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21. Isolation and Identification of Thermoalkalophilic Bacillus Species from Unkeshwar - Hot Water Spring for Production of Antimicrobial Compound

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Abstract

The gram positive bacterium Bacillus subtilis produces a large number of antibiotics, which are classified as ribosomal or non-ribosomal. The high proportion of antimicrobial compounds producing strains may be associated with ecological role, playing a defensive action to strains into an established microbial community. Micro-organisms are highly efficient in their ability to produce many kinds of bioactive compounds. A large number of antibiotics have been shown to be produced by various types of bacteria, such as actinomycetes. Screening bacteria from alkaline habitats or those grown under extreme cultural conditions remains a profitable area for investigation. Some new antibiotics were produced by certain bacteria when an alkaline medium with high alkalinity (pH 9 to 10.5) was used. Thus, this study is taken with the objective of isolation of Thermoalkalophilic Bacillus subtilis from Water sample Hot Water Spring – Unkeshwar, Tq. Kinwat, Dist. Nanded (Maharashtra) to produce the antimicrobial compound.

Keywords: Thermoalkalophilic, antimicrobial compound

Introduction

Microorganisms have been found living in almost every place explored so far, from hydrothermal vents in the deepest trenches of the Pacific Ocean to frozen Antarctic ice, and deep in the earth's lithosphere. Under conditions that represent the extreme ranges of physical and chemical conditions that permit cellular survival, microorganisms termed extremophiles represent the most radical adaptations that allow survival and growth. The concept of normal or mild conditions is of course relative. However, we can generalize that life, at least as we know it on earth, depends on the availability of liquid water as the most important solvent (Rothschild and Mancinelli, 2001). Thermophiles (literally heat lovers) are microorganisms that thrive at

temperatures above the mesophilic range of 25°C to 40°C that characterizes the mainstream of life. While thermophiles are an eclectic bunch, these organisms share a common theme: they exist at the fringes, where high temperature excludes all but the hardiest of inhabitants. Every component of these small, prokaryotic cells (typically about 1 μm in diameter) is exposed continually to the high temperatures of their environments and must be adapted to function under these conditions. Thus, all molecules, ranging from cell surface complexes, cytoplasmic membrane (Itoh et al., 2001), and ribosomes, down to metabolic enzymes and intermediary metabolites (Robb and Maeder, 1998), must cope with the threat of unfolding or decomposition (Russell, 2003). These double or triple extremophiles may be able to extend the limit of one extreme because of the effects of another. (Madigan et al., 2003) Starting with the invention of the polymerase chain reaction (PCR) amplifcation method and its reliance on thermostable DNA polymerases, thermophiles have contributed to many areas of economic development (food processing, biofuels, and so on) (Vieille and Zeikus, 2001).

Many alkaliphiles have been isolated from various sources. These include aerobic spore-formers, anaerobic non-spore-formers, halophiles, thermophiles including archaea, psychrophiles, piezophiles and others. The most concentrated and widespread occurrences of organisms are generally observed in "moderate" environments. It has also been known that there are "extreme" environments on earth which were thought to prevent the existence of life (Horikoshi; 1991). In these habitats, environmental conditions such as pH, temperature and salinity concentrations are extremely high or low. Extreme environments are populated by groups of organisms that are specifically adapted to these particular conditions and these types of extreme micro-organisms are usually referred to as alkaliphiles, halophiles, thermophiles and acidophiles, reflecting the particular type of extreme environment which they inhabit (Horikoshi; 1991).

The spread of resistance to antibiotics undermines the therapeutic utility of Anti-infective drugs in current clinical use (Bax etal; 2000). For example, Staphylococcus aureus, a major cause of community and hospital acquired infections, has developed resistance to most classes of antibiotics, and isolates exhibiting such resistance is drawing great concern. Methicillin - resistant Staph. aureus (MRSA) strains appeared in the hospital environment after introduction of the semi-synthetic Penicillin, Methicillin, leaving Vancomycin as the last line of defense for MRSA treatment (Enright; 2003). Thus, new antibiotic and therapy options are urgently needed to improve the management of bacterial infections (Saimann etal; 2001), and a major challenge is to find drugs that act against Methicillin-resistant Staph. aureus (MRSA). Most bacteria

produce antimicrobial compounds such as broad spectrum classical antibiotics, metabolic products viz. organic acids and lytic agents such as lysozyme (El-Banna; 2003). The gram positive bacterium Bacillus subtilis produces a large number of antibiotics, which are classified as ribosomal or non-ribosomal. The high proportion of antimicrobial compounds producing strains may be associated with ecological role, playing a defensive action to strains into an established microbial community. Micro-organisms are highly efficient in their ability to produce many kinds of bioactive compounds. A large number of antibiotics have been shown to be produced by various types of bacteria, such as actinomycetes. Screening bacteria from alkaline habitats or those grown under extreme cultural conditions remains a profitable area for investigation. Some new antibiotics were produced by certain bacteria when an alkaline medium with high alkalinity (pH 9 to 10.5) was used (Sato et al; 1983).

The alkaliphilic actinomycete Nocardiopsis strain, a producer of phenazine, successfully grew at pH 10.0 in culture medium (Tsai et al; 1995). In a recent research study, microorganisms isolated from the alkaline saline Lake Ac›göl in Turkey were screened for their activity against other micro-organisms. The preliminary results indicated that alkaline-saline lake isolates exhibited antimicrobial activity against Bacillus subtilis, Staphylococcus aureus, Micrococcus luteus, Mycobacterium smegmatis, and Candida albicans. The preliminary results have encouraged further research work to identify the metabolites produced by alkaliphilic bacteria (Eltem et al;1998).

The discovery of these bioactive compounds provides evidence that organisms from such environments are also capable of producing antibiotic-type compounds. Alkaliphilic producers of novel bioactive agents still await exploitation, Thus, this study is taken with the objective of isolation of Thermoalkalophilic Bacillus subtilis from Water sample Hot Water Spring – Unkeshwar, Tq. Kinwat, Dist. Nanded (Maharashtra) to produce the antimicrobial compound.

Material & Methodology

Collection of Soil Samples

Isolates of Bacillus species were obtained by screening water sample, collected from Hot Water Spring – Unkeshwar, Tq. Kinwat, Dist. Nanded (Maharashtra). (Tambekar et al.,2010). Water sample I and II are collected in sterile water sampling bottles from the site. Water samples are kept in an icepack cabinet maintained at temperature below 10^0C. The pH & temperature of the water was recorded in 2007(Data Not Published In this paper). Same sample was used for further study (Narayan etal;2008, Abou-Shanab etal; 2007)

Isolation of Bacterial Species

The collected water samples were added in Nutrient broth and NG medium adjusted to pH 9 (containing 10 gm (Gram) Nutrient broth; 10 gm Glucose; 2 gm Sodium chloride; 5 mg (miligram) of CuSO4.5H2O; 7.5 mg of FeSO4.7H2O; 3.6 gm of MnSO4.5H2O; 15 mg of CaCl2.2H2O; and 9 mg of ZnSO4.7H2O; (per liter)) supplemented with 50 µg for tryptophan per ml at 45^0C for 24 hours (Hosoya etal; 1998) for enrichment. The enriched culture was re-inoculated on Nutrient Agar and NG Agar medium adjusted to pH 9 for isolation of bacteria and incubated at 45^0C for 24 hours. Colonies showing characteristic feature were selected and confirmed by colony character and biochemical test. These strains were selected for further study.

Identification of Bacterial Species

The subcultured cultures on slants were used for identification of cultures using screening for Antimicrobial Compound production (Data Not Published In this paper), biochemical analysis using Bergey's manual determinative bacteriology (Beregy, 1994).

Results and Discussion

Twenty cultures were isolated form Hot Water Spring – Unkeshwar, Tq. Kinwat, Dist. Nanded (Maharashtra). Out of these isolates most isolates were identified as Bacillus species by performing different biochemical tests on the isolates and were confirmed by using Bergey s manual of determinative bacteriology (Bergey, 1994) (Table 1). Out of these isolates two isolates showed growth in pH range 8.5 & 9 and in temperature range 45 & 50°C. So, the selected Two isolates were screened for Antimicrobial Compound production using NG Agar medium plate (containing 10 gm (Gram) Nutrient broth; 10 gm Glucose; 2 gm Sodium chloride; 5 mg (miligram) of CuSO4.5H2O; 7.5 mg of FeSO4.7H2O; 3.6 gm of MnSO4.5H2O; 15 mg of CaCl2.2H2O; and 9 mg of ZnSO4.7H2O; (per liter)) supplemented with 50 µg for tryptophan per ml. These isolates were selected and confirmed again by biochemical analysis as Bacillus species. From the above result it was confirmed that out of twenty bacterial isolates from Hot Water Spring – Unkeshwar, Two isolates were Thermoalkaliphilic Bacillus species identified by classical biochemical tests are capable of producing antimicrobial compound (Data Not Published In this paper) & will be used for production of antimicrobial compound.

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Table 1: Identification of the Bacterial Isolates

Sr. No.	Test	Bacterial isolates	
		BAT08	BAT14
1	Endospore	Central	Central
2	Gram nature	+	+
3	Catalase	+	+
4	Oxidase	+	±
5	Amylase	+	+
6	Gelatinase	+	+
7	Urease	+	-
8	Indole	-	-
9	Methyl Red	+	+
10	VP	-	-
11	Citrate	±	+
12	Glucose	+	+
13	Xylose	+	+
14	Mannitol	+	+
15	Lecithinase	-	-
16	Growth at 45°C	++	++
17	Growth at 65°C	+	+