ANTIBACTERIAL ACTIVITY AND BIOTECHNOLOGICAL POTENTIAL OF ENDOPHYTIC BACTERIA ISOLATED FROM SEAWEED

ABSTRACT:

Seaweeds are indeed fascinating and diverse organisms found in coastal areas around the world. They play a significant role in marine ecosystems and have various uses for both humans and other marine life. They are categorized into three main groups based on coloration: red, green, and brown seaweeds. Each group has its unique characteristics and uses.Endophytic bacteria are significant contributors to the plant microbiome and are essential for the survival, development, and growth of the plant. Seaweed samples are collected and selected based on their morphology and colour.After the collection of seaweed they are cultured in TSA agar plates to obtain pure colonies.This method is usually done for the isolation of endophytic bacteria. Gram staining procedure are done to study about the morphological characteristics of the isolated bacteria.Followed by gram staining bacterial confirmation test is performed for the rapid and certain bacterial identification. Antimicrobial analysis is performed and the zone of inhibition is measured. The isolated bacteria are inoculated into the culture to produce PHB. The Sudan Black B staining method is used to detect the presence of polyhydroxybutyrate (PHB) granules in bacterial cells.Amylolytic and Proteolytic activity is also done. FTIR analysis is performed to analyze the chemical composition of substances by measuring their infrared absorption spectra.The study states that the maximum zone of inhibition of *E.coli* and *S.aureus* is 17mm and 15mm.The PHB production using endophytic fungi from seaweed was foundto produce PHB up to 900 mg of its dry cell weight.

KEYWORDS: Seaweed, Endophytic bacteria, Polyhydroxybutyrate (PHB),Antimicrobial activity, enzymatic activity and FTIR analysis.

1.INTRODUCTION

Seaweeds are a group of photosynthetic non-flowering plant-like organisms (called macroalgae) that live in the sea. They belong to three major groups based on their dominant pigmentation: red (Rhodophyta), brown (Phaeophyta) and green (Chlorophyta). Seaweeds were traditionally and are currently still used as food in China, Japan and the Republic of Korea.[1]Endophytes are an endosymbiotic group of microorganisms that can be readily isolated from any microbial and plant growth medium and they may colonize in plants and microbes. Endophytic organism plays a key role in discovery of novel bioactive secondary metabolites, these metabolites are alkaloids, quinones, steroids, saponins, tannins, terpenoids and phenolic acid. Bioactive secondary

metabolites of endophytic organisms serve as a vital sources for antimicrobial, anti-insect, anticancer and many more properties.[2]

The endophytic microorganisms can be bacteria, fungi, actinomycetes, or viruses. While they express a variety of symbiotic lifestyles ranging from parasitism to mutualism. Terrestrial endophytic organisms – chiefly fungi and bacteria which reside the living interior parts of plant tissue without causing any harmful effect to the host.[3] Marine endophytes – fungi have a firm relations with soft-bodied marine organisms, these organism does not have a clear structural protection mechanisms, and thus rely on chemical protection by the production of secondary metabolites. Fungi found in almost all the corners of marine habitat, namely marine plants such as algae, driftwood, mangrove plants and in marine invertebrates - sponges, corals, ascidians, holothurians and vertebrates (mainly fungi)[4]

PHB materials are typically stiff and brittle in nature, with low thermal stability and a high degree of crystallinity. Many PHB plastics have properties that are similar to the petroleum polymers polypropylene (PP) and polyethylene (PE). Feedstocks for PHB biopolymer production include renewable and sustainable sources such as food waste. These factors, combined with its biocompatibility and predisposition to biodegradation on exposure to designated active biological environments make PHB a leading candidate as an alternative to synthetic polymers such as PP and PE. Poly-3-hydroxybutyrate (PHB) is a copolymer of the polyhydroxyalkanoate family. It accumulates in the intracellular granules of bacteria under nitrogen limiting environment. The PHB producing bacteria was isolated from farm soil by serial dilution method. The Sudan Black B staining and Nile Blue A staining were carried out to confirm that the isolated strain was capable of producing PHB. Further, it was subjected to morphological, biochemical and molecular characterization. The 16S rRNA sequencing and phylogenetic analysis confirmed the organism was Bacillus cereus BB613 with accession number LN613102. The growth parameter and usage of low cost substrate for production was optimized. The maximum PHB accumulation was found at 30°C and pH 7.0 after 48h incubation. For the production of PHB, 1% sucrose was used as a carbon source. Sucrose was then substituted by low cost substrates such as mosambi peel, orange peel, banana peel and molasses which gave yields 35.2%, 26.7%, 32.1% and 33.9% respectively.[5]

2. MATERIALSANDMETHODS:

2.1 COLLECTION OF SAMPLES

The seaweed sample was collected from the Sathyabama University, Tamil nadu .Seaweeds were first observed, then collected according to differentiation in morphology and color. Collected seaweed samples are then transferred gently into Polythene zip-lock sample cover. Collected seaweed samples were quickly taken to a laboratory and then seaweed was gently washed in sterile water. Seaweed samples are then gently pressed with a sterile tissue paper to remove wetness from seaweed samples. Seaweed samples were further sterilized to remove external microorganisms for the isolation ofendophytic bacteria.[6]

2.2 BACTERIAL ISOLATION AND IDENTIFICATION

Endophytic bacteria was isolated and identified from the seaweed.Healthythallus of the seaweeds were thoroughly washed in seawater followed by running tap water, then surface sterilized by a modified method of, The selected thallus segments were immersed in 95% ethanol for 30 sec, 4% sodium hypochlorite solution for 3 min and 95% ethanol for 30 sec followed by rinsing with sterile distilled water three times and allowed to surface dry under sterile conditions.[7] After drying, each thallus segment was cut into approximately 0.5 cm and placed on petri plates containing tryptic soy agar medium (TSA) supplemented with ketoconazole (100 mg/L) to suppress the fungal growth. Petri plates were sealed with parafilm and incubated at 37°C for 24 hours in the incubator. They were monitored after the incubation period for growth of endophytic bacterial colonies. Bacteria growing out from the samples were subsequently transferred onto fresh TSA plates to isolate pure colonies

3.3 MORPHOLOGICAL CHARACTERISTICS

The colony characteristics such as size, shape, color, elevation, texture, margin and opacity were recorded. Furthermore, the growth cycle of the individual isolate was examined using a light microscope. Briefly, all actinobacterialmorphotypes were grown in LB broth and incubated at 28±2 °C at 120 rpm. The Gram staining was performed every 24 h up to 10 days to observe the growth.The slides were observed in a light microscope under 100X magnification.[8]

3.4 MICROSCOPIC EXAMINATION:

Identification of bacteria was done through the microscopic examination through gram staining technique to find out the morphological structure of the isolated bacteria.

3.5 BIOCHEMICALTEST:

Identification of bacteria was done through the biochemical test to find out the characteristic of the bacteria that include indole,MR,VP,Citrate utilization test,catalase test.

3.6.ANTIBACTERIAL ANALYSIS:

Antibacterial activity was performed by the Agar well diffusion method on Muller Hinton Agar (MHA). After incubation, the diameter of the zone of inhibition (mm) was measured and recorded.[9]

3.7.PHB PRODUCTION:

The Endophytic Bacterial culture was isolated and inoculated into the culture to produce the phb. All smears that resulted as positive were detected for the production of PHB granules using the Sudan black B solution (30%).Cells containing blue-black cytoplasmic granules were considered PHB producers, preserved in 2% glycerol vials for preservation and further analysis.[10] Stained slides were observed to determine the cellular characteristics of the isolates. The bacterial cells after 48 h of incubation were harvested by centrifugation and were air dried. The dried PHB was weighed and stored for further studies. The obtained PHB extraction was analyzed by Fourier transform infrared(FTIR).[11]

3.8.ENZYMATIC ACTIVITY:

3.8.1.AMYLOLYTIC ACTIVITY:

The starch hydrolysis test was carried out in order to assess the bacterial isolates' capacity for amylase. Starch hydrolysis agar, the selective medium, was made and sterilized for 15 minutes at 121°C and 15 lb pressure. Hydrolysis zones can be seen within the sight of dull blue foundation.[12]

3.8.2.PROTEOLYTIC ACTIVITY

On blood agar media,,the activity of proteolytic bacteria was qualitatively tested. Clear zones around the colon show that the bacteria are able to integrate protein.[13]

4.RESULT AND DISCUSSION:

4.1 IDENTIFICATION OF SEAWEED:

The identified seaweed is the Sargassum species. It has a highly branched thallus with hollow

berrylike floats (pneumatocysts). The numerous fronds are generally small and leaflike with toothed edges. Most species reproduce sexually, but the pelagic species reproduce by fragmentation. The largest members can reach several meters in length.

4.2 ISOLATION OF ENDOPHYTIC BACTERIA FROM SEAWEED:

The seaweed sample was collected from Sathyabama University. Then it was cut into small pieces to inoculate into TSA plates.*Bacillus cereus* Bacterial strain was identified. Bacterial endophytes promote the growth of host plants and enhance their resistance toward various pathogens and environmental stresses.

4.3 ANTIBACTERIAL ACTIVITY AGAINST UTI PATHOGENS:

In Antibacterial activity tests the *E.coli* and *Staphylococcus aureus* were used. *E.coli*and *Staphylococcus aureus*pathogens are swabbed into plates and the 20ul,50ul,75ul bacterial broth was inoculated into well diffusion method .In *E.coli* the zone of inhibition was 9mm,13mm,17mm. In *Staphylococcus aureus* the zone of inhibition was 10mm,12mm,15mm. In this method streptomycin antibiotic was used as a positive control. In *E.coli* and *Staphylococcus aureus* the zone of inhibition was 14mm and 15mm.

4.4 PHB PRODUCTION:

4.4.1.SUDAN BLACK B STAINING:

Results from the initial Sudan Black B staining on test isolate cultured in petri dishes showed that colonies' colors altered to a dark greenish-blue hue when the dye was poured into the dishes. This symptom is taken to indicate that test isolates have PHB accumulations.

4.4.2. PHB EXTRACT:

Bioplastics are gaining importance due to their biocompatible and biodegradable nature which may have a great impact as an alternative for petroleum based plastics. The bacterium isolated from the seaweed was identified as Bacillus cereus and was found to produce PHB up to 900 mg of its dry cell weight.

4.4.3.FTIR RESULT:

The phb extraction powder was analyzed by Fourier transform infrared (FTIR). The PHB powder

functional group was observed by FTIR peaks. The alkanes (C-H) group were located in 2924.09. They show alkanes consist of single-bonded carbon and hydrogen atoms. In 1743.65 peaks shows the ketones (C=O) group were located and carbonyl groups are composed of a carbon atom double-bonded to an oxygen atom. Alkenes groups were located in 1643.35. In alkene, carbon carbon double bond (C=C) is the functional group. Nitro compounds also have been presented. NO2 is strongly present in the extract polymer. In 1442.75 the aromatic compounds (C=C) were observed. The functional group that contains only carbon and hydrogen is an aromatic ring which is a six-carbon ring with alternating double bonds.Alkyl& Aryl Halides groups also presented.When a mixture of alkyl halide and aryl halide is treated with sodium in dry ether alkyl arene is formed.

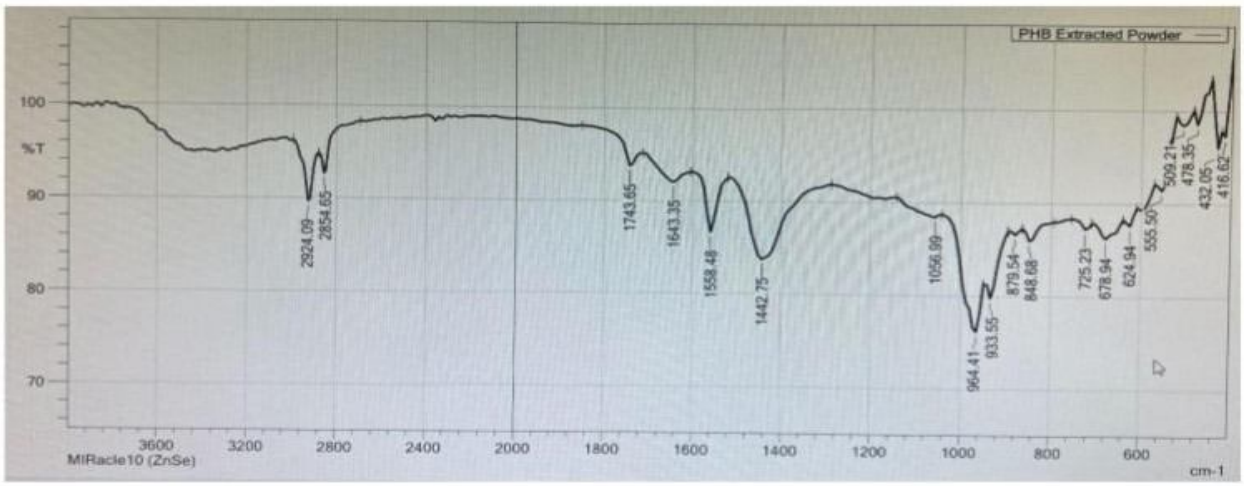

Figure 8 : FTIR report of PHB powder

4.5 AMYLOLYTIC ACTIVITY:

The isolated bacteria showed prominent growth on starch hydrolyzing, a zone of inhibition was observed after the addition of iodine.

4.6 PROTEOLYTIC ACTIVITY:

The isolate bacteria showed prominent growth on blood agar by the formation of zone of clearance after incubation

5.DISCUSSION

The bacterium isolated from the seaweed was identified as *Bacillus cereus*. The antibacterial actiWhen compared to previous finding of bacterium isolated from oil soaked cloth was found to produce PHB of 490 mg of dry cell weight .The PHB production using endophytic fungi from seaweed was foundto produce PHB up to 900 mg of its dry cell weight.The PHB extraction powder was analyzed by Fourier transform infrared(FTIR). The PHB powder functional group was observed by FTIR peaks. The alkanes (C-H) group were located in 2924.09. In 1743.65 peaks shows the ketones (C=O) group. Alkenes groups were located in 1643.35. In alkene, carbon carbon double bond (C=C) is the functional group. Nitro compounds also have been presented. NO2 is strongly present in the extract polymer. In 1442.75 the aromatic compounds (C=C)were observed.

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