Chapter - V
Discussion
5. DISCUSSION

Among the Asian countries, India is perhaps the only one that has a long record of inventories of coastal and marine biodiversity dating back to at least two centuries. However, these are so diverse in space, time and taxon that it is almost impossible to review all records and reports. In terms of marine environment, India has a coastline of about 8000 km, an Exclusive Economic Zone of 2.02 million km adjoining the continental regions and the offshore islands and a very wide range of coastal ecosystems such as estuaries, lagoons, mangroves, backwaters, salt marshes, rocky coasts, sandy stretches and coral reefs, which are characterized by unique biotic and abiotic properties and processes. Seaweeds are macroscopic marine algae attached to solid substratum, growing in the shallow waters of the sea. Since the present study was concerned with the isolation and characterization of bacteria associated with the chosen some seaweeds found along the coast of Thondi, Ramanathapuram District, India, the seaweeds were collected from the coast of Thondi.

Generally, the different varieties of seaweeds are abundant in the coast of Gulf of Mannar where as they are scarce in Palk Bay, especially in the coast of Thondi. In the present study, red seaweeds such as Jania rubens (Linnaeus) Lamouroux and Gracilaria corticata (J.Agardh), brown seaweeds such as Dictyota dichotoma (Hudson) Lamouroux, and Chnoosphora implexa (Herring) J.Agardh and green seaweeds such as Enteromorpho intestinalis (Linnaeus) Nees and Caulerpa racemosa (Forsskal) J.Agardh were collected. The above said seaweeds were randomly distributed. These seaweeds were very rare in Thondi coast. The availability of seaweeds might have been come from the coast of Gulf of Mannar due to the strong water current. Because, they were seen rare and attached loosely with the substratum.
Endophytic and epiphytic bacteria growing on the surfaces of/in seaweed live in a healthy competitive environment where space and access to nutrients are limited. These bacteria can produce secondary metabolites which inhibit the settlement of potential competitors such as invertebrate larvae and can antagonize other bacteria (Holmstrom and kjelleberg, 1999).

In the present study, eighteen endophytic as well as epiphytic bacterial isolates were isolated from six seaweeds. Out of which twelve isolates were endophytic bacteria (ARS-1-01, ARS-1-02, ARS-2-03, ARS-3-04, ARS-4-05, ARS-5-06, ARS-6-07, ARS-6-08, ARS-7-09, ARS-8-10, ARS-8-11 and ARS-12-18) and the six were epiphytic bacteria (ARS-9-12, ARS-9-13, ARS-10-14, ARS-10-15, ARS-11-16, and ARS-12-17). From the above said eighteen isolates, twelve bacteria such as ARS-1, ARS-2, ARS-3, ARS-4, ARS-5, ARS-6, ARS-7, ARS-8, ARS-9, ARS-10, ARS-11 and ARS-12 were identified. Among these twelve bacteria, four were gram positive rods; genus, *Bacillus* (ARS-1-01, ARS-4, ARS-7 and ARS-8) and the rest of them were gram negative rods; genus, *Idiomarina* (ARS-2 and ARS-3), *Vibrio* (ARS-5, ARS-6, ARS-9 and ARS-11), *Rhodopirellula* (ARS-10) and *pseudomonas* (ARS-12). All the bacterial isolates were identified based on their cultural characterization, microscopical characterization, Gram’s staining, biochemical characterization and 16s rRNA gene sequencing. In addition to those, antifungal susceptibility, antibacterial susceptibility and antibiotic susceptibility were also tested against selected three fungi, three poultry and three cattle pathogens and five antibiotic discs.

The bacterial isolates, ARS-1-01 and ARS-1-02 were isolated as endophytic from the red seaweed, *Jania rubens* and green seaweed, *Enteromorpha intestinalis* respectively. The colonies of these two isolates of ARS-1 were moderate, yellow, circular, entire and raised. And the cells were short rod, motile and Gram positive.
They showed positive results for Catalase, ONPG, nitrate reduction, citrate utilization, Voges-Proskauer’s, esculin hydrolysis, saccharose, trehalose, glucose and oxidase tests. And the isolates had negative results for lysine utilization, ornithine utilization, urease, phenyl alanine deamination, H2S production, methyl red, indole, malonate utilization, arabinose, Xylose, adonitol, rhamnose, cellobiose, melibose, raffinose and lactose. Bacterial isolate ARS-1-01 and ARS-1-02 showed the maximum antifungal activity against the fungus, *Fusarium oxysporum* (36 mm) where as the minimum activity against *Rhizoctonia solani* (12 mm) and no activity against *Pyricularia oryzae*. These isolates exhibited the average antibacterial activity (5mm) against two poultry pathogens such as *Eicheria coli* and *Pasteurella multocida* and slightly higher (7mm) against *Salmonella pullorum*, the poultry pathogen. These isolates ARS-1-01 and ARS-1-02 had highest antibacterial activity (10mm) against the cattle pathogen, *Pseudomonas aeruginosa*, the medium (8mm) against *Staphylococcus aureus* and the minimum (6mm) against the cattle pathogen, *Eicheria coli*. These isolates showed the maximum sensitive (30 mm) to the antibiotic, *Cefoperozone-75mcg* and the medium (25mm and 20mm) against the antibiotics, *Amikacin-30mcg* and *Gentamycin-10mcg* respectively. They had resistance to the antibiotics such as *Cefpodoxime-10mcg* and *Ceftazidime-30mcg*. The bacterial isolates ARS-1-01 and ARS-1-02 were sequenced using 16s rRNA gene sequencing and the sequences of genes were compared with the Blast analysis for the closest match of the bacteria (Database, NCBI) and the distance tree for the same was obtained. Based on the result of Blast analysis and distance tree of the isolates ARS-1-01 and ARS-1-02, the closest matched bacterium was *Bacillus subtilis*. And the sequences of the same were deposited in NCBI, GenBank for getting accession number. The GenBank accession number for the isolated bacterium, *Bacillus subtilis* (Isolate ARS-1) is KF688211.
The bacterial isolate, ARS-2-03 was isolated as endophytic also from the red seaweed, *Jania rubens*. The colony of this isolate was small, off-white, circular, entire and flat. The cells of the bacterial isolate, ARS-2-03 were short rod, non-motile and Gram negative. It had positive results for the tests of Catalase, ornithine utilization, citrate utilization, Voges-Proskauer’s, methyl red, malonate utilization, arabinose, saccharose, trehalose, glucose, and oxidase and the negative results for the tests of ONPG, lysine utilization, nitrate reduction, urease, phenyl alanine deamination, H₂S production, indole, esculin hydrolysis, Xylose, adonitol, rhamnose, cellobiose, melibiose, raffinose and lactose. Bacterial isolate ARS-2-03 had highest antifungal susceptibility (60mm) against the fungus, *Fusarium oxysporum* whereas medium (15mm, 12mm) against the fungi *Rhizoctoni solani* and *Pyricularia oryzae* respectively. This isolate showed no antibacterial activity against the poultry pathogen, *Eicheria coli* and minimum activity (7mm, 5mm) against the poultry pathogens, *Pasteurella multocida* and *Salmonella pullorum* respectively. It showed medium antibacterial activity (8mm) against the cattle pathogen, *Eicheria coli*, minimum (6mm) against the cattle pathogen, *Staphylococcus aureus* and no response against the cattle pathogen, *Pseudomonas aeruginosa*. In the case of antibiotic susceptibility, this isolate exhibited maximum sensitivity (25mm) against both the antibiotics, *Amikacin-30mcg* and *Cefoperozone-75mcg*. It had medium sensitivity (22mm) against the antibiotic, *Gentamycin-10mcg*, minimum activity (10mm) against *Cefpodoxime-10mcg* and no response against *Ceftazidine-30mcg*. The bacterial isolates ARS-2-03 was sequenced using 16s rRNA gene sequencing and the sequences of genes were compared with the Blast analysis for the closest match of the bacteria (Database, NCBI) and the distance tree for the same was obtained. Based on the result of Blast analysis and distance tree of the isolate ARS-2-03, the closest
matched bacterium was *Idiomarina marina*. And the sequences of the same were deposited in GenBank-NCBI for getting accession number. The GenBank accession number for the isolated bacterium, *Idiomarina marina* (Isolate ARS-2) is KF688212.

The bacterial isolate, ARS-3-04 was isolated as endophytic from the red seaweed, *Gracilaria corticata*. The colony of this isolate was small, off-white, circular, entire and flat. The cells of the isolate were slightly curved rod, motile and Gram negative. It showed positive results for the tests of Catalase, ornithine utilization, citrate utilization, Voges-Prokauer’s, malonate utilization, saccharose, trehalose, glucose, and oxidase and the negative results for the tests of ONPG, lysine utilization, urease, phenyl alanine deamination, nitrate reduction, H₂S production, methyl red, indole, esculin hydrolysis, arabinose, Xylose, adonitol, rhamnose, cellobiose, melibose, raffinose and lactose. The bacterial isolate, ARS-3-04 showed the maximum antifungal activity (40mm) against the fungi, *Fusarium oxysporum*, the minimum (13mm) against *Rhizoctonia solani* and no activity against *Pyricularia oryzae*. This isolate exhibited an average antibacterial activity against the poultry pathogens, *Echeria coli* (8mm), *Pasteurella multocida* (7mm) and *Salmonella pullorum* (9mm). It had the antibacterial activity only against the cattle pathogen, *Echeria coli* (10mm) and no activity against both the cattle pathogens, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. This isolate showed antibiotic susceptibility as maximum (25mm) against the antibiotic, *Cefoperozone-75mcg* and the susceptibility of 23mm, 20mm, 15mm and 10mm against the antibiotics, *Amikacin-30mcg*, *Gentamycin-10mcg*, *Ceftazidine-30mcg* and *Cefpodoxime-10mcg* respectively. The bacterial isolates ARS-3-04 was sequenced using 16s rRNA gene sequencing and the sequences of genes were compared with the Blast analysis for the closest match of the bacteria (Database, NCBI) and the distance tree for the same was
obtained. Based on the result of Blast analysis and distance tree of the isolate ARS-2-03, the closest matched bacterium was *Idiomarina loihiensis*. And the sequences of the same were deposited in GenBank-NCBI for getting accession number. The GenBank accession number for the isolated bacterium, *Idiomarina loihiensis* (Isolate ARS-3) is KF688213.

The bacterial isolate, ARS-4-05 was isolated as endophytic from the red seaweed, *Gracilaria corticata*. The colony of this isolate was moderate, yellow, circular, entire and raised. The cells of the isolate were short rod, motile and Gram positive. This isolate exhibited positive results for the tests of Catalase, ONPG, ornithine utilization, phenyl alanine deamination, nitrate reduction, citrate utilization, malonate utilization, esculin hydrolysis, cellobiose, saccharose, trehalose, glucose, lactose and oxidase and the negative results for the tests of lysine utilization, urease, H$_2$S production, Voges-Proskauer’s, methyl red, indole, arabinose, Xylose, adonitol, rhamnose, melibiose and raffinose. This isolate showed the maximum antifungal activity (34mm) against the fungus, *Fusarium oxysporum*, the minimum (17mm) against *Rhizoctonia solani* and no activity against *Pyricularia oryzae*. With reference to antibacterial activity, this isolate showed 9mm against the poultry pathogen, *Salmonella pullorum* and no activity against both the pathogens, *Eicheria coli* and *Pasteurella multocida*. And it had antibacterial activity of 7mm and 5mm against the cattle pathogens, *Eicheria coli* and *Pseudomonas aeruginosa* respectively. But, there was no antibacterial activity against the cattle pathogen, *Staphylococcus aureus*. This isolate ARS-4-05, showed the antibiotic susceptibility of 27mm, 28mm, 10mm, 15mm and 15mm against the antibiotics, *Amikacin-30mcg*, *Gentamycin-10mcg*, *Cefoperozone-75mcg*, *Cefpodoxime-10mcg* and *Ceftazidime-30mcg* respectively. The bacterial isolates ARS-4-05 was sequenced using 16s rRNA gene sequencing and the
sequences of genes were compared with the Blast analysis for the closest match of the bacteria (Database, NCBI) and the distance tree for the same was obtained. Based on the result of Blast analysis and distance tree of the isolate, ARS-4-05 the closest matched bacterium was *Bacillus megaterium*. And the sequences of the same were deposited in GenBank-NCBI for getting accession number. The GenBank accession number for the isolated bacterium *Bacillus megaterium* (Isolate ARS-4) is KF688214.

The bacterial isolate, ARS-5-06 was isolated as an endophytic from the brown seaweed, *Dictyota dichotoma*. The colony of this isolate was moderate, colourless, circular, entire and raised. The cells of the isolate were short rod, motile and Gram negative. Isolate ARS-5-06 showed positive results for the tests of Catalase, lysine utilization, ornithine utilization, urease, nitrate reduction, $H_2S$ production, citrate utilization, methyl red, indole, saccharose, trehalose, glucose, and oxidase and the negative results for the tests of ONPG, phenyl alanine deamination, Voges-Proskauer’s, malonate utilization, esculin hydrolysis, arabinose, Xylose, adonitol, rhamnose, cellobiose, melibiose, raffinose and lactose. This isolate showed the antifungal activity against only one fungus namely, *Rhizoctoni solani* (10mm) but no activity against the remaining two fungi namely, *Fusarium oxysporum* and *Pyricularia oryzae*. It had antibacterial activity against the poultry pathogens such as *Eicheria coli* (10mm0, *Pasteurella multocida*(8mm) and *Salmonella pullorum* (7mm).This isolate showed the equal level of antibacterial activity (6mm) against the cattle pathogens such as *Staphylococcus aureus* and *Eicheria coli* and no activity against *Pseudomonas aeruginosa*. The isolate ARS-5-06 showed highest antibiotic susceptibility (33mm) against the antibiotic, *Cefoperozone-75mcg* and the medium activity of 25mm and 23mm against the antibiotics, *Amikacin-30mcg* and *Gentamycin-10mcg* respectively. It showed minimum activity of 17mm and 15mm
against the antibiotics, Ceftazidime-30mcg and Cefpodoxime-10mcg respectively. The bacterial isolate ARS-5-06 was sequenced 16s rRNA gene sequencing and the sequences of genes were compared with the Blast analysis for the closest match of the bacteria (Database, NCBI) and the distance tree for the same was obtained. Based on the result of Blast analysis and distance tree of the isolate, ARS-5-06 the closest matched bacterium was *Vibrio alginolyticus*. And the sequences of the same were deposited in GenBank-NCBI for getting accession number. The GenBank accession number for the isolated bacterium *Vibrio alginolyticus* (Isolate ARS-5) is KF688215.

The bacterial isolates, ARS-6-07 and ARS-6-08 were isolated as endophytic from the brown seaweed, *Dictyota dichotoma* and from the green seaweed, *Enteromorpha intestinalis*. The colonies of the isolates were moderate, colourless, circular, entire and raised. The cells of the isolates were short rod, motile and Gram negative. Isolates ARS-6-07 and ARS-6-08 showed positive results for the tests of Catalase, lysine utilization, urease, nitrate reduction, H2S production, citrate utilization, saccharose, trehalose, glucose and oxidase and the negative results for the tests of ONPG, ornithine utilization, phenyl alanine deamination, Voges-Proskauer’s, methyl red, indole, malonate utilization, esculin hydrolysis, arabinose, Xylose, adonitol, rhamnose, cellobiose, melibiose, raffinose and lactose. They showed antifungal activity as 38mm against the fungus, *Fusarium oxysporum* and 20mm against *Rhizoctoni solani* and no activity against *Pyricularia oryzae*. They exhibited the maximum antibacterial activity (10mm) against the poultry pathogen, *Salmonella pullorum* and the minimum (6mm) against *Pasteurella multocida*. But, they did not show any activity against *Eicheria coli*. They had the antibacterial activity of 9mm and 6mm against the cattle pathogens, *Eicheria coli* and *Staphylococcus aureus* respectively and no activity against *Pseudomonas aeruginosa*. The isolates showed
the antibiotic susceptibility against the antibiotics, *Amikacin-30mcg* (25mm), *Gentamycin-10mcg* (26mm), *Cefoperozone-75mcg* (30mm) and no susceptibility against the remaining two antibiotics such as *Cefpodoxime-10mcg* and *Ceftazidime-30mcg*. The bacterial isolates ARS-6-07 and ARS-6-08 were sequenced using 16s rRNA gene sequences and the sequences of genes were compared with the Blast analysis for the closest match of the bacteria (Database, NCBI) and the distance tree for the same was obtained. Based on the result of Blast analysis and distance tree of the isolates, ARS-6-07 and ARS-6-08 the closest matched bacterium was *Vibrio sp.*. And the sequences of the same were deposited in GenBank-NCBI for getting accession number. The GenBank accession number for the isolated bacterium *Vibrio sp.* (Isolate ARS-6) is KF688216.

The bacterial isolate, ARS-7-09 was isolated as endophytic from the brown seaweed, *Chnoosphora implexa*. The colony of the isolate was moderate, yellow, circular, entire and raised. The cells of the isolates were short rod, motile and Gram positive. The bacterial isolate, ARS-7-09 showed positive results for the tests of Catalase, lysine utilization, nitrate reduction, citrate utilization, Voges-Proskauer’s, esculin hydrolysis, Xylose, cellobiose, saccharose, raffinose, trehalose, glucose and oxidase and the negative results for the tests of ONPG, ornithine utilization, urease, phenyl alanine deamination, H₂S production, methyl red, indole, malonate utilization, arabinose, adonitol, rhamnose, melibiose, and lactose. The isolate gave the higher antifungal activity (40mm) against the fungus, *Fusarium oxysporum* and medium activity (22mm) against *Rhizoctoni solani*. But, there was no activity against *Pyricularia oryzae*. It showed the higher antibacterial activity (12mm) against the poultry pathogen, *Pasteurella multocida* and the medium activity against *Eicheria coli* (5mm) and *Salmonella pullorum* (4mm). This isolate showed the maximum level
of antibacterial activity against the cattle pathogen, *Staphylococcus aureus* (23mm), medium activity against *Pseudomonas aeruginosa* (9mm) and the minimum activity against *Eicheria coli* (5mm). It exhibited the antibiotic susceptibility against only three antibiotics such as *Amikacin-30mcg* (28mm), *Gentamycin-10mcg* (24mm) and *Cefoperozone-75mcg* (25mm) and no susceptibility against the remaining two antibiotics such as *Cefpodoxime-10mcg* and *Ceftazidime-30mcg*. The bacterial isolate ARS-7-09 was sequenced using 16s rRNA gene sequencing and the sequences of genes were compared with the Blast analysis for the closest match of the bacteria (Database, NCBI) and the distance tree for the same was obtained. Based on the result of Blast analysis and distance tree of the isolate, ARS-7-09 the closest matched bacterium was *Bacillus amyloliquefaciens*. And the sequences of the same were deposited in GenBank-NCBI for getting accession number. The GenBank accession number for the isolated bacterium, *Bacillus amyloliquefaciens* (Isolate ARS-7) is KF688217.

The bacterial isolates, ARS-8-10 and ARS-8-11 were isolated as endophytic from the brown seaweed, *Chnoosphora implexa* and the green seaweed, *Caulerpa racemosa* respectively. The colonies of the isolates were moderate, yellow, circular, entire and raised. The cells of the isolates were short rod, motile and Gram positive. The bacterial isolates, ARS-8-10 and ARS-8-11 showed positive results for the tests of Catalase, ONPG, lysine utilization, ornithine utilization, nitrate reduction, citrate utilization, Voges-Proskauer’s, indole, arabinose, adonitol, rhamnose, cellobiose, saccharose, raffinose, trehalose, glucose, lactose and oxidase and the negative results for the tests of urease, phenyl alanine deamination, H₂S production, methyl red, malonate utilization, esculin hydrolysis, Xylose and melibiose. The isolates showed highest antifungal activity against the fungus, *Fusarium oxysporum* (66mm) and the
lowest against *Rhizoctoni solani* (16mm) and no activity against *Pyricularia oryzae*. The isolates had higher antibacterial activity (30mm) against the poultry pathogen, *Salmonella pullorum*, the medium (18mm) against *Eicheria coli* and the lower activity (8mm) against *Pasteurella multocida*. They exhibited antibacterial activity of 8mm against the cattle pathogen, *Eicheria coli*, 6mm against *Pseudomonas aeruginosa* and no activity against *Staphylococcus aureus*. The isolates showed antibiotic susceptibility against three antibiotics such as Amikacin-30mcg (28mm) Gentamycin-10mcg (30mm) and Cefoperozone-75mcg (34mm) and no susceptibility against the remaining two antibiotics such as Cefpodoxime-10mcg and Ceftazidine-30mcg. The bacterial isolates ARS-8-10 and ARS-8-11 were sequenced using 16s rRNA gene sequencing and the sequences of genes were compared with the Blast analysis for the closest match of the bacteria (Database, NCBI) and the distance tree for the same was obtained. Based on the result of Blast analysis and distance tree of the isolates ARS-8-10 and ARS-8-11, the closest matched bacterium was *Bacillus tequilensis*. And the sequences of the same were deposited in GenBank-NCBI for getting accession number. The GenBank accession number for the isolated bacterium, *Bacillus tequilensis*. (Isolate ARS-8) is KF688218.

The bacterial isolates ARS-9-12 and ARS-9-13 were isolated as epiphytic from the red seaweed, Jania *rubens* and the brown seaweed, *Chnoosphora implexa* respectively. The colonies of these isolates were moderate, colourless, circular, entire and raised and the cells of the isolates were short rod, motile, and Gram negative. The isolates showed positive results for tests of Catalase, ONPG, nitrate reduction, citrate utilization, malonate utilization, esculin hydrolysis, arabinose, rhamnose, cellobiose, saccharose, trehalose, glucose, and oxidase and the negative results for the tests of lysine utilization, ornithine utilization, urease, phenyl alanine deamination, H₂S.
production, Voges-Proskauer’s, methyl red, indole, Xylose, adonitol, melibose, raffinose and lactose. The isolates showed the antifungal activity only against the fungus, Rhizoctoni solani (17mm) and no activity against the fungi, Fusarium oxysporum and Pyricularia oryzae. These isolates exhibited the same antibacterial activity value (10mm) against three poultry pathogens such as Eicheria coli, Pasteurella multocida and Salmonella pullorum. The isolates showed the same antibacterial activity (8mm) against the cattle pathogens such as Eicheria coli and Pseudomonas aeruginosa. And they had 9mm activity against Staphylococcus aureus. The isolates exhibited the antibiotic susceptibility against all five antibiotics such as Amikacin-30mcg (28mm), Gentamycin-10mcg (24mm), Cefoperozone-75mcg (30mm), Cefpodoxime-10mcg (12mm) and Ceftazidine-30mcg (15mm). The bacterial isolates ARS-9-12 and ARS-9-13 were sequenced using 16s rRNA gene sequencing and the sequences of genes were compared with the Blast analysis for the closest match of the bacteria (Database, NCBI) and the distance tree for the same was obtained. Based on the result of Blast analysis and distance tree of the isolates, ARS-9-12 and ARS-9-13 the closest matched bacterium was Vibrio natriegens. And the sequences of the same were deposited in GenBank-NCBI for getting accession number. The GenBank accession number for the isolated bacterium, Vibrio natriegens. (Isolate ARS-9) is KF688219.

The bacterial isolates ARS-10-14 and ARS-10-15 were isolated as epiphytic from the seaweed, Gracilaria corticata and the green seaweed, Caulerpa racemosa respectively. The colonies of the isolates were moderate, yellow, circular, entire and raised and the cells of the isolates were ovoid, motile and Gram negative. The isolates showed positive results for the tests of Catalase, lysine utilization, nitrate reduction, esculin hydrolysis, arabinose, Xylose, rhamnose, cellobiose, melibiose, saccharose,
raffinose, trehalose, glucose, lactose and oxidase and the negative results for the tests of ONPG, ornithine utilization, urease, phenyl alanine deamination, H2S production, citrate utilization, Voges-Proskauer’s, methyl red, indole, malonate utilization, and adonitol. The isolates showed antifungal activity against the fungi, Rhizoctoni solani (10mm) and Fusarium oxysporum (8mm) and no activity against Pyricularia oryzae. The isolates showed same value (7mm) of antibacterial activity against the poultry pathogens such as Eicheria coli and Salmonella pullorum and no activity against Pasteurella multocida. The isolates exhibited the antibacterial activity against the cattle pathogens such as Staphylococcus aureus (8mm) and Eicheria coli (9mm). And no activity against Pseudomonas aeruginosa. These isolates showed antibiotic susceptibility against the antibiotics such as Amikacin-30mcg (25mm), Gentamycin-10mcg (30mm) and Cefoperozone-75mcg (35mm). But, no susceptibility was seen against Cefpodoxime-10mcg and Ceftazidime-30mcg.

The bacterial isolates ARS-10-14 and ARS-10-15 were sequenced using 16s rRNA gene sequencing and the sequences of genes were compared with the Blast analysis for the closest match of the bacteria (Database, NCBI) and the distance tree for the same was obtained. Based on the result of Blast analysis and distance tree of the isolates, ARS-10-14 and ARS-10-15 the closest matched bacterium was Rhodopirellula baltica. And the sequences of the same were deposited in GenBank-NCBI for getting accession number. The GenBank accession number for the isolated bacterium, Rhodopirellula baltica (Isolate ARS-10) is KF688220.

The bacterial isolate ARS-11-16 was isolated as epiphytic from the brown seaweed, Dictyota dichotoma. The colony of the isolate was moderate, colourless, circular, entire and raised. The cells of the isolate were strait rod, motile and Gram negative. The isolate showed positive results for tests of Catalase, lysine utilization,
H$_2$S production, methyl red, esculin hydrolysis, glucose, lactose and oxidase and the negative results for the tests of ONPG, ornithine utilization, urease, phenyl alanine deamination, nitrate reduction, citrate utilization, Voges-Proskauer’s, indole, malonate utilization, arabinose, Xylose, adonitol, rhamnose, cellobiose, melibiose, saccharose, raffinose and trehalose. The isolate showed higher antifungal activity (34mm) against the fungus, *Fusarium oxysporum* and lower activity (17mm) against the fungus, *Rhizoctoni solani* and no activity against *Pyricularia oryzae*. The isolate exhibited the maximum antibacterial activity (8mm) against the poultry pathogen, *Eicheria coli* and medium activity (7mm) against *Pasteurella multocida*. There was minimum activity (5mm) against *Salmonella pullorum*. The isolate had antibacterial activity against the cattle pathogens such as *Staphylococcus aureus* (8mm) and *Pseudomonas aeruginosa* (9mm). But, there was no activity against *Eicheria coli*. The isolate ARS-11-16 showed antibiotic susceptibility against three antibiotics only namely *Amikacin-30mcg* (22mm) *Gentamycin-10mcg* (15mm) and *Cefoperozone-75mcg* (15mm). And there were no activities against the rest of the antibiotics namely *Cefpodoxime-10mcg* and *Ceftazidime-30mcg*. The bacterial isolate ARS-11-16 was sequenced using 16s rRNA gene sequencing and the sequences of genes were compared with the Blast analysis for the closest match of the bacteria (Database, NCBI) and the distance tree for the same was obtained. Based on the result of Blast analysis and distance tree of the isolates, ARS-11-16 the closest matched bacterium was *Vibrio sp.2*. And the sequences of the same were deposited in GenBank-NCBI for getting accession number. The GenBank accession number for the isolated bacterium, *Vibrio sp.2*. (Isolate ARS-11) is KF688221.

The bacterial isolates ARS-12-17 and ARS-12-18 were isolated as epiphytic and endophytic respectively from the seaweeds, *Enteromorpho intestinalis* and
Caulerpa racemosa respectively. The colonies of the isolate were moderate, colourless opaque, circular, entire and raised. The cells of the isolate were short rod, motile and Gram negative. The isolates showed positive results for the tests of Catalase, lysine utilization, ornithine utilization, nitrate reduction, citrate utilization, saccharose, glucose and oxidase and the negative results for the tests of ONPG, urease, phenyl alanine deamination, H₂S production, Voges-Proskauer’s, methyl red, indole, malonate utilization, esculin hydrolysis, arabinose, Xylose, adonitol, rhamnose, cellobiose, melibiose, raffinose, trehalose and lactose. The isolates showed antifungal activity against all three fungi such as Fusarium oxysporum (8mm), Rhizoctoni solani (7mm) and Pyricularia oryzae (5mm). The isolates exhibited same antibacterial activity (6mm) against the poultry pathogens such as Eicheria coli, and Pasteurella multocida and higher activity (12mm) against Salmonella pullorum. The isolates exhibited maximum antibacterial activity (7mm) against the cattle pathogen, Pseudomonas aeruginosa and minimum activity (6mm) against Eicheria coli and there was no activity against Staphylococcus aureus. The isolates were sensitive to three antibiotics namely Amikacin-30mcg (22mm), Gentamycin-10mcg (17mm) and Cefoperozone-75mcg (18mm) and resistant to two antibiotics namely Cefpodoxime-10mcg and Ceftazidine-30mcg. The bacterial isolates ARS-12-17 and ARS-12-18 were sequenced using 16s rRNA gene sequencing and the sequences of genes were compared with the Blast analysis for the closest match of the bacteria (Database, NCBI) and the distance tree for the same was obtained. Based on the result of Blast analysis and distance tree of the isolates, ARS-12-17 and ARS-12-18 the closest matched bacterium was Pseudomonas knackmussii. And the sequences of the same were deposited in NCBI for getting accession number. The GenBank accession
number for the isolated bacterium, *Pseudomonas knackmussii*. (Isolate ARS-12) is KF688222.

The word, ‘symbiosis’ is derived from the Greek word, which means ‘living together’. Symbiosis could be a close, prolonged or temporary, intimate association between two organisms, which are widely separated phylogenetically. In symbiosis, growth and survival of both the organisms are benefited and neither of them can survive under natural conditions without each other (Odum, 1959). In the association of two organisms the larger one is the host and it harbours the mutual partner- the symbiont, typically providing nourishment and shelter. Thus, microorganisms occur as useful guests in numerous animals and plants. Symbiosis may be divided into two distinct categories, ectosymbiosis and endosymbiosis.

In Ectosymbiosis the symbiont lives on the body surface of the host including internal surfaces such as lining of the digestive tube and ducts of glands. Marine invertebrates living closer to the sea bottom, bivalve molluscs have established symbiosis with chemosynthetic bacteria that use inorganic reduced compounds as an electron source, in regions where sulfide and oxygen are present in the water perfusing the sediments. The sulfur-oxidizing epibacteria found on nematodes living in marine sands, at the oxic- anoxic interface; the nematodes appear to meet their carbon requirements directly by feeding on their symbionts, and they move down the chemocline (chemical gradient in the water column) to provide their symbionts with both sulfide and oxygen (Dimijian, 2000).

In Endosymbiosis, the symbiont lives within the cells or intracellular space of the host. Such a symbiont is called an endosymbiont. Occurrence of endosymbiosis is well documented in the hydrothermal vent systems (Cary et al., 1993; Feldman et al., 1997; Hurtado et al., 2003). The biodiversity of hydrothermal systems include a large
assemblage of invertebrate organisms from large tubeworms to a varied species of crabs (Van Dover et al., 2001; Guinot and Hurtado, 2003). The deep-sea hydrothermal vent sites are characterized by high concentration of reduced sulfur compounds (Baker, 1994). Here, life is supported by the growth of chemolithoautotrophic bacteria, capable of oxidizing hydrogen sulfide to generate energy that is used to fuel carbon dioxide fixation into macromolecules (Compere et al., 2002 and Garcia et al., 2003). Such symbiotic associations are not confined to very special habitats like hydrothermal venting sites. The bacteria-animal symbiosis may vary from transient, nonspecific symbionts in many aquatic organisms to highly specific, non-culturable, intracellular symbionts as in pyrosomes (Nealson et al., 1981). In the present study, out of eighteen bacterial isolates, twelve isolates were endophytic bacteria (ARS-1-01, ARS-1-02, ARS-2-03, ARS-3-04, ARS-4-05, ARS-5-06, ARS-6-07, ARS-6-08, ARS-7-09, ARS-8-10, ARS-8-11 and ARS-12-18) and the six were epiphytic bacteria (ARS-9-12, ARS-9-13, ARS-10-14, ARS-10-15, ARS-11-16, and ARS-12-17). From the above said eighteen isolates, twelve bacteria such as ARS-1, ARS-2, ARS-3, ARS-4, ARS-5, ARS-6, ARS-7, ARS-8, ARS-9, ARS-10, ARS-11 and ARS-12 were identified. Among these twelve bacteria, four were gram positive rods; genus, Bacillus (ARS-1, ARS-4, ARS-7 and ARS-8) and the rest of those were gram negative rods; genus, Idiomarina (ARS-2 and ARS-3), Vibrio (ARS-5, ARS-6, ARS-9 and ARS-11), Rhodopirellula (ARS-10) and Pseudomonas (ARS-12).

Most of the sea bacteria belong to gram-negative (Zobell and Upham, 1944). Gram positive bacteria are less than 10% of the total bacterial population. Now evidences indicate that Gram positive bacteria do occur at higher percentage in sediments. The actinomycetes (Order: Actinomycetales) and related diverse group are gram positive filamentous forms. The other Gram positive genera like
Arthrobacter and endospore producing forms *Bacillus* and *Clostridium* (family Bacillaceae) have also been isolated. Especially *Bacillus* species readily grow in medium containing nutrients. In the present study, out of twelve bacteria eight were Gram negative (ARS-2 (*Idiomarina marina*), ARS-3 (*Idiomarina loihiensis*), ARS-5 (*Vibrio alginolyticus*), ARS-6 (*Vibrio sp. 1.*), ARS-9 (*Vibrio natriegens*), ARS-10 (*Rhodopirellula baltica*), ARS-11 (*Vibrio sp. 2.*) and ARS-12 (*Pseudomonas knackmussii*)) and four were Gram positive (ARS-1 (*Bacillus subtilis*), ARS-4 (*Bacillus megaterium*), ARS-7 (*Bacillus amyloliquefaciens*) and ARS-8 (*Bacillus tequilensis*)) which belong to the genus, *Bacillus*. In the present study, the cells of the bacterium ARS-1, ARS-4, ARS-7, ARS-8, ARS-9 and ARS-12 were short rods. The cells of the bacterium ARS-3 were slightly curved. The cells of the bacterium ARS-5, ARS-6 and ARS-11 were straight rod where as the cells of the bacterium ARS-10 and ARS-10 were ovoid shaped.

In the present study, all the bacterial isolates were positive result for Catalase and oxidase tests. All the bacterial isolates except isolate ARS-11 were positive for saccharose test. Citrate utilization was positive in all isolates except two isolates, ARS-10 and ARS-11. Phenylalanine Deamination was positive only one isolate ARS-4. The test Urease was positive only in two isolates, ARS-5 and ARS-6.

Many marine free-living and sediment-inhabiting marine bacteria have been shown to produce secondary metabolites that display antibacterial properties (Burgess et al., 1991). This antibacterial activity has been widely exploited and for the past 50 years antibiotics have revolutionized medicine by providing cures for formerly life-threatening diseases. Despite the immense clinical significance of antibiotics in health care, little is understood on the ecology of the organisms that produce them. The first antibiotic from a marine bacterium was identified and characterized by Burkholder et
al. (1966). The marine environment harbours a wide range of microbes capable of exhibiting bacteriolytic and antibiotic activity. Bacteriolytic activities were found to be higher in the zooplankton than in sea water and the major group isolated were the gram negative bacteria, particularly the *Vibrio parahaemolyticus* followed by the gram positive strain, *Staphylococcus aureus* (Nair et al., 1985). There are also reports of such bacteria from nutrient-rich algal surfaces (Jensen and Fenical, 1994; Bernan et al., 1997) seaweeds (Imamura et al., 1997) to large biofilms on the surfaces of marine organisms (Lemos et al., 1986). The bacteriolytic bacteria inhabit mainly in the places where the organic matter is high, and contribute to its decomposition (Nair et al., 1985). The present study resulted that all the bacterial isolates showed antifungal activity against one fungus, namely *Fusarium oxysporum* out of three fungi, especially the isolate, ARS-8 revealed maximum activity (66mm) against it. Next, they showed better activity against *Rhizoctoni solani*. The isolate ARS-7 showed maximum activity (22mm) against it. But, majority of the isolates except two (ARS-2 and ARS-12) showed no activity against *Pyricularia oryzae* (Figure.46). This may be because of *Pyricularia oryzae* had resistant ability against the isolates and also it may have genotypical similarity with the isolates.

Bacteria promote interspecies antagonism; bacteriocins are responsible for intra specific antagonism. Colicin produced by E.coli has been studied extensively. Similarly, there are brevicin, nisin, pediocin produced by different groups. Bacteriocin produced by Halobacterium mediterranei ATCC 33500, has been shown to be active against many other halobacteria (Meseguer et al., 1985). Bacteriocin -producing bacteria could be made to change their strategy from anti- to pro-biotic depending on the environment. Thus, a brevicin- producer could be skillfully used as probiotic to ward off unwanted microbes or to mitigate pathogenesis. A deep sea pigmented
Brevibacterium spp. has been shown to produce linocin-like compound that could be used as probiotic in aquaculture feeds. The extracts of this bacteria have not only been suggested to be useful in prolonging shelf-life of dairy products but the cultures could be used as probiotics and as feed additives in aquaculture (Loka Bharathi et al., 2003). In the present study, all the isolates exhibited the antibacterial activity against three poultry pathogens such as Eicheria coli, Pasteurella multocida and Salmonella pullorum. Especially, the isolate ARS-8 showed highest activity both the poultry pathogens, Eicheria coli (18mm) and Salmonella pullorum (30mm) and the isolate ARS-9 had same activity (10mm) against all three poultry pathogens (Figure.47). Hence, all the isolates have the secondary metabolites which have the ability to suppress the above said three poultry pathogens. In the case of antibacterial activity against cattle pathogens, the present study exhibited that the isolate ARS-7 had maximum activity (23mm) against Staphylococcus aureus and this may be suitable for the control of cattle pathogen, Staphylococcus aureus. All other isolates had mild activity against the remaining pathogens (Figure.51 & 54).

A number of surface-associated marine bacteria have also been found to produce antibiotics. Trischman et al. (1994) isolated a species of Streptomyces from the surface of a jellyfish. A predominant response by the bacteria towards antagonism is antibiotic resistance. However, strains of bacteria have recently emerged that are virtually unresponsive to antibiotics. Strains having antibacterial activity are found to be lesser susceptible to inhibitory substances. Resistance of bacteria to antibiotics like chloramphenicol, tetracycline and streptomycin even at higher concentrations were attributed to the pigments present in them (Nair et al., 1992). In the present study, Majority of the isolates were susceptible and highly sensitive to three particular antibiotics namely Amikacin-30mcg, Gentamycin-10mcg, Cefoperozone-75mcg. But,
only a few isolates were sensitive to the remaining antibiotics namely Cefpodoxime-10mcg and Ceftazidine-30mcg (Figure. 52). This study revealed that the isolates may have powerful antibiotics in their own structure than that of the inactive antibiotics which may be called as probiotics.

Among the identified 12 bacteria out of 18 bacterial isolates isolated from the six seaweeds in this study, the bacterium Bacillus subtilis was associated with the red seaweed, Jania rubens and the green seaweed, Enteromorpha intestinalis. The bacterium Bacillus megaterium was associated with the red seaweed, Gracilaria corticata and the bacterium, Bacillus amyloliquefaciens was associated with the brown seaweed, Chnoospora implexa. The bacterium, Bacillus tequilensis was associated with the brown seaweed, Chnoospora implexa and the green seaweed, Caulerpa racemosa. The bacterium, Idiomarina marina was associated with the red seaweed, Jania rubens and Idiomarina loithiensis with the red seaweed, Gracilaria corticata. The bacterium, Vibrio natriegens was associated with red seaweed, Jania rubens and Vibrio alginolyticus with the brown seaweed, Dictyota dichotoma. The bacteria, Vibrio sp.1 was associated with the brown seaweed, Dictyota dichotoma and the green seaweed, Enteromorpha intestinalis. The bacterium, Vibrio sp.2 was associated with the brown seaweed, Dictyota dichotoma. The bacterium, Rhodopirellula baltica was associated with the red seaweed, Gracilaria corticata and the green seaweed, Caulerpa racemosa. The bacterium, Pseudomonas knackmussii was associated with the green seaweeds, Enteromorpha intestinalis and Caulerpa racemosa.

In recent years, 16S rRNA sequence analysis has been used extensively for the reconstruction of bacterial phylogeny. Earlier studies based on 16S rRNA analysis demonstrated that all the Yersinia species form a phylogenetically coherent cluster
exhibiting sequence similarities of 96.9 to 99.8% within the genus (Ibrahim, A., et al. 1993,1997). In this study, the bacterial isolates were involved for 16s rRNA gene sequencing (Figure.6). And the sequences so obtained were compared with the Blast analysis (NCBI, Database). Out of eighteen isolates isolated from six seaweeds, twelve bacteria were identified. Neighbour-joining tree for the 16s rRNA genes sequences of total 12 bacterial strains isolated from the six seaweeds found along the coast of Thondi, Ramanathapuram District, India, was drawn by using Mega5 software. The sequences were aligned by Clustal W. The bootstrap analysis was done to check the reliability of the tree. The bootstrap values (%) are given in the nodes. The values below 22% are omitted. By analysing the Neighbour-joining tree, it was cleared that the bacterium Pseudomonas knackmussi was entirely distanced from other bacteria which gave two main branches namely, Rhodopirellula baltica and other remaining bacteria. The bacterium Bacillus tequilensis was branched with the distance of 59% to give Bacillus subtilis. Both the species were branched with the distance of 83% to give Bacillus amyloliquefaciens. These three species were branched with the distance of 90% to give Bacillus megaterium. All those bacteria were branched with the distance of 68%. In the other main branch, Idiomarina marina was branched with the distance of 85% to give Vibrio species and Idiomarina loihiensis. The bacterium Idiomarina loihiensis was branched with the distance of 22% to give Vibrio species. The bacterium Vibrio sp.2 and Vibrio natriegens were branched with the distance of 94% to give Vibrio alginolyticus and Vibrio sp.1. The bacterium Vibrio sp.2 was branched with the distance of 42% to give Vibrio natriegens and Vibrio sp.1. was branched with distance of 28% to give Vibrio alginolyticus (Figure.57).
The sequences of 16s rRNA gene sequences of all isolates were submitted in GenBank for getting accession numbers. The GenBank accession numbers are as follows: ARS-1 (*Bacillus subtilis*) - KF688211, ARS-2 (*Idiomarina marina*) - KF688212, ARS-3 (*Idiomarina loihiensis*) - KF688213, ARS-4 (*Bacillus megaterium*) - KF688214, ARS-5 (*Vibrio alginolyticus*) - KF688215, ARS-6 (*Vibrio sp.1.*) - KF688216, ARS-7 (*Bacillus amyloliquefaciens*) - KF688217, ARS-8 (*Bacillus tequilensis*) - KF688218, ARS-9 (*Vibrio natriegens*) - KF688219, ARS-10 (*Rhodopirellula baltica*) – KF688220, ARS-11 (*Vibrio sp.2.*) - KF688221, ARS-12 (*Pseudomonas knackmussii*) – KF688222 ((Figure.55).

In conclusion, the present study revealed that the bacterial strains associated with the red seaweeds and brown seaweeds had higher amount of antibacterial compounds compared with the green seaweeds. Among all those isolates, the endophytic bacterial strains had higher activity than the epiphytic bacterial strains. And the bacteria isolated in this study are having the resistance to some antibiotics. So, these bacteria may have some effective compound and the same may lack in the antibiotics which are not able to suppress these bacteria. From the present study, it is also cleared that the bacteria associated with the seaweeds are having the antibacterial compounds and they may be used for treating the diseases of poultry as well as the cattle.

“Nothing in biology makes sense except in the light of evolution” - Theodosius Dobzhansky.

In animals’ relationship too, the associations are not static and but are constantly evolving in response to their environment. It could include all degrees of repulsion and antagonisms to affinity, from tight symbiosis of bacteria with their host eukaryotic cells to more loosely bound partnerships between entirely separate
organisms. So, the findings of present work is a little bit of research on marine microbiology and further studies on the seaweed associated bacteria are needed in the aspects of habit, habitat, and detailed characterizations and for the invention of novel antifungal, antimicrobial and antioxidant compounds for the betterment of mankind.