

# A study on Isolation of *Bacillus Tequilensis* and its Antimicrobial Activity

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**Abstract:** Various mangrove soils were tested for antibacterial bacterial cultures. In a sheep blood haemolysis test, just three of 80 bacterial isolates from mangrove soils throughout India were determined to be non-toxic. These cultures' antimicrobial activity was discovered to be extracellular. After a 35-hour incubation period, the highest activity was detected. Though after treatment using proteinase K and temperatures as much as 75°C, the culture's activity remained steady. The cultures of *Bacillus tequilensis* were studied

**Keywords:** Bacillus, Antimicrobial. Mangrove soil

## 1. Introduction

The rise of multidrug-resistant (MDR) and extreme drug-resistant (XDR) bacteria in the last decade has increased mortality and healthcare costs. Pathogens develop resistance by a variety of biochemical and physiological mechanisms, including as changing the target location so that antibiotics are rendered ineffective, and quickly pumping drugs out of the cell. [1]. Antibiotic overuse and a dearth of new antibacterial substances could be important causes throughout this predicament. This involves the monitoring of antibiotic usage as well as the identification of new antibiotic molecules [2]. Mangrove soils could act as a reference of microorganisms with something like a broad variety of activities, particularly anti-inflammatory and antimicrobial activity, due to the evolving conditions. [3]. Mangrove soils provide a variety of ecological habitats for the growth of various microorganisms such as actinomycetes, fungus, bacteria, macro and micro algae, secondary medicinal metabolite production and efficacy against common pathogenic microbes. [4,5].

## 2. Materials & Methods

Sediment samples were taken from the rhizosphere of *Avicennia marina*, a prominent mangrove plant in Mumbai's coastline regions, at a depth of 5-10 cm. After collecting the sample and eliminating any debris, it was delivered to the laboratory in sterile polyethylene bags. Soil sample (1g) was mixed thoroughly and serially diluted in 100 ml of sterile distilled water. After that, dilutions (10<sup>-3</sup> and 10<sup>-6</sup>) were spread plated on starch casein agar (SCA) and cultured for 48 hours at 37°C. For preservation, morphologically different colonies were separated, purified, and inoculated on yeast manitol agar (YMA). Cultures were cultivated in yeast manitol broth for about 70 hours before being utilised in all experiments. Isolated cultures were spot injected on sheep blood agar medium and cultured at 37°C for 24 hours before being examined for a haemolytic pattern on a sheep blood agar plate. [6,7]. The DNA was extracted by using genomic DNA isolation kit. This sequence was compared to National Biotechnology Information Center GenBank entries using the BLAST algorithm. [8,9]. Samples were obtained at various incubation intervals up to 120 hours after cultures were put in a growth media. The well penetration was utilised to govern supernatant behaviour while the optical density at 600nm was employed to assess cultural evolution. Isolates (1 OD, 1%) were

introduced into YMB and cultivated for 48 hours at 35 degrees Celsius and 120 revolutions per minute. Centrifugation was used to obtain culture supernatant at 4°C. The supernatant was aggressively mixed with different solvents (methanol, acetone, chloroform, and ethyl acetate) at a ratio of 1:1 v/v for 2 hours at 20°C. To precipitate, the solvent was evaporated at 50°C. The antibacterial function was then tested by immersing it in 1 ml of filtered water. The indicated samples were inoculated with changing pH in 100 ml broth (YMB) and incubated with the shaker incubator at 25 degree Centrigade. (120 rpm). Isolates were inoculated in a pH-optimized medium and incubated at various temperatures to determine the best temperature. Following the incubation of cultures, the diffusion approach against pathogenic cultures was examined. [10,11].

### 3. Characterization of The Antimicrobial Compound

After solvent extraction, the precipitate (1mg) was subjected to different temperatures (40 to 80 degree centigrade) for 20 minutes before being tested for activity against pathogens on MH agar plate. Similarly, the precipitate was incubated in phosphate buffer for 30 minutes with Proteinase K (1mg/ml). Supernatant without treatment, buffer, or Proteinase K served as the control. The inhibitory zone was measured and tracked. [12].

### 4. Results

Morphological colonies were isolated from various mangrove soil samples. Furthermore, these cultures were employed for antibacterial activity well-diffusion regulation. Twenty cultures were examined for inhibitory effectiveness against the majority of harmful bacteria. In 16s rRNA sequencing Culture *Bacillus tequilensis* were identified. After about 36 hours of incubation, the culture activity was at its peak. The antibacterial activity of cultures was tested using various solvent extracts, with methanol extract being employed for pathogenic microbes (Table 1). After processing with proteinase K, the efficacy of culture supernatant was almost comparable to that of supernatant, but it was nearly obliterated after heat treatment at 80°C.

**Table: 1 Well diffusion techniques for extracts' - antimicrobial activity.**

Pathogens	Jf6	JF14+1	MP15(2)
<i>B. subtilis</i>	0.7 cm	1.2cm	0.5 cm
<i>S. aureus</i>	0.8 cm	0.9 cm	0.7 cm
<i>S. typhi</i>	0.9 cm	0.8 cm	0.7 cm
<i>S. paratyphi</i>	0.8cm	0.7 cm	0.9 cm
<i>P. aeruginosa</i>	0.6cm	0.3 cm	0.7 cm
<i>E.coli</i>	1.1 cm	0.7 cm	0.7 cm
<i>Shigella</i>	1.6	0.7	0.4 cm
<i>Xanthomonas</i>	0.2cm	2.2cm	1.1cm

### 5. Conclusion

The current demand for novel antibacterial compounds has driven scientists all around the globe to look into new sources and creatures. Mangrove soil has a diverse microbial composition, with *Bacillus* spp. being the second most prevalent species after actinomycetes [6]. *Bacillus* bacteria are known to generate antibacterial and antifungal chemicals that aid in the battle against harmful microorganisms. Only three cultures, JF6, JF14+1, and MP15(2), were able to suppress the proliferation of the aforementioned pathogens. Molecular identification of JF6, JF14+1, and MP15 (2) as *Bacillus tequilensis* was achieved using 16S rRNA sequencing. There have been findings of antibacterial and antifungal properties in mangrove soil microbial communities [1,13,14]. treated with various solvents [15,16], with the fraction extracted with methanol showing only antibacterial activity. The activity was not lost when the extract was treated with proteinase K,

however it was lost completely when heated to 80°C. It denotes the antibacterial compound's non-protein origin. Mangrove soils are an excellent source of many microorganisms. Three *Bacillus* strains isolated from mangrove soils on the Indian coast were found to be non-hemolytic and capable of inhibiting usual harmful bacteria. Maximum antibacterial activity was identified after 36 hours of incubation at 30°C in medium with a pH of 6.0. Methanol extraction was used to isolate the activity, which was identified in the extracellular fraction. At 80 degrees C, the active compounds were found to be immune to proteinase K activity and temp sensitive. This suggests that in nature, the active fraction is non-proteinous. Because *Bacillus* species may produce substantial amounts of extracellular fractions, the crops suggested are promising for future antibacterial characterisation.

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