Research Article

Antibacterial Activity of Honey against *Bacillus Sp*. EU847311

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Accepted on: 29-03-2013; **Finalized on:** 31-05-2013.

ABSTRACT

Soil is the main reservoir of the genus *Bacillus*. Due to their ability to form spores and withstand a range of variable environmental conditions, *Bacillus sp*. adapts easily to diverse habitats and cause diseases. So, it is essential to find a compound which is having antimicrobial property, there by killing *Bacillus* an opportunist pathogen. In the present study, isolation of bacillus from the soil sample was performed and identified by 16s rRNA sequencing and submitted in genbank under the accession number EU847311. Antibacterial potential of honey was tested by disc diffusion method and compared with standards. The results obtained with honey were found to be moderate in its antibacterial activity when compared to standards.

Keywords: *Bacillus*, Pathogen, Antimicrobial activity, Honey.

INTRODUCTION

Bacillus is a genus of Gram positive rod shaped bacteria and a member of the phylum firmicutes. It is an opportunistic pathogen since the late nineteenth century. Under stressful environmental conditions, the cells produce oval endospores that can stay dormant for extended periods. Estimates of endospore longevity range from thousands to millions of years, although it is more likely on the lower end of that range, a number of factors are responsible for this robustness including dehydration of the spore core and compaction of chromosomal DNA[1]. The process by which these dormant cells reinitiate growth is called germination and it occurs in response to environmental signals including amino acids and cell wall peptidoglycan muropeptides derived from growing cells[2]. *Bacillus sp.* are considered medically significant: *Bacillus anthracis,* which causes anthrax and *Bacillus cereus* causing a food borne illness similar to that of *Staphylococcus*. Anthrax is a disease of great antiquity and historical interest, it may have been responsible for two of the biblical plagues of Egypt in 1491 BC and its symptoms in animals were clearly described in 29 BC by Virgil in the Georgics. As at least one cause of "Black Bane" and "Murrain" in Saxon and Medieval times, it caused heavy losses of cattle and sheep in Britain and Europe and it has been suggested that it contributed to the Black Death that swept Europe in the mid-fourteenth century[3]. In many countries, anthrax remains a major problem, it is enzootic in China, Iran, parts of Africa, India and South America, and it is virtually enzootic in many other areas. Worldwide, there may be as many as 10,000 animal outbreaks each year and perhaps, 9000 human cases. The interruption of animal vaccination in Zimbabwe by insurgency rapidly gave rise to a major bovine epizootic, a subsequent epidemic with more than 6000 human cases, most of them cutaneous and up to 100 deaths in a period of only six months[4]. Honey is gaining acceptance by the medical profession for use as an antibacterial agent for the treatment of ulcers and bed sores, and other surface infections resulting from burns and wounds. The strawberry tree *(Arbutus unedo)* honey of Sardinia is valued for its therapeutic properties[5], in India lotus *(Nelumbium sceciosum)* honey is said to be a panacea for eye diseases [6]. Hence, present study was aimed to isolate *Bacillus sp* from the soil sample at Salem District, Tamilnadu, India and to find a antibacterial substance that kills *Bacillus*. So, Natural and marketed honey was used to test the antimicrobial property against the isolated *Bacillus sp*.

MATERIALS AND METHODS

Isolation

One gram of soil sample was dissolved in 100ml of distilled water. Serial dilution technique was adopted for isolation. 0.1ml from the serially diluted tube was spread plated on nutrient agar medium and incubated at 30˚C for 24 - 48hr. The colonies that came up on agar plates were stored at -80˚C in nutrient broth containing 30% glycerol. Routine Gram staining and biochemical tests were performed for the identification[7].

16S rRNA sequence analysis

16S rRNA gene sequences were performed. Analysis was done by Acme Progen Biotech Private Limited, Salem. The sequences containing 1306bp were aligned using Bio Edit software.

Antimicrobial assay

The inhibition of *Bacillus Sp*. by honey was tested by standard disc diffusion technique[8]. The honey samples were first inoculated separately on standard nutrient media with no test organisms so as to evaluate their possible contamination. Thereafter, solidified nutrient agar plates were separately flooded with the liquid inoculums of the test organisms using the pour plate method. The plates were drained and allowed to dry at 37˚C for 30mins after which wells were created on the

plates. Different concentrations of the honey samples were separately placed in the wells with 1ml sterile syringe. The plates were allowed to stay for 15 mins for pre-diffusion to take place followed by an overnight incubation that lasted for 24 hrs at 37˚C. The resulting zones of inhibition were measured and recorded. Similarly, conventional antibiotics like streptomycin, ampicillin and rifamicin were tested.

RESULTS AND DISCUSSION

Identification

According to morphological and biochemical results, the isolated organism was found to be Gram positive, Rod shaped *Bacillus Sp*.

16s rRNA gene sequence analysis

Final confirmation was done by 16s rRNA gene sequencing. 1306bp of 16s rRNA gene of *Bacillus sp*. was isolated using universal primer. The amplified product was purified by perfect prep@Gel cleanup kit cells. The sequence was aligned using BioEdit software and 1306bp long 16s rRNA gene of *Bacillus sp.* was obtained. The sequence was submitted to Genbank obtained accession number. EU847311. Similarity search using BLAST program showed 99% similarity with *Bacillus sp*. (accession number.DQ079006). The sequence was also aligned and analysed with the existing sequences in ribosomal database project 11 and the genus *Bacillus sp*. was confirmed by RDP classifier. This analysis showed 100% similarity to the genus *Bacillus sp*. DQO79007. Phylogenetic tree was constructed using maximum likelihood program version 3.6A2.1 of phylip available on Bio Edit suite.

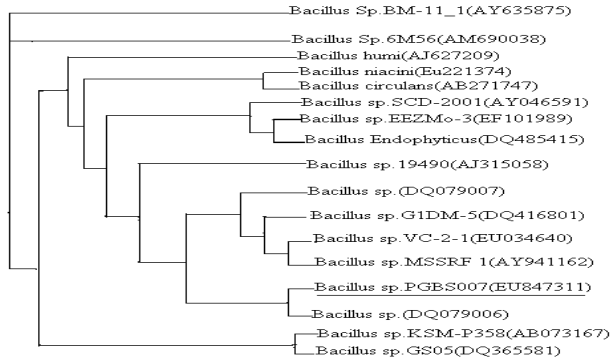

Figure 1: Phylogenic tree showing related Bacillus sp.

Antimicrobial potential of honey, standard antibiotics

Antibiotics are chemical compounds used to kill or inhibit the growth of infectious organisms. Results of antimicrobial activity of honey and standard antibiotics were depicted in Table 1.

Antimicrobial activity of undiluted natural honey was found to be more effective at concentration of both 5 and 10µl. whereas, the antimicrobial activity of undiluted marketed honey was low at 5µl and found to be moderate at 10µl. The antimicrobial activity of synthetic agents like Streptomycin, Ampicillin, Rifampicin was also tested. Among the synthetic agents used ampicillin showed highest zone of inhibition whereas, the zone was less with rifampicin. Even though, the zone of inhibition of honey was less when compared to synthetic agents, it might be a better agent having antimicrobial property at higher concentrations. Honey has a valuable role in traditional medicine for centuries. Honey is recognized as an efficacious topical antimicrobial agent in the treatment of burns and wounds[9,10]. More recently, honey has been reported to have an inhibitory effect to around 60 species of bacteria including aerobes and anerobes. Honey acts as a highly viscous barrier preventing bacterial penetration and colonization of wound surface. It has often been assumed that this is entirely due to the osmotic effect of its high sugar content[11], pH[12], activity of glucose oxidase, Hydrogen peroxide [13,14], Non peroxide substances[15,16], Presence of propolis which contain flavonoid[15,17] and volatile antibacterial substances[18]. It has been reported that honey contains lysozyme, a well known antibacterial agent[19]. However, in another study no lysozyme activity was found[20].

Table 1: Antibacterial property of honey

Samples / Synthetic agents	Antibacterial activity (cm)	
	5µl	10µl
Natural Honey	***	***
Marketed Honey	*	**
Streptomycin	2.2	3.0
Ampicillin	2.8	3.5
Rifampicin	1.5	2.0

*Low, **Medium, ***High,

CONCLUSION

Pathogenic organisms are increasing more and more. Hence, it is essential to develop antimicrobial agents from natural sources for better therapeutic effects to get rid of infection, diseases etc. In this study, antimicrobial activity is less in honey when compared to standard and in addition to that, honey is a natural therapeutic agent. The difference observed with other reports might be due to the variation in honey with respect to nature and type, place, source. Honey clears infection by stimulating the body's immune system to fight infection.

Acknowledgement: The authors thank Honourable. Vice chancellor Avl, Respected Registrar Avl, Periyar University Salem, Tamil Nadu, India for their support. The authors also thank Dr. Ganesan V, Acme Progen Biotech Private Limited, Salem for providing lab facilities and in identifying the organism.

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Source of Support: Nil, **Conflict of Interest:** None.