IJPSR (2018), Volume 9, Issue 3 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 02 June, 2017; received in revised form, 22 August, 2017; accepted, 29 August, 2017; published 01 March, 2018

STUDY OF MICROBIAL DIVERSITY IN *ULVA LACTUCA* FROM NORTH WEST COAST OF GUJARAT, INDIA

Ghanshyam G. Patel, Vaidehi Patel and Mukund Chandra Thakur*

Department of Biotechnology, Ashok and Rita Patel Institute of Integrated Study and Research in Biotechnology and Allied Sciences, New Vallabh Vidyanagar - 388121, Gujarat, India.

Keywords:

Ulva lactuca, Epiphytes, Endophytes, Microbial diversity, DNA sequencing

Correspondence to Author:
Dr. Mukund Chandra Thakur

Assistant Professor,
Department of Biotechnology,
Ashok and Rita Patel Institute of Integrated Study and Research in Biotechnology and Allied Sciences,
New Vallabh Vidyanagar - 388121,
Gujarat, India.

E-mail: mukundthakur@aribas.edu.in

ABSTRACT: Seaweeds form economically important component of marine ecosystems worldwide. A wide range of beneficial and detrimental interactions exists between seaweeds and epiphytic as well as endophytic bacteria. Diversity study can be used to retrieve ecological information about community partner that relates the degree of stability of that community. The present work deals with the study the microbial diversity of *Ulva lactuca*. Epiphytic and endophytic bacteria were isolated from *U. lactuca*. Total of 4 bacterial isolates were obtained from this seaweed. Among them 2 (VP2 and VP4) were endophytic bacteria and 2 were epiphytic bacteria (VP6 and VP7). Gram staining and biochemical tests for identification of micro-organisms were performed. On the basis of colony morphology and biochemical tests, VP2, VP4 and VP6 were identified as *Enterobacter aerogenes*, *Bacillus cereus* and *Staphylococcus aureus* respectively. Out of 4, 1 isolate could not be identified on the basis of biochemical tests performed. Hence, molecular characterization was attempted by targeting 16S rDNA sequencing for the fourth isolate, VP7. The amplified product was identified as *Salinicoccus* sp. Further bioactive compounds can be obtained from this seaweed associated bacteria for industrial applications.

INTRODUCTION: Biodiversity defined as the range of significantly different types of organisms and their relative abundance in an assemblage or community. Microbial diversity is defined by the number of species or different groups of microbes living in a certain environment, as well as the evenness of the species abundance distribution [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.9(3).1201-12

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.9(3).1201-12

Diversity studies can be of stability of that community. Well organized communities that contain a certain level of diversity are stable [2]. If some kind of stress is introduced to this community, the stability may collapse and the diversity will change. Microorganisms are the dominant component of any ecosystem on Earth and many ecosystems are exclusively microbial.

Microbes are found in all viable habitats from deep sea sediments and bed rock to high up in the atmosphere diverse than eukaryotic diversity [1]. There are many eukaryotes present which are closely associated with bacteria which aids in increasing their physiological capacities.

There has been increasing interest in defining macroalgae-associated bacterial communities and macroalgal development and morphogenesis [3]. Epibiosis is a process by which bacteria frequently colonizes on biotic surface. This biotic surface provides diverse and rich environment for process and development of various bacterial species [4]. In the marine environment, complex biofilms are formed when microorganism present colonize with every exposed surface of substrate present [5]. Many marine macroalgae harbour surface communities that differ both quantitatively and qualitatively from their surrounding environment despite the high epibiotic colonization pressure [6].

There are stable associations with marine eukaryotes and bacterial community which rely on them for their growth, development and protection from predation and colonization [7 - 10]. Bacteria are capable of regulating mineralization of organic matter, nutrient cycling and energy transfer through bacterial metabolism and utilization of their molecules [11]. Bacterial communities associated with seaweeds can be species specific, varieties specific [12, 13] or they can also be made of different bacteria but with similar metabolic functions [14]. The associated bacterial colonies are efficient producers of bioactive compounds promoting algal growth, quorum sensing signals and other substances promoting algal development and survival [15].

Macro-algae exhibit great varieties of bacterial communities [16]. However the bacterial communities associated with red and brown algae are *Firmicutes, Actinobacteria* and *Planctomycetes* while in green algae, *Bacteroidetes* and *Alphaproteobacteria* are associated with them [17]. Epibiotic bacterial community also showed temporal and spatial variability on red macroalgae [18]. Culture independent studies also reported the presence of planctomycetes in bacterial communities of macroalgae [19, 20].

Seaweeds are one of the diverse and large ecosystems which plays a role in development of specific bacterial communities as marine algae provides protective and nutrient rich conditions substrates for the bacterial growth [21]. As compared to other multicellular organisms, seaweeds have a rich diversity of associated microorganisms.

Marine microorganisms have been large source of bioactive compounds. Since last three decades more than 50,000 bioactive natural products have been found and more than 8,000 had bactericidal activity [22]. Macroalgae and epi- and endosymbiotic bacteria possess a wide range of beneficial and detrimental interactions that reside either on the surface or within the algal cells [17]. Marine Ulvacean algae are colonized by the dense microbial communities present in marine Ulvacean algae are expected to have a significant role in the development, defence as well as metabolic activities of the plant [23]. Surface colonization is abundant in the marine environment and macroalgal surfaces are most prominent.

A large and discrete group of bacteria are present on marine macroalgae with densities varying from 10^2 to 10^7 cells cm^{-2} depending on the species of macroalgae, type of thallus and season [24, 25]. One of the study by image analysis of the microbial community associated with the surface of *Ulva australis* indicates that bacterial density increases from the thallus tips (10^6 cells cm^{-2}) to the algal base (10^7 cells cm^{-2}) [26]. Growth and morphogenesis of different Ulva species such as *U. mutabilis, U. linza*, *U. pertusa* and *U. fasciata* were controlled by variety of marine bacterial species such which included *Proteobacteria, Bacteroidetes* and *Firmicutes* [27, 28, 29]. The effects of 38 bacterial strains, isolated from three species of Ulva, on the growth rate and morphological development of *U. linza*axenic plantlets treated with antibiotics, was assessed for 28 days by Marshall *et al*., (2006) [30].

The results showed that not a single bacterium was able to completely restore normal morphology to axenic *U. linza*, in contrast to results obtained in *U. mutabilis* applying bacteria isolated from *U. rigida* [31]. Grueneberg *et al.*, (2016) also demonstrated that Ulva can benefit from bacterial sources other than its own epiphytes, as diffusible waterborne morphogens which affect the development in Ulva. Epiphyte is an organism which grows on a living plant. Epiphytic bacterial communities are fast colonizers consisting of complex bacterial assemblages growing on the surfaces of seaweeds, capable of rapid metabolization of algal exudates [12, 15, 32, 33]. These organisms are not parasites, which mean they do not depend on the host plant for water and nutrition.

Sometimes, they may destroy the host plant due to their shading. Epiphytism is present among many groups, such as fungi, algae and plants and in associations such as the lichens. Epiphytes are part of the canopy community and major importance for nutrient cycling of forests and important for creating niche habitat for several animal species. Epiphytes are usually found on aquatic species developing on algae or on aquatic flowering plants [34]. Endophytic, derived from Greek word Endon (within) and phyton (plant). This organism is used in wide range of potential hosts and inhabitants. It includes bacteria, fungi, plants, insects in plants and algae. These organisms are defined as the inconspicuous infections. Endophytic are the bacteria and fungi which are identified at a specific period within the tissues of healthy host plants [35].

MATERIALS AND METHODS:

Algal Sample Collection and Identification: The green macroalgae *Ulva lactuca* was collected near Okha coast of Gujarat at the depth of 0.5 m during February and March 2016. The sample was then brought to the laboratory in plastic bags. The samples were washed thrice with distilled water to remove dirt and debris from the seaweed. The algae was identified on the basis of their taxonomical characters based on the literature published by Jha *et al*., (2009) [36]. The samples were then used for further experiments.

Isolation of Epiphytic Bacteria and Endophytic Bacteria: The collected seaweed was thoroughly washed thrice with sterile seawater to remove the macro element attached with seaweed. A sterile cotton swab was used to rub the seaweed surface and the removed bacteria present in the cotton swab were inoculated on sea water complex agar plates. The inoculated plates were incubated for 24 hours at 37 °C or until colonies were observed. After 24 hours at 37 °C, different colonial morphologies of bacteria were chosen and purified by successive re-streaking (four flame method). The pure bacterial cultures obtained were maintained on Nutrient Agar slants and can be used in future experiments. The collected seaweed was thoroughly washed thrice with sterile sea water to remove the macro element attached with seaweed. To isolate endophytic bacteria, seaweed was first treated with different concentration of Betadine for different time interval followed by washing with sterile seawater. Treated seaweed sample was grinded in sterilized mortar and pestle. The paste was diluted to 10 ml by using sterile sea water and serially diluted with the sterile sea water up to 10^{-4} dilutions. Each dilution was spread on the labelled petriplates containing 15 ml of autoclaved seawater complex agar plates. The plates were kept for incubation at 37 °C for 24 hours or until the colonies were observed. After incubation, different colonial morphologies of bacteria were chosen and purified by successive re-streaking (four flame method). The pure bacterial cultures obtained were maintained on Nutrient Agar slants and can be used in future experiments.

Colony Characterization: The bacterial colonies were characterized on the basis of their size, shape, texture, margin, opacity, and pigmentation. It is usually done by observing the colony with the unaided eye (*i.e.* not necessarily through microscopy). Colony morphology is one of the means in identifying bacterial species [37].

Gram Staining: Thin smear was prepared on glass slide from fresh bacterial growth (older or low viable cultures may not stain with gram stain characteristics).

Primary Stain: Smear was stained with crystal violet (Hucker's stain) for 1 min. The slide was washed in tap water, no longer than 2 seconds, to remove liquid Hucker's stain. The smear was flooded with gram's iodide and allowed to remain for 1 minute. Then smear was decolorized with 95% ethyl alcohol.

Counter Stain: Smear was stain with saffranin and allowed it to stain for 1 min. The smear was washed with tap water, blotted and dried gently between sheets of lint-free paper towel. Smear was examined under 100X oil immersion lens. Morphologies and staining patterns observed were recorded.

Biochemical Studies of Epiphytic and Endophytic Bacteria:

Catalase Test: One - two drops of hydrogen peroxide solution was placed on a glass microscopic slide. Isolated single colony was picked up and transferred onto the drop of hydrogen peroxide with a nicrome wire loop. Production of the gas bubbles was observed.

Coagulase Test: Test culture was inoculated into the nutrient broth and incubated at 37 °C for 18 - 24 hours. Two-three drops of rabbit plasma was added on test culture and observed the clot formation.

Methyl Red (M - R) Test: Culture was inoculated in glucose phosphate broth (GPB) and incubated at 37 °C for 48 - 72 hours. After incubation, 5 drops of methyl red indicator was added to the medium. The positive result gives the development of red color.

Voges-Proskauer (V - P) Test: Culture was inoculated in glucose phosphate broth (GPB) and incubated at 37 °C for 24 - 48 hours. After incubation 0.6 ml of α-naphthol and 0.2 ml of KOH solution per ml of culture broth were added. After adding each reagent, all the tubes were shaken properly and kept in slanted position to increase aeration. Color change was observed after 15 - 60 minutes.

Citrate Utilization Test: Culture was streaked on surface of the agar slant and incubated the slant at 37 °C for 24 - 48 hours. A color change of the slant was observed after incubation.

Indole Production Test: Culture was inoculated in the Tryptone broth and incubated at 37 °C fir 24 hours. After incubation, 3 - 4 drops of xylene were added in the medium and shaked it vigorously. Layers were allowed to separate. Then slowly 1 ml of Ehrlich's reagent was added to it so as to form the layer on the surface of xylene. The formation of pink colored ring at the lower surface of the xylene layer is observed. Kovac's reagent (1 ml) was added on top of the broth and observed for the pink ring.

Starch Hydrolysis Test: The culture was inoculated on the plates as spot or line and incubated at 37 °C for 24 - 72 hours. The plates were flooded with Lugol's iodide after observing the transparent zone surrounding the colony and read immediately, because the blue color fades rapidly.

Ammonia Production Test: A loopful culture was inoculated into peptone nitrate broth. A red litmus paper strip was placed in the mouth of the culture tube in such apposition that ¼ to ½ of the strip projects below the cotton plug. The culture was incubated at 37 °C for 24 hours and the color change will be observed from red to blue litmus after incubation.

Nitrate Reduction Test: The cultures were inoculated in to PNB with and incubate the medium at 37 °C. The Reagent A (0.5 ml) and reagent B (0.5 ml) were added in to the culture in this order. After adding and mixing the test reagent, the development of red color will be observed within 30 seconds after adding test reagent.

MacConkey's Agar Plates: The culture was inoculated on the Macconkey's plates by streaking, and incubated it at 37 °C for 24 hours. (MacConkey agar: Peptone 20 g, Lactose 10.0 g, NaCl 5. 0 g, Bile salts 3.0 - 5.0 g, Neutral red 30.0 mg, Crystal violet 10.0 mg, Distilled water 1000.0 ml, Agar 30.0 g, pH 7.4). The pH was adjusted to 7.4 and sterilized by autoclaving at 15 psi (121 °C) for 15 minutes. The colour change of the colony was observed after incubation.

Identification of the Isolates by Molecular Characterization (Epiphytic and Endophytic Bacteria): The isolated colonies obtained were characterized by biochemical tests. Molecular characterization of the isolate was done which could not be identified by biochemical identification tests. The DNA was isolated and amplified by PCR (Polymerase Chain Reaction) to determine the nucleic acid sequence by16SrRNA targeted 16S rDNA sequence. The isolates were sent to Xcelris, Ahmedabad, Gujarat for sequencing. The primers 5'-AGAGTTTGATCCTGGCTCAG-3' and 5'-AC GCTACCTTGTTACGACTT-3' were used as forward and reverse primer for the 16S rRNA gene amplification. The sequences were submitted to NCBI.

RESULTS:

Algal Sample Collection and Identification: Gujarat has two major gulfs namely Gulf of Khambhat and Gulf of Kutch which embrace diverse coastal habitats as well as biota of ecological significance. The Gulf of Kutch is the biggest gulf of the west coast of India in the Arabian Sea. The seaweed was collected from Okha coast, Gujarat. The taxonomic characters of the collected seaweed were soft, leafy, membranous, light green in colour, thallus broad with undulated margins imparting lettuce type

morphology (**Fig. 1**). Thus on the basis of the taxonomic characters described by Jha *et al*., 2009, the plant was identified as *Ulva lactuca*.

Isolation and Screening of Epiphytic and Endophytic Bacteria: From the collected seaweed (*Ulva lactuca)* sample, 4 different bacterial isolates were found. Among them, 2 isolates were endophytic bacteria and were named as isolate VP2 and VP4 while VP4 and VP7 were epiphytic isolates (**Fig. 2**).

FIG. 1: IMAGE OF *ULVA LACTUCA*

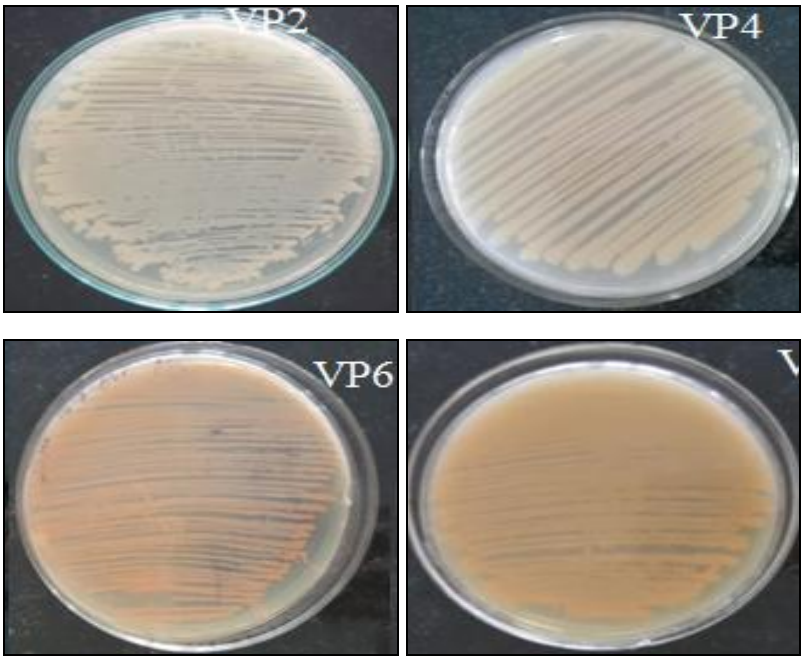


FIG. 2: IMAGE OF ENDOPHYTIC (VP2 AND VP4) AND EPIPHYTIC BACTERIA (VP6 AND VP7)

Colony Characterization: Naureen Akhtar *et al*., reported that identification of bacterial species was done by observing colony morphological features which includes (color, shape, size, texture, margins and odour *etc.*) and cell microscopic characters such as color, cell wall, contents, shape, arrangement, material, Gram stain, capsular material and motility and, he concluded that all the strains were rod-shaped, Gram negative and motile bacteria. Isolate VP2 was found amoeboid in shape and with medium size colony. The margin and texture of VP2 was Lobate and smooth respectively, while the pigmentation of this isolate was white and opaque. The isolate VP4 colony was found irregular in shape and with large size colony. The margin and texture of VP4 was rhizoidal and rough respectively, while the pigmentation of this isolate was greyish white and opaque. Isolate VP6 colony was found round in shape and medium size colony.

The margin and texture of VP6 colony was entire and smooth respectively, while the pigmentation of this isolate was golden yellow and translucent. The isolate VP7 colony was found round in shape and medium size colony. The margin and texture of VP7 colony was entire and smooth respectively, while the pigmentation of this isolate was orange and opaque.

Gram's Staining of the Isolates: Gram's staining was performed and as a result is shown in **Fig. 3**. The endophyte VP2 was gram negative, short rods and non-spore forming organism. VP4 was gram positive, large, straight or slightly curved rods which are commonly appear in short to long chains. Epiphyte VP6 was gram positive cocci having round shape, entire edge, convex, soft, glistening colonies with golden yellow pigment. VP7 was gram negative bacteria.

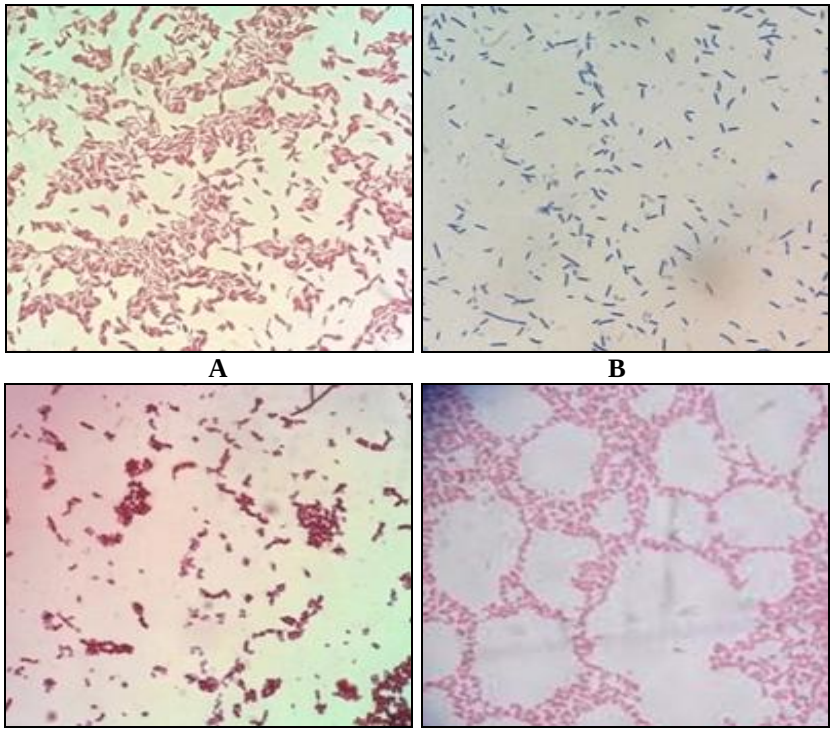
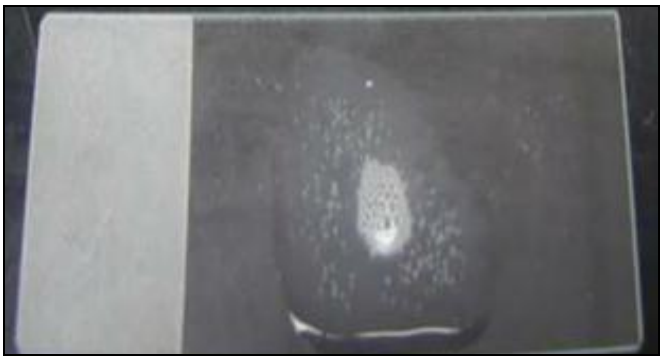


FIG. 3: GRAM STAINING OF THE ENDOPHYTE (A) VP2: GRAM NEGATIVE SHORT RODS, (B) VP4: GRAM POSITIVE LARGE RODS, EPIPHYTES (C) VP6: GRAM POSITIVE ROUND SHAPED COCCI, (D) VP7: GRAM NEGATIVE RODS

Biochemical Tests for Bacteria: Biochemical tests were performed for the isolated bacteria (VP2, VP4, VP6 and VP7). Naureen Akhtar *et al*., suggested that, bacteria can be identified on the basis of biochemical tests. Such tests actually show the ability of a specific enzyme to utilize a biomolecule as substrate such as amino acids, proteins, lipids *etc*. His results of citrate test suggested that none of the isolate was capable of citrate transport into the cell for fermentation. Nitrite was detected for strain B2, B3 and B5 in the reaction tube that was an indicative of nitrate conversion to nitrite by nitrate reductase.

Catalase Test: Microorganisms living in the oxygenated environments produce the enzyme catalase to neutralize toxic form of metabolites such as hydrogen peroxide (H_2O_2). It breaks down hydrogen peroxide into oxygen and water. Catalase positive test is evident by immediate effervescence or copious bubble formation. Catalase positive indicates the presence of genus *Staphylococcus* and catalase-negative indicates the presence of genera *Enterococcus* or *Streptococus*. The bacterial isolates VP4, VP6 and VP7 showed positive results in Catalase test. The active gas bubbles were observed in these isolates.

On the other hand, in the isolates VP2, no gas bubbles were found, hence were observed negative (**Fig. 4**).

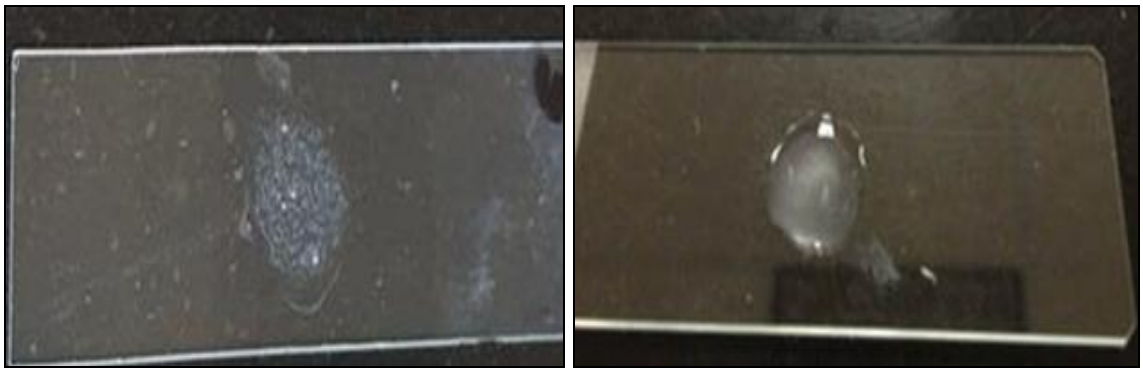


A

B

FIG. 4: CATALASE TEST (A) POSITIVE: ACTIVE BUBBLES FORMATION ON ADDITION OF H_2O_2 WITH ISOLATE VP4, VP6 AND VP7 (B) NO BUBBLES FORMATION IN ISOLATE VP2

Coagulase Test: Coagulase is an enzyme produced by *Staphylococcus aureus* used to convert insoluble fibrinogen into soluble fibrin in plasma. Coagulase test is used to differentiate *S. aureus* positive into coagulase negative *S. aureus*. The bacterial isolate VP6 showed positive result in Coagulase Test shown in (**Fig. 5**). Clot formation was observed after addition of rabbit plasma in bacterial culture. Clot formation is observed very rapidly within 10-15 seconds after the addition of culture suspension with plasma.VP2, VP4 and VP7 were found negative results, *i.e.* no clot was formed by these isolates.



A B

FIG. 5: COAGULASE TEST (A) POSITIVE: CLOT FORMATION ON ADDITION OF RABBIT PLASMA IN BACTERIAL CULTURE FOR ISOLATE VP6 (B) NEGATIVE

No clot formation on addition of rabbit plasma in bacterial culture for isolate VP2, VP4 and VP7.

Methyl-Red Test: The positive result in Methyl-Red Test shows the production of stable red color after addition of methyl red indicator on GPB. Methyl red is a pH indicator having a range between 6.2 (yellow) to 4.4 (red), so pH at which methyl red detected acid was considerably less than the pH for other indicators used in bacteriological media. Appearance of red color indicates the positive test and presence of genus *Escherichia coli, Salmonella* and *Proteus*. No color change indicates the negative result and presence of genus *Enterobacter, Klebsiella*. The bacterial isolates VP2, VP4, VP6 and VP7 showed negative results in Methyl-Red Test. The color of medium had not changed into red color (**Fig. 6**).

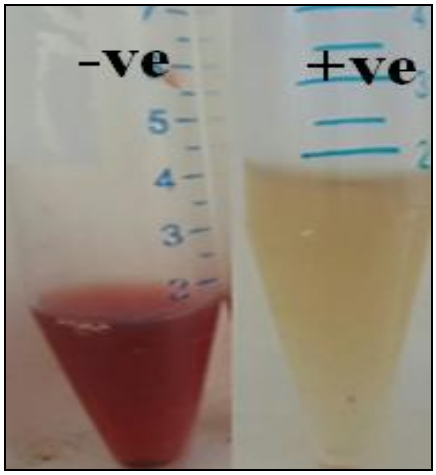


FIG. 6: METHYL RED TEST

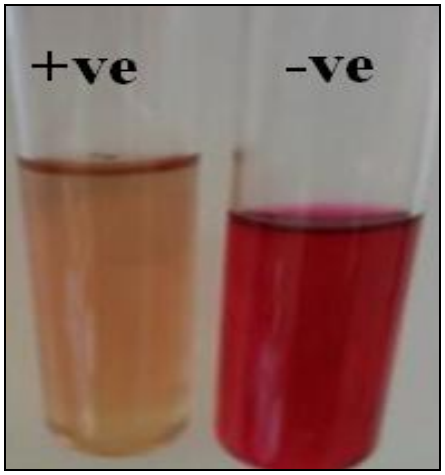


FIG. 7: VOGES-PROSKAUER TEST

Voges- Proskauer Test: Voges- Proskauer Test is generally used to distinguish VP-negative *E. coli* to VP-positive *Klebsiella Enterobacter* groups. The bacterial isolates VP2 showed positive result in Voges- Proskauer Test as shown in **(Fig. 7**). The color of medium was changed to red because the medium might be alkaline. Acetoin was oxidized to diacetyl in presence of air and as a result proteins of peptone lead pink color. The bacterial isolates VP4, VP6 and VP7 showed negative results in Voges- Proskauer Test, shown in **Fig. 11**. The color of medium did not changed to red in presence of alkali and air acetoin do not oxidize to diacetyl. As a result, further reaction with proteins of peptone did not occur and no pink color was produced.

Citrate Utilization Test: Citrate is a sole source of carbon and energy which is transported to bacterium by citrate permease. Oxygen is necessary for citrate utilization. Bacteria oxidize citrate and liberate CO_2, which combines with sodium and water to form sodium carbonate and alkaline product which raised pH and the pH indicator turns out to blue in color. Positive test indicates the presence of *Klebsiella pneumoniae* and negative test indicates the presence of *E. coli*. The bacterial isolates VP2, VP4 and VP6 showed positive results in Citrate utilization test (**Fig. 8**). The color of slant changed to blue. The bacterial isolate VP7 showed negative results in Citrate utilization test, shown in figure. The color did not change to blue.

Indole Production Test: The positive result shows the development of bright pink color appeared after the addition of xylene and Kovac's reagent. The bacterial isolates VP2, VP4, VP6 and VP7 showed negative result in Indole test. The pink color did not appear after adding xylene and Kovac's reagent.

FIG. 8: CITRATE UTILIZATION

FIG. 9: INDOLE PRODUCTION

Starch Hydrolysis Test: The bacterial isolates VP4 and VP6 showed positive results in Starch hydrolysis test, shown in figure. Transparent zone surrounding the colony was observed after the flooding the plates with Lugol's iodine. The bacterial isolates VP2 and VP7 showed negative results in Starch hydrolysis test. Transparent zone surrounding the colony did not appear.

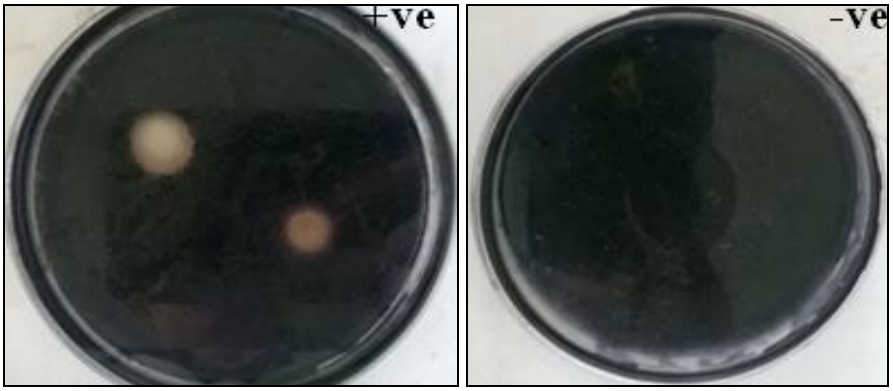


FIG. 10: STARCH HYDROLYSIS TEST (A) POSITIVE: CLEAR TRANSPERANT ZONE OBSERVED SURROUNDING THE COLONY SHOWING THE HYDROLYSIS OF STARCH; (B) NEGATIVE: ABSENCE OF ZONE SURROUNDING THE COLONY INDICATING THE ABSENCE OF STARCH HYDROLYSIS

Ammonia Production Test: All bacterial isolates VP2, VP4, VP6 and VP7 showed positive results in Ammonia production test. The change of red litmus to blue color was observed (**Fig. 11**).

Nitrate Reduction Test: The bacterial isolates VP2, VP4 and VP7 showed positive results in Nitrate reduction test. The red color was observed within 30 seconds after the addition of α-naphthylamine and sulphanilic acid reagent. The bacterial isolate VP6 showed negative results in Nitrate reduction test. The change of red color did not observe within 30 seconds (**Fig. 12**).

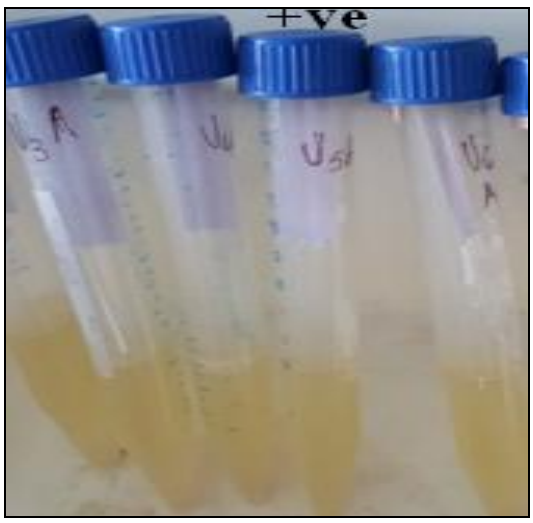


FIG. 11: AMMONIA PRODUCTION TEST

FIG. 12: NITRATE REDUCTION TEST

TABLE 1: BIOCHEMICAL TESTS OF VP2, VP4, VP6 AND VP7 ISOLATES

Sr. no.	Biochemical tests	Isolates			
		VP2	VP4	VP6	VP7
1	Catalase Test	–	+	+	+
2	Coagulase Test	–	–	+	–
3	Methyl-Red Test	–	–	–	–
4	Voges- Proskauer Test	+	–	–	–
5	Citrate Utilization Test	+	+	+	–
6	Indole Production Test	–	–	–	–
7	Starch Hydrolysis Test	–	+	+	–
8	Ammonia Production Test	+	+	+	+
9	Nitrate Reduction Test	+	+	–	+

"+" indicates positive test and "–" indicates negative test.

Identification of the Isolates by Molecular Characterization: The isolate VP7 could not be identified on the basis of biochemical tests performed. Hence, molecular identification of this epiphytic isolate was required. Thus DNA from this isolate was isolated by bacterial DNA isolation kit (Genei). The isolated DNA was further amplified by 16S rRNA sequencing using universal primers for 16S DNA (**Fig. 13**). The sequences obtained by 16S rRNA sequences were then blasted and submitted to NCBI with accession number ARVP7.

DISCUSSION: On the basis of the biochemical tests, out of 4 isolates, 3 isolates (2 endophytic isolate VP2 and VP4 and one epiphytic isolate VP6) were identified. VP2 was gram negative, short rods and non-spore forming organism. VP2 grew rapidly on nutrient agar producing intermediate size of mucoid and glistening colony. On Macconkey's agar plate organism was formed pink colored colony. VP2 showed Voges-Proskauer test, Citrate utilization test, Ammonia production test and Nitrate reduction test positive. Methyl-Red test and Indole production test gave negative results. Thus from the results the isolate VP2 may be *Enterobacter aerogenes* bacteria.

VP4 was gram positive, large, straight or slightly curved rods which are commonly appear in short to long chains. VP4 produced oval, nonbulging spores at central or sub-terminal positions. On nutrient agar, VP4 produced large, spreading raised, dull,

opaque, greyish white colonies with rhizoidal margin. VP4 showed Catalase test, Citrate utilization test, Starch hydrolysis test, Ammonia production test and Nitrate reduction test positive. M-R test, V-P test and Indole test gave negative results. Thus from the results the isolate VP4 was identified as *Bacillus cereus* bacteria.

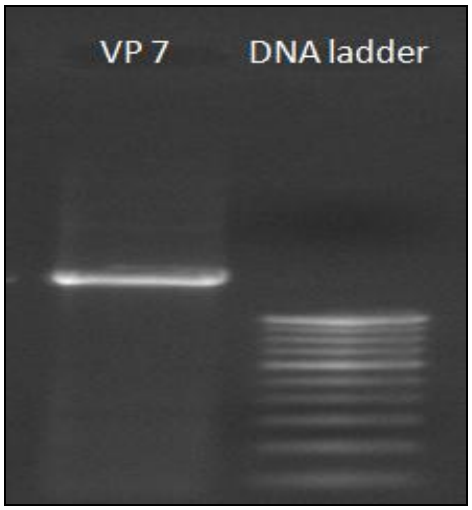


FIG. 13: DNA BANDS OF THE ISOLATES AFTER PCR AMPLIFICATION

VP6 was gram positive cocci having round shape, entire edge, convex, soft, glistening colonies with golden yellow pigment. VP6 also produced coagulase which was able to clot citrated plasma. It also produced the enzymes catalase. Thus from the results the isolate VP6 was identified as *S. aureus* bacteria. Further, identification of isolate VP7 was identified as Salinicocus sp. by nucleotide blast.

Algariphagus sp. and Polaribacter sp. have been isolated from *Ulva mitabilis* that could rescue complete morphology in *U. mutabilis*. This study revealed that strains of same genus (UL19 and EC19), can harbour different functional traits [31]. Some microorganisms isolated were similar in identity with *Marinobacter xestospongiae* that belongs to category of oil degrading bacteria that were isolated from *Xestospongia testudinaria* collected from Red Sea [38] (Lee *et al.,* 2012).

Very few studies have been reported for the study of species specificity of *Ulva*-bacterial interaction [31, 39, 40]. This study is reported on cross testing of potentially morphogenesis inducing bacteria isolated from Ulva species, between *U. mutabilis* and *U. intestinalis.* Marshal *et al*., 2006 isolated phylogenetically well characterized bacterial strains and performed complementary bioassay which revealed the test-strains replaced first one, and then after the other bacterium in the tripartite *U. mutabilis* - *Roseovarius maribacter* community [28, 41]. Many species like Bacillus, Halomonas, Paenebacillus and Clostridium spp. are salt tolerant, which have salt tolerance level above 10% NaCl.

A number of bacteria such as Bacillus and Paenebacillus have been also reported from other seaweeds [42, 43]. Ramalingam and Amutha isolated 11 endophytic and epiphytic bacteria from red seaweeds. Among them three endophytic bacterial strains namely AR-ST I, AR-ST II and AR-ST III and two epiphytic bacterial strains namely AR-ST 1 and AR-ST V were isolated from the red seaweed, Janiarubens and three endophytic bacterial strains namely AR-ST VI, AR-ST VII and AR-ST VIII and three epiphytic bacterial strains namely AR-ST IX, AR-ST X and AR-ST XI were isolated from the red seaweed, *Gracilaria corticata*. Bacillus is widely scattered genus found in terrestrial as well as in aquatic environments [44]. Enterobacteriaceae (*Enterobacter asburiae* and *Enterobacter hormaechei*) and Bacillaceae (*Bacillus subtilissubtilis* ATCC 6051) were identified in the seaweed *L. digitata*. A diverse group of species were isolated from the surface of *L. saccharina* which include *B. subtilissubtilis* ATCC 6051, *Acinetobacter genomospecies* 9, *Bacillus subtilisspizizenii, Paenibacillus ehimensis* and *Bacillus licheniformis* [45].

CONCLUSION: The results indicate that the marine algae *Ulva lactuca* collected from the Okha coast of Gujarat inhabit different species of microbes. The occurrence of epiphytic and endophytic microorganisms reflects the microbial diversity on seaweed. These algae are hosts to many organisms which possess potent anti-microbial and antifungal activity. These organisms are also known to contain many bioactive compounds which can be used as drugs in the treatment of several diseases.

ACKNOWLEDGEMENT: We thank Charutar Vidya Mandal, Vallabh Vidyanagar for providing facility and financial support to undertake the research work. The authors thank the Director, Ashok and Rita Patel Institute of Integrated Study and Research in Biotechnology and Allied

Sciences, New Vallabh Vidyanagar, for providing us lab facilities and equipment for research work.

CONFLICT OF INTEREST: Nil

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How to cite this article:
Patel GG, Patel V and Thakur MC: Study of microbial diversity in *Ulva lactuca* from North West coast of Gujarat, India. Int J Pharm Sci & Res 2018; 9(3): 1201-12. doi: 10.13040/IJPSR.0975-8232.9(3).1201-12.