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Antimicrobial Potentials of Bioactive Compound P roduced by Thermoalkalophilic Bacillus species iso lated from Unkeshwar - Hot water spring.

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ABSTRACT:

Both ribosomal and non-ribosomal antibiotics are produced in enormous quantities by the gram-positive bacterium Bacillus subtilis. It's possible that the large percentage of strains that produce antimicrobial chemicals has an ecological function, acting as a barrier against new strains entering an established microbial community. Microorganisms are incredibly effective at producing a wide variety of bioactive substances. Actinomycetes are among the many species of bacteria that have been demonstrated to produce a wide range of antibiotics. It is still profitable to investigate the screening of bacteria from alkaline habitats or those cultivated under harsh cultural settings. When specific bacteria were cultured in an alkaline medium with high alkalinity (pH 9 to 10.5), certain novel antibiotics were developed. Therefore, the purpose of this study is to investigate the antimicrobial potential of a bioactive compound produced by thermoalkalophilic Bacillus subtilis from a water sample at a hot spring in Unkeshwar, Tq. Kinwat, Dist. Nanded (Maharashtra) against a variety of microorganisms, including P. aeruginosa, Aspergillus flavus, Aspergillus species, E. coli, and Staphylococcus aureus.

Keywords: *Thermoalkalophilic, bioactive compound, actinomycetes.*

Introduction:

Almost every location that has been investigated to date has been shown to support life, including frozen Antarctic ice, deep into the earth's lithosphere, and hydrothermal vents in the deepest reaches of the Pacific Ocean. Microorganisms known as extremophiles represent the most extreme adaptations that allow life and growth under settings that represent the extreme ranges of physical and chemical conditions that permit cellular survival. Naturally, what constitutes normal or mild circumstances varies. Nonetheless, it may be broadly stated that the existence of liquid water is the most essential solvent for life, at least as we know it on Earth (Rothschild and Mancinelli, 2001). Microorganisms known as thermophiles—literally, "heat lovers"—dwell at temperatures higher than the mesophilic range of 25°C to 40°C, which is typical for most living things. Despite their diversity, organisms that are thermophiles all have one thing in common: they live on the edge, where temperatures so extreme that only the hardiest of inhabitants can survive. Each part of these tiny, prokaryotic cells—which usually have a diameter of around 1 μm—must be modified to function in these circumstances since they are constantly exposed to high temperatures. Consequently, every molecule, including complexes on the cell surface and cytoplasmic membranes (Itoh et al., 2001)

Numerous alkaliphiles have been isolated from different environments. These comprise halophiles, thermophiles (including archaea), psychrophiles, piezophiles, aerobic spore-formers, and anaerobic non-spore-formers. In "moderate" habitats, organisms are typically found in the most concentrated and ubiquitous forms. Additionally, it is recognized that certain "extreme" environments occur on Earth, which were once assumed to be incompatible with life (Horikoshi, 1991). Environmental factors including pH, temperature, and salinity concentrations are either abnormally high or low in these ecosystems. Groups of organisms that have evolved specifically to survive in extreme environments are known as alkaliphiles, halophiles, thermophiles, and acidophiles, respectively, to reflect the specific type of extreme environment that these organisms inhabit (Horikoshi, 1991).

The therapeutic value of anti-infective medications in current clinical use is threatened by the emergence of antibiotic resistance (Bax et al., 2000). As an illustration, Staphylococcus aureus, a common source of hospital and community-acquired infections, has evolved resistance to the majority of antibiotic classes, and isolates showing this resistance are causing serious worry. Vancomycin is the last line of defense for treating Methicillin-resistant Staph. aureus (MRSA) strains, which emerged in the hospital setting following the introduction of Methicillin, a semi-synthetic Penicillin (Enright, 2003).

Therefore, it is imperative to develop novel antibiotics and treatment alternatives to enhance the management of bacterial infections (Saimann et al., 2001). One significant obstacle is developing medications that target Methicillin-resistant Staph. aureus (MRSA). The majority of bacteria generate lytic agents like lysozyme, metabolic products like organic acids, and broad range classical antibiotics, among other antimicrobial substances (El-Banna, 2003). Antibiotics are produced in vast quantities by the gram-positive Bacillus species and are categorized as either ribosomal or non-ribosomal. It's possible that the large percentage of strains that produce antimicrobial chemicals has an ecological function, acting as a barrier against new strains entering an established microbial community. Microorganisms are incredibly effective at producing a wide variety of bioactive substances. Actinomycetes are among the many species of bacteria that have been demonstrated to produce a wide range of antibiotics. It is still profitable to investigate the screening of bacteria from alkaline habitats or those cultivated under harsh cultural settings. When an alkaline medium with high alkalinity (pH 9 to 10.5) was utilized, certain bacteria developed novel antibiotics (Sato et al; 1983).

These bioactive chemicals have been discovered, which shows that organisms from similar habitats can also produce molecules similar to antibiotics. Novel bioactive compounds produced by alkaliphilic producers are yet to be used. Therefore, the purpose of this study is to investigate the antimicrobial potential of a bioactive compound produced by thermoalkalophilic Bacillus subtilis from a water sample at a hot spring in Unkeshwar, Tq. Kinwat, Dist. Nanded (Maharashtra) against a variety of microorganisms, including P. aeruginosa, Aspergillus flavus, Aspergillus species, E. coli, and Staphylococcus aureus.

Material & Methodology:

Collection of soil samples: By screening water samples taken from Hot Water Spring - Unkeshwar, Tq. Kin wat, Dist. Nanded (Maharashtra), isolates of Bacillus species were obtained. (Tambekar and others, 2010). In sterile water sampling bottles, water samples I and II are taken from the location. Samples of water are stored in an icepack cabinet that is kept at a temperature lower than 100C. The water's temperature and pH were not ed. For additional research, the same sample was used (data not included in this work). (Abou-Shanab et al., 2007; Narayan et al., 2008)

Isolation & Identification of Bacterial species: The water samples that were collected were added in nutrie nt broth and NG medium that had been adjusted to pH 9. The nutrient broth contained 10 gm (grams) of gluc ose, 2 gms of sodium chloride, 5 mgs (miligrams) of CuSO4.5H2O, 7.5 mg of FeSO4.7H2O, 3.6 gms of Mn SO4.5H2O, 15 mg of CaCl2.2H2O, and 9 mg of ZnSO4.7H2O (per liter). Additionally, the broth was supple mented with 50 µg of tryptophan per milliliter at 450C for a 24-hour period (Hosoya et al., 1998) for enrichm ent. After being re-inoculated on NG Agar and Nutrient Agar medium that had been pH-adjusted to 9 for bac terial isolation, the enriched culture was incubated at 450C for a full day. Colonies exhibiting distinguishing

characteristics were chosen and validated using colony character and biochemical testing (information not in cluded in this paper). These isolates were then chosen for additional investigation (Beregy, 1994).

Production of Bioactive compound: NG media was used as the inoculum and production medium for the id entified isolates of Bacillus species in order to screen them for the production of bioactive compounds. The i noculated production medium was then incubated at 450C for 72 hours in order to produce bioactive compou nds. Cells were then extracted from the incubated production medium after it had been centrifuged for 10 mi nutes at 10,000 rpm using a cooling centrifuge REMI.

Three times, 10 milliliters of 50% n-butanol were used to extract the contents of the cells. Each time, the aqueous layer was collected and evaporated to concentrate at room temperature (Tamehiro et al, 2002). The extract was again extracted using ethyl acetate and utilized for further purification after being resuspended in 4 milliliters of methanol. Lipid extraction technique was used to carry out the purification (Katz and Demain, 1977).

Bioassay of Bioactive compound: Using the Kirby-Bauer Diffusion Method, the antimicrobial compound's crude extract and ethyl acetate were employed for the bioassay. On sterile Muller Hinton (MH) agar, sterile s wabs were used to streak the cultures of test organisms, including E. Coli, Staphylococcus aureus, P. aerugin osa, Candida tropicalis, Aspergillus flavus, and Aspergillus species, which were all 24 hours old. Next, MH a gar wells were created. One hundred microliters of crude extracts of bioactive compounds were added to eac h well. The plates were refrigerated to allow the chemical to diffuse. Following a 24-hour incubation period a t 35±0.5 0 C, the diameter of the inhibitory zone was measured on the plates.

Purification of phospholipid antimicrobial compound: One milliliter of the crude extract was combined w ith 3.75 milliliters of 1:2 (v/v) CHCl3, methanol, and vortexing thoroughly. In order to create a two phase sy stem, 1.25 cc of distilled water was added last, thoroughly mixed, and centrifuged at 1000 rpm for five minut es at room temperature. After removing the bottom phase, silica gel was used to perform TLC, or thin layer c hromatography. The bottom phase was used to spot the plates, then CHCl3, methanol, and water (65:25:04 v /v) were used to develop the plates. By putting the plates in an iodine chamber and treating them with iodine vapour, the phospholipid spots were found on the chromatogram. Spots of the isolated Bacillus species' bioac tive compounds were found, removed, and extracted using CHCl3:Methanol (Katz and Demain, 1977).

Antimicrobial activity of purified bioactive compound: After being well-prepared and inoculated with E. Coli, Staphylococcus aureus, P. aeruginosa, Candida tropicalis, Aspergillus flavus, and Aspergillus species, the extracted purified bioactive component was placed into Muller-Hinton agar plates. The plates were then incubated at 35±0.5 ° C for 24 hrs. The zone of inhibition's diameter was measured after incubation.

Results and Discussion: The goal of the current study was to maximize the circumstances that led to the syn thesis of bioactive microbial metabolites. produced by a type of thermoalkalophilic Bacillus that was isolated from a hot spring in Unkeshwar. Bergy's Manual of Systematic Bacteriology was used to isolate and identify twenty isolates (Bergey et al, 1994).

Four of these isolates demonstrated action against a range of pathogens, including E. Coli, Staphylococcus aureus, P. aeruginosa, Candida tropicalis, Aspergillus flavus, and Aspergillus species. These isolates were evaluated for antimicrobial activity of crude bioactive component. The isolates showed reduced activity against Candida tropicalis and P. aeruginosa and stronger activity against Gram positive Staphylococcus aureus, Gram negative E. coli, Aspergillus species, and Aspergillus flavus (Table 1). Consequently, the manufacture of bioactive compounds was carried out using these four isolates, and the resulting purified bioactive substance underwent testing against Aspergillus flavus, E. coli, and Staphylococcus aureus.

Table 1: Screening of Bacterial Isolates for Bioactive Compound

Bacterial Isolates	**Antimicrobial Potential of Bioactive Compound**					
	E. coli	*Staphylococcus aureus*	*P. aeruginosa*	*Candida tropicalis*	*Aspergillus flavus*	*Aspergillus species*
BAT 02	++	+++	-	-	++	+++
BAT 06	++	+++	-	-	++	+++
BAT 09	++	++	-	-	++	++
BAT 11	++	++	-	-	++	++

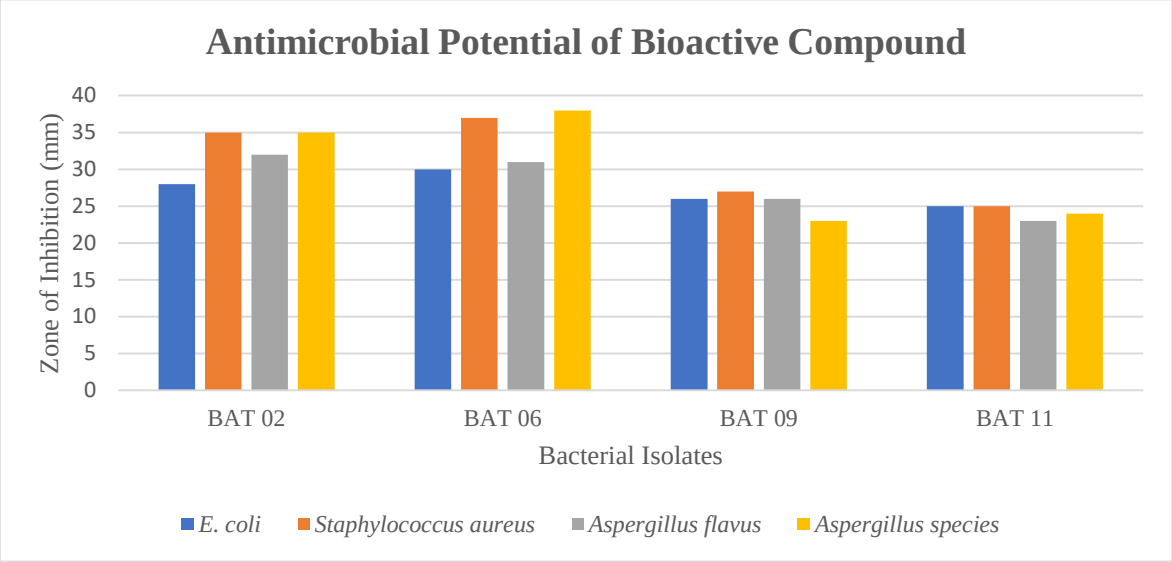
Researchers looking for antibiotics made by Bacillus species have discovered that Bacillus pumilus (MSH) produces a substance that inhibits Aspergillus and Mucoraceae. Additionally, a variety of experts have documented the inhibition of diverse species (Maraheil et al, 1997). A strain of Bacillus subtilis C126 was identified by researchers from the fermentation of sugar cane. This strain produced bacitracin, a polypeptide antibiotic that prevented Micrococcus flavus from growing (Tamehiro et al, 2002). It was discovered that the Bacillus licheniformis strain 189, which was isolated from a hot spring environment in the Azores, Portugal, produced a peptide antibiotic that significantly inhibited the development of Gram-positive bacteria (Mendo et al, 2004).

Thin layer chromatography was used in the current investigation to further purify the crude bioactive component from the isolates. The chemical was fractionated once the site was identified. It was observed that the bioactive compound from the isolated four thermoalkalophilic Bacillus species, of which isolates BAT 02 & BAT 06 exhibited better broad spectrum activity against Staphylococcus aureus, E. coli, and Aspergillus flavus as compared to other two isolates BAT 09 BAT 11, when the antimicrobial activity of the collected fraction was tested against the most sensitive organisms, Staphylococcus aureus, and E. coli. (Table.2 & Graph 1). This suggests that, in comparison to BAT 09 & BAT 11, the Thermoalkalophilic Bacillus species isolates BAT 02 and BAT 06 are creating a higher active broad range bioactive chemical. Antibiotic identification in unfractionated material is evidently complicated by the multiplicity of antibiotic synthesis. Chromatography, however, can be used to detect similarities or differences between known and unknown antibiotics even in complete cultures (Snell et al, 1955).

Table 2: Antimicrobial Potential of Bioactive Compound

Bacterial Isolates	**Antimicrobial Potential of Bioactive Compound (in mm)**			
	E. coli	*Staphylococcus aureus*	*Aspergillus flavus*	*Aspergillus species*
BAT 02	28	35	32	35
BAT 06	30	37	31	38
BAT 09	26	27	26	23
BAT 11	25	25	23	24

Graph 1: Antimicrobial Potential of Bioactive Compound



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