 7 days. The broth in flasks were observed daily for turbidity and subculture were made from those flasks showing turbidity on to Nutrient Agar plates and incubated at 57^0 C sealed in plastic bags to prevent drying.

Colonies on Nutrient Agar were purified by serial transfers.

Morphology and motility of the bacterium was observed by Gram's stain and hanging drop method respectively. The hot spring isolate was subjected for various physiological and biochemical characterization in order to detect the difference if any by following the conventional methods. For different biochemical tests, the test strains was grown on nutrient agar plates and a single colony was picked up for inoculation into specific medium for detection of Cytocrome oxidase, Tryptophanase, Urease, Catalase, Coagulase, Nitrate reduction and utilization of Glucose, Sucrose, Lactose and Citrate. Amylase activate of the bacteria was tested by the starch agar-iodine method[5]; Proteases activity was tested on skim milk agar[6]; lipases activity was tested using Tween 20 and Tween 80 (Sigma Chemical Company, U. S. A.) as substrates in phosphate buffer[7].

The growth temperature range of strain was examined by measuring optical density (OD) at 405nm of the culture incubated at 57^0 C -100^0 C through 75^0 C in 100ml of Nutrient broth in Orbital shaker. The P^H range for growth was examined by measuring the O.D. at 405nm of the culture incubated at ambient temperature and P^H 6.0-10.0.

Antimicrobial susceptibility was tested by the modified Kirby-Bauer disk diffusion technique[8] with commercially available discs (Himedia, Mumbai, India) ampicillin (10µg), chloramphenicol (30µg), co-trimoxazole (25µg), ciprofloxacin (5µg), furazolidone (100µg), gentamicin (10µg), neomycin (30µg), nalidixicacid (30µg), norfloxacin (10µg), streptomycin (10µg), tetracycline (30µg), rifampicin (5mcg), kanamycin (30mcg), cephotaxime (30mcg), carbenicillin (100mcg), azithromycin (15mcg), piperacillin (100mcg), and doxycyclin (30mcg). Characterization of strains as being susceptible or resistant was based on the size of inhibition zone around each disc according to manufacturer's instruction, which matched interpretive criteria recommended by the WHO[9].

RESULTS AND DISCUSSION

The temperature of the hot spring at Tarabalu was 57^0 C. The P^H of the spring water was 7.0. The strain formed round, flat, smooth, grayish white colonies with 5-6mm diameter. It was motile gram negative, rod shaped and aerobic. The shape and size of the cell were observed by light microscope.

The growth P^H range of strain was 6. 5 - 9. 5 and the optimum P^H was 7. 0. The growth temperature range was 65^0 C -100^0 C and the optimum temperature was 75^0 C which indicates that it was thermo stable bacteria. Several biochemical characteristics such as utilization of Glucose, lactose & manitol; reduction of Nitrate; absence of Gas & Hydrogen Sulphide in TSI Agar medium; and absence of Cytochrome Oxidase, Catalase, Coagulase, Urease, Lipase, Protease and Glutamase enzymes were observed. The presence of the enzyme Amylase was detected at 57^0 C.

The hot spring isolate was susceptible to ampicillin, chloramphenicol, co-trimoxazole, furazolidone, gentamicin, neomycin, nalidixicacid, streptomycin, tetracycline, norfloxacin, ciprofloxacin, rifampicin, kanamycin, azithromycin, piperacillin, doxycyclin and resistant to carbenicillin, and cephotaxime

Phenotypically our hot spring strain is completely different from strain isolated at Atri[3, 4]. Detection of Amylase evidenced as a very useful enzyme which has wide industrial application[10]. It is used in starch and detergent related industries. In this study our hot spring is amylase positive at 57^0C. To the best of our knowledge this is first report of isolation and characterization thermophilic bacteria from a new hot spring at Tarabalu which has considerable significance. Further genetic analysis is required to find its genus and species position.

ACKNOWLEDGEMENTS

We thankfully acknowledge Indian Council of Medical Research, New Delhi, for it's funding to conduct the study.

REFERENCES

1. Adiguzel. A. Ozkan. H. Baris O. Inan K. Gulluce M. Sahin F., *J. Microbiol. Methods*., In press. (2009).
2. Sharma. A. Pandey. A. Shouche Y. S. Kumar. B. and Kulkarni, G. *J., Basic Microbiol.,* **49(2)**: 187-94 (2009).
3. Rath, C.C. and Subramanyam V. R. J., *Micobes.,* **86**: 157-61 (1996).
4. Panda, S. K.. Jyoti V. Bhadra. B. Nayak. K. C. Shivaji. S. Rainey. F.A. Das. S. K., *Int J Syst Evol Micrbiol.,* **59(9)**: 2171-5 (2009).
5. Modi. D. C. Bhatt. Y. B. Shaikh. M. K. G. Subramanyam V. R. Dias. F. F*., J. Appl. Bact*., **61**: 315-8. (1986).
6. Dias. F. F. Shaikh. M. K. G. Bhatt. Y. B. Modi. D. C. Subramanyam, V. R. J. *Appl. Bact*., **60**: 271-5. (1986)
7. Colin. C. H. Lyne, P. M. Grange. J. M. Microbiological Methods., Sixth editn. *Butterworth,* London (1989).
8. Bauer. A.W. Kirby. W. M. N. Sherri J. C. Turek. M., *Amer. J. Clin. Path.,* **45**: 493-6.(1966).
9. World Health Organization. Guidelines for cholera control. World Health Organization. Geneva, Switzerland (1993).
10. Fogarty W. M. Applied Science., Publishers Ltd. London. (1983).