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## Research Article



### Isolation and characterization of thermophilic bacteria of a hot water spring source, Balbal

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#### Abstract

The purpose of this study was to isolate and characterize the thermophilic bacteria obtained from the hot sulphur spring source, Balbal, of Chatra district of Jharkhand. Water sample from this source was isolated at 55<sup>0</sup>C and pH 7.5. Colonies were grown and their morphological and biochemical characterizations were performed to identify the basic surface profile of these bacterial colonies including their size, shape, consistency, colour, motility etc. and amylase, protease and catalase activity respectively. Further there broth cultures were prepared at different pH values (from 5.5 to 7.5) and their turbidity were measured using spectrophotometer at 600nm to obtain the optimum growth condition of these bacteria.

**Keywords:** hot spring; balbal; thermophilic bacteria; characterization.

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#### Introduction

Microorganisms can be grouped into broad categories according to their temperature ranges for growth; psychrophyles, mesophiles, thermophiles and hyperthermophiles.

Thermophiles are heat loving, with an optimum growth temperature of 55°C or more. Since thermophilic microorganisms prefer living at temperatures not commonly found in nature, but in hot springs and water heaters, their preference for hot temperatures has given rise to some speculation about their evolution. One theory suggested that the thermophiles were among the first living things on this planet, developing and evolving during the primordial birthing days of earth when surface temperatures were quite hot, and thus had been called the “Universal Ancestor” [1]. Estimated at 3.6 billion years old, they are said to be as abundant as to comprise as much as half of all living things on the planet [2].

As thermophiles grow at high temperature, so they must contain metabolites that can function at high temperature. The enzymes isolated from some extremophiles have proven to be of great use in the modern fields of biological sciences (for example heat stable DNA polymerases for polymerase chain reaction), medicine and in surfactants as they are able to do work under such conditions that would denature enzymes taken from most "normal" organisms [3]. The importance of thermostable biomolecules in the growing field of biotechnology has spurred research into organisms capable of growth at high temperatures.

During the past few years, most research on the microbes of hot springs has concentrated on the cultivation and isolation of extreme thermophiles [4]. Thermophiles belonging to the bacterial domain have received attention for their potential in the

bioconversion of substrates of plant origin to end products such as lactate and ethanol, compounds with potential for the production of bulk chemicals and fuels [5].

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<sup>o</sup>c and 40<sup>o</sup>c. This allows thermophiles to grow at high temperatures by providing the right degree of fluidity need for membrane function. The presence of chaperons which refold denatured proteins increase the stability of thermophilic proteins [6]. Also, thermophilic proteins appear to be smaller and in some cases more basic, which may also result in increased stability [7].

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 finger printing, etc. The enzyme 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 stearthermophilus* (temperature maximum 75<sup>o</sup>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 etc. [8].

Hence keeping all such importance of thermophiles in mind, the current study was carrying out with the objective to isolate and characterize the various aspects of thermophilic bacteria from Balbal site.

## Materials and Methods

### Collection of water

Water was collected aseptically from the mouth of the hot spring source of Balbal in an autoclaved sterile screw capped glass bottle. The temperature and pH of the water source was also noted.

### Bacterial isolation

Thermophilic bacteria were isolated and cultured on thermus agar medium (ATCC medium 697) containing 0.5% NaCl, 0.5% peptone, 0.4% beef extract, 0.2% yeast extract and 2% agar, the pH of the medium was adjusted to 7.0 before autoclaving plates were incubated at 60<sup>o</sup> for 24 hrs.

Isolation of pure culture was done using spread plate and streak plate method [9] and purity of the colonies were checked microscopically[10] (fig 1).

### Characterization of the bacterial isolates

#### Morphological studies

Morphological studies of the water sample were investigated by using 18 hrs old cultures on thermus agar plates and were summarized in the Table-1.

#### Biochemical Characterizations :

##### Starch hydrolysis test

Amylase are a class of enzymes that are capable of digesting these glycosidic linkages found in starches. Amylase are present in all living organisms, but the enzymes vary in activity, specificity and requirements from species to species and even from tissue to tissue in the same organism. Alpha- amylase(1,4 D-glucanohydrolase) acts upon large polymers of starch at internal bonds and cleaves them to short glucose polymers. Bacterial alpha-amylase is particularly suited for industrial usage since it is inexpensive and isothermally stable.

Starch agar is an example of differential medium which tests the ability of an organism to produce certain alpha-amylase and oligo-1,6- glucosidase that hydrolyse starch. Starch molecules are too large to enter into the bacterial cells, so some bacteria will secrete exogenous enzyme that will degrade starch into subunits that can be then easily utilized by the organism. Starch agar is a simple nutritive medium with starch added. Since no colour change occurs in the medium when organisms hydrolyse starch, iodine solution is added to the plate after incubation. Iodine turns blue in the presence of starch. A clearing around the bacterial growth shows that the organism has hydrolysed starch [11](fig. 2).

### **Hydrolysis of skimmed milk (casein)**

The ability of bacterial isolates to hydrolyse casein was tested by streaking on skimmed milk agar plates containing (1.0% skimmed milk, 0.2% yeast extract, 0.01%  $\text{KH}_2\text{PO}_4$ , 0.03%  $\text{K}_2\text{HPO}_4$ , 0.5% NaCl and 2% agar) and incubation at 55°C for 72 hrs after maintaining the pH 6.5. The production of a clear halo around the colonies indicated casein hydrolysis [12](fig.3).

### **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 clean cavity slide. A loopful of the isolate was transferred to the centre 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 cover slip 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 [13]. Motile cells came in view and were seen moving rapidly in the field.

### **Optimization of growth conditions:**

#### **Determination of optimum pH**

Optimum pH for the growth of thermophilic bacteria from balbal region was determined by producing broth culture of these bacteria under five different sets of pH

ranges (5.5, 6.0, 6.5, 7.0 and 7.5). The exact pH values were confirmed by using digital pH meter. These broth cultures were then incubated at 55°C for 24 hrs. Their turbidity value were finally assessed by using UV-Visible spectrophotometer at 600nm wavelength after every 30 mins of interval.

## **Results and Discussion**

A general analysis of the morphological features of the bacterial isolates, from Balbal shows some noticeable points and could be categorized as thermophilic since they required a temperature of about 55°C for optimum growth. Our next focus of the study was to characterize biochemically the bacterial isolates. The following results were observed after performing various biochemical tests.

### **Starch hydrolysis test**

The thermophilic bacteria was supposed to secrete extracellular amylase enzyme as positively indicated by the clear white zones around the bacterial colonies (that might hydrolyse the starch agar medium) (fig.2).

### **Casein hydrolysis test**

Clear halos were observed in the skimmed milk agar culture media, that clearly indicates the release of extracellular proteases from the bacterial isolates which could digest the skimmed milk agar medium around them.

### **Motility test**

As freely movable bacterial cells, obtained from the broth culture could be seen by using hanging drop method, it undoubtedly proved that the bacteria cells are motile and their motility may be provided by their flagella.

### **Optimization of growth conditions at different pH**

As the graphical representation shown (in fig.4) indicates that the optimal pH for maximum bacterial growth is undoubtedly at 7.5. It means that the culture was not only thermophiles but also alkalophiles (i.e alkali loving). Therefore they could be collectively named as thermoalkalophiles.



Figure 1. Bacterial colonies obtained after spread plate technique at 55 °C.

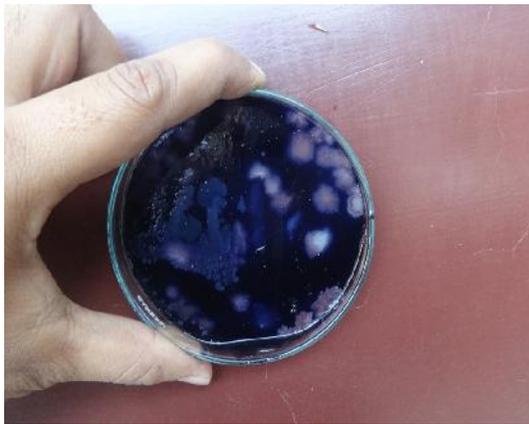


Figure 2. Clear white bacterial colonies in a starch agar medium.

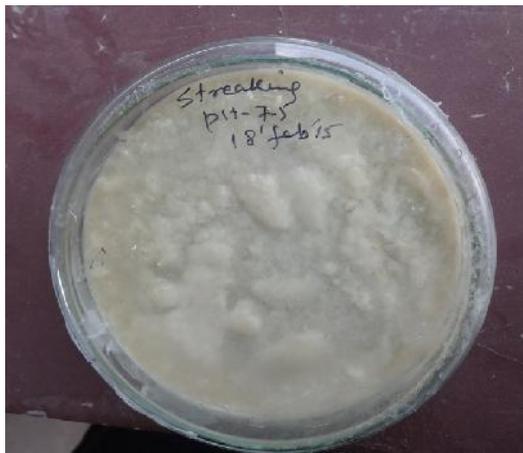


Figure 3. Clear halos around the bacterial isolates in a skimmed milk agar plate.

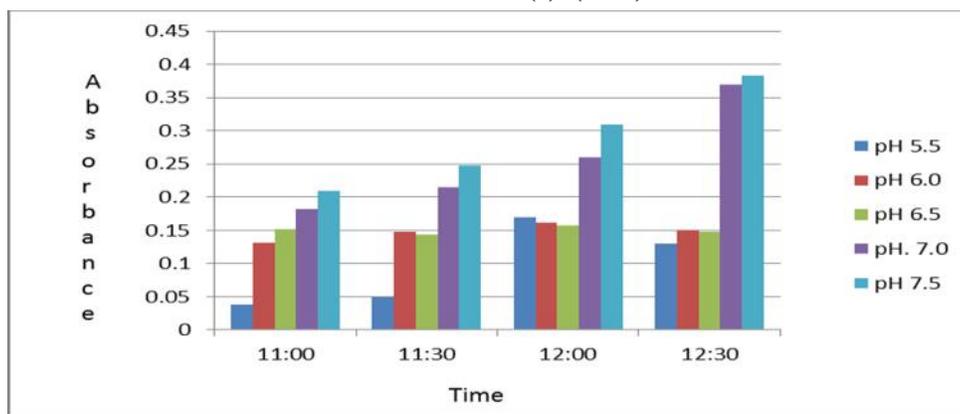


Figure 4. Effect of pH on the growth of thermophilic bacteria.

Table1: Colonial morphological features of bacterial colonies isolated from sources

Source	Colonial shape	Colonial edge	Colonial opacity	Colonial colour	Colonial size(cm)	Colonial surface	Colonial elevation	Colonial pattern
Balbal	irregular	fibrous	Opaque	Off white	0.1-0.2	smooth	flat	moderate

### Conclusion

From our experiment we were able to isolate thermophilic bacterial colony that are having 0.1-0.2 cm size, fibrous edge, off white in color, alkalophilic, motile, able to produce extra cellular amylase, and proteases, These organisms may therefore be used in the production of enzymes like proteases and amylases which are having a great industrial and biotechnological importance. We feel that continued investigation in this field may provide various new insights that unfold some more hidden mysteries which could aid in the welfare of human beings either directly or indirectly in the coming future.

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### References

[1] Doolittle W.F. (1999) Phylogenetic classification and universal tree. Science, Vol (284): 2124-2128.  
 [2] Woese C.R. (1998) The universal ancestor, Proc. Natl.

Acad. Sci. USA (95): 6854-6859.  
 [3] Mattila P., Korpela T., Tenkanen and K. Pitkanen K. (1991). Fidelity of DNA synthesis by the *Thermococcus litoralis* DNA polymerase, an extremely heat stable enzyme with proofreading activity. Nucleic Acids Res., Vol 19(18): 4967-4973.  
 [4] Belkova N.L., Tazaki K., Zakharova J.R., Parfenova V.V.(2007). Activity of bacteria in water of hot springs from Southern and Central Kamchatskaya geothermal provinces, Kamchatka Peninsula. Russ. Microbiol. Res.,Vol 162(2): 99-107.  
 [5] Wiegel J. and Ljungdahl L.G., (1981). *Thermoanaerobacter ethanolicus* gen. nov., sp.nov., a new extreme thermophilic, anaerobic bacterium. Arch. Microbiol.Vol(128):343-348.  
 [6 ] Jaenicke R. et al. (1996).Structure and stability of hyperstable proteins. Adv. Protein Chem. Vol(48):181–269.  
 [7] Kumar S and Nussinov R(2001). How do thermophilic proteins deal with heat? Cell. Mol. Life Sci. Vol 58:1216-1233.  
 [8] Stetter K.O., (1996); Kristjansson J.K and Hreggvidsson G.O.,(1995), Bertoldo C and Antranikian G,(2001).Adv. Protein Chem. Vol (48):181-269.  
 [9] Rath CC, Subramanyam V.R(1998). Isolation of thermophilic bacteria from hot springs of Orissa,India.Geobios,25 (2-3):113-119.

- [10] Khali A. M, Salim and Sallal A.K. (1998). Enumeration of thermotolerant bacteria from recreational thermal ponds in Jordan", *Cytobios.* 57-63.
- [11] Fawole, M.O. and Oso, B.A. (2001) 'Laboratory manual of microbiology' Spectrum Books Limited, Ibadan, Nigeria.
- [12] Fujio Y. and Kume S. (1991). Characteristics of highly thermostable neutral protease produced from *Bacillus stearothermophilus*. *World Journal of Microbiology and Biotechnology.* Vol(7):(12-16).
- [13] Olutiola P.O., Famurewa O. and Sonntag H.G., (2000). *An Introduction to microbiology, a Practical Approach.* Tertiary Text Book series.