Research & Reviews: Journal of Hospital and Clinical Pharmacy

Isolation and Screening on Antagonistic Secondary Metabolites from Seaweeds Surface Associate Bacterial Strains and In Vitro Pharmaceutical Approach

Henson jebajose
jebajose@gmail.com

Extended Abstract

Abstract

The present study explores the pharmaceutical potentials of marine bacterial strains isolated from the surface of seaweeds (macroalgae) collected from Kanyakumari coast of Tamilnadu. Totally six different seaweeds namely Chaetomorpha indica, Ulva fasciata (Red algae), Sargassum wightii, Padina gymnosperma (Brown algae) and Hypnea musciformis, Gracilaria edulis (Red algae) were collected and the total epiphytic bacterial isolates (CFU/g) on the surface of each seaweed species were assessed. Results on total epiphytic bacterial load revealed that they were in the following order: brown algae (19 to 23 x 105 CFU/g) < green algae (24 to 27 x 105 CFU/g) < red algae (30 to 37 x 105 CFU/g). Gram's reaction of epiphytic bacterial strains inferred red algae possess equal proportion (50%) of gram positive and gram-negative bacteria; nonetheless green and brown algae had higher percentage of gram positive rather than gram negative bacteria. Polyphasic characteristics showed presence of 19 different bacterial genera. Amongst which Bacillus sp. dominated higher percentage of occurrence (22 to 45.47%) in all the seaweed species. But other bacterial genera showed a consistent variation in its occurrence. Primary screening through cross-streak method inferred Bacillus sp., Pseudomonas sp., Alteromonas sp., Vibrio sp., Shigella sp., Pseudoalteromonas sp. and Shewanella sp. strongly deterred growth of all the human bacterial pathogens. Further to scrutinize and identify the potent bacterial strain (s), a secondary screening test was conducted using agar well diffusion method and it inferred that Pseudoalteromonas sp. (SW13) and Vibrio sp. (SW07) had profound antagonistic activity the other tested bacterial genera. Antibacterial assay using crude ethyl acetate extracts of both epiphytic bacterial strains indicated Pseudoalteromonas sp. had elevated inhibitory zone (23 to 27mm) than Vibrio sp. (13 to 19mm).

The bank of Tamil Nadu bears rich development of kelp. In excess of 200 types of ocean growth have been found here. Indian Ocean growth ventures rely upon this coastline for crude materials for the creation of agar and sodium alginate. In the present investigation the study the biopotentials of epiphytic bacteria strains present on the surface of selected seaweeds species were collected from Kanyakumari coast was in detail. In details, generally occurrence of seaweeds in this coastal region is between high tide to low tide and in the sub-tidal region up to a depth were 0.01% photosynthetic light is available. However distribution of seaweed species generally depends on plant pigments, light, exposure, depth, temperature, tides and the shore characteristic which combine together and create appropriate environment for the survival classified into three seaweeds (or) algae are basically classified in three different categories based on the pigment namely green (Chlorophyta), brown (Phaeophyta) and red (Rhodophyta) algae. In the present investigation six different seaweeds were collected from study area and identified using standard keys. Based on the keys they were found to be the representative of Chlorophyta (Chaetomorpha indica, Ulva fasciata), Phaeophyta (Sargassium wightii, and padina gymnosphora) and Rhodophyta (Hypnea musciformis, Gracillaria edulis). Besides, occurrences of epiphytic bacterial strain on the surface of selected seaweeds were determinaded through standard plate count methods.

Result of epiphytic bacterial density on selected seaweeds showed significant ($P<0.05$) variation. Amongst the seaweeds species red algae had pronounced bacterial density in on the surface of namely H. musciformis (37×105 CFU/g) and G. edulis (24×105 CFU/g) whereas phase (19 to 24 ×105 CFU/g) displayed llesser bacterial density of different seaweeds studied. The surface of red algal thallus displayed maximum of 142 distinct epiphytic isolates Gram's reaction that of the 142 isolates 50.7% were gram negative and 49.2% gram+ve belong to gram positive bacterial strains. Brown algal surface showed presence of 142 distinct bacterial isolates with 52.42% gram negative and 47.57%-gram positive bacterial strains. Green algal surface registered least number (84) of isolated with maximum of 60.7 gram negative and 39.3%-gram positive bacterial strains, respectively.

In the present study, the isolated epiphytic bacterial strains were individually subjected to primary screening through cross-streak method. Results showed that, Bacillus sp. (SW04), Pseudoomonas sp. (SW05), Alteromonas sp. (SW06), Vibrio sp. (SW07), Shigella sp. (SW08), Pseudoalteromonas sp. (SW13), and Shewanella sp. (SW16) were the most predominant epiphytic bacteria strains which significantly inhibited the growth of all the tested (100%) pathogenic bacteria strains with percentage of inhibition.

Followed by this, Arthrobacter sp (SW15), Aeromonas sp (SW20), Marinobacter sp (SW01), Serratia sp (SW08), Citrobacter sp (SW17), and Micrococcus sp (SW09) showed moderate growth inhibition (66.6 to 83.3%) of bacterial pathogens.

Further secondary screening was performed using most predominant result rendering epiphytic bacterial strains to substrate its antagonistic efficacy and also to identify the promising bacterial strain. Accordingly results of secondary screening inferred that CFU of Vibrio sp (17 to 20mm) and Pseudoalteromonas sp. (22 to 26mm) had profound antagonistic activity than the other epiphytic bacterial strains studied.

The result of morphological, physiologicaly and biochemical characteristics of seaweeds epiphytic bacterial strains revealed that isolated bacterial strains belongs to twenty different bacterial genera viz. Marinobacter sp., Staphyloccoccus sp., Brevibacterium sp., Bacillus sp., Pseudomonas sp., Alteromonas sp., Vibrio sp., Serratia sp., Micrococcus sp., Shigella sp., Rhodococcus sp., Kelbsiella sp., Pseudoalteromonas sp., Alcaligens sp., Arthrobacter sp., Shewanella sp., Citrobacter sp., Acinetobacter sp., Microbacterium sp. and Aeromonas sp. Percentage distribution of bacterial genera showed significant difference in its occurrence in the thallus of seaweeds. Here, Bacillus sp., (15.4 to 22%) and Stapahylococcus (8.6 to 11.5%) were found to be motst dominant genera in all the seaweeds surfaces. However, percentage occurrence of other seaweed epiphytic bacterial genera was markedly lesser and moreover it showed remarkable differences in its occurrence.

Based on the primary and secondary screening results both these two epiphytic bacterial isolates (SW07 and SW13) were subjected to molecular characterization through 16S rRNA sequencing. Sequencing results showed presence of 1454 bp in Pseudoalteromonas sp., (SW13) and 1440 bp in Vibrio sp. (SW07) when tested through BLAST hit. Based on phylogenetic analysis both epiphytic bacterial strains were submitted to NCBI Genbank as Vibrio sp. (MH908766) and Pseudoaltermonas flavipulchra (MH908650) and its respective Genbank numbers were obtained.

Both Vibrio sp. (SW07) and P. flavipulchra (SW13) were mass cultivated, centrifuged and the supernatant obtained was partitioned with ethyl acetate. The ethyl acetate fraction was separated and concentrated under vacuum. The ethyl acetate extraction of both the bacterial strains was tested for antibacterial efficacy against bacterial pathogens in comparison with standard antibiotic. It was found that both strains exerted significant ($P<0.05$) variation in inhibitory zones. However, between the two different bacterial strains. P. flavipulchra rendered higher growth inhibitory activity ranged between 20 and 29mm. But Vibrio sp., exerted relatively lesser antagonistic activity in the range of 13 to 19mm. On comparison positive control 22 to 27mm displayed inhibitory zones ranged between 23 and 27mm.

Bacteriostatic (MIC) and bactericidal (MBC) activities of crude ethyl acetate extracts of the promising epiphytic bacterial strains Vibrio sp., and P. flavipulchra showed notable variations in exerting bioactivity. For instance, P. flavipuchra registered meager concentration of 12.5 To 50µg/ml towards retarding (MIC) growth of bacterial pathogens and marginally increased concentration of 25 to 75µg/ml towards killing (MBC) the test bacterial strains. On the same instance, Vibrio sp., recorded a consistent increase in concentration of extract ranged between 50 and 75µg/ml towards inhibiting growth of bacteria (MIC) and 75 to 100µg/ml to eradicate bacterial (MBC) pathogens.

In the present study, the antiradical scavenging activity of ethyl acetate extract of the selected seaweed epiphytic bacterial strains were performed by DPPH assay. The DPPH radical scavenging activity of crude ethyl acetate extract of Vibrio sp. inferred that it had markedly lesser scavenging activity than P. falvipulchra and positive control.