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Production of enzymes and siderophores by epiphytic bacteria isolated from the marine macroalga *Ulva lactuca*

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ABSTRACT: Macroalgae provide important habitats for microorganisms, with surfaces that are rich in nutrients and a microbial niche for isolation of different bacterial groups. In this study, we evaluated the production of enzymes (amylases, lipases, cellulases and agarases) and siderophores by strains isolated from the macroalga *Ulva lactuca* growing in the Santa Marta region of the Colombian Caribbean Sea. We taxonomically identified a subset of the bacteria and tested 207 bacterial isolates. Of these, 25 (12%) and 121 (58%) produced agarases and siderophores, respectively. Out of the isolated bacteria with amylolytic (97), cellulolytic (118) and lipolytic (26) activity, 31 (32%), 78 (66%) and 4 (15%) strains, respectively, produced a zone of clearance >1.5 cm, indicating substantial activity. Enzymatic activities and siderophore production were statistically different between years of sampling, and principal component analysis showed grouping for samples from the same year. These activities and production were recorded in bacterial strains belonging to the genera *Vibrio*, *Pseudomonas* and *Bacillus*. The results show that marine bacterial cultures isolated from the macroalga *U. lactuca* are a source of enzymes and siderophores that may have potential for biotechnological and industrial processes.

KEY WORDS: Epiphytic bacteria · Siderophores · Cellulases · Lipases · Amylases · Agarases · Macroalgae

INTRODUCTION

Macroalgae influence sediment structure and water trajectories and have a large surface area that may be colonized by microbial communities (Martin et al. 2014). These surfaces are nutrient-rich environments and provide differentiated habitats for microorganisms, with which they experience a range of interactions. This association is important for growth and development of macroalgae and may protect the macroalgal surfaces from colonization by species that interfere with macroalgal growth and survival (Egan et al. 2008, 2013).

Epiphytic bacteria (macroalgae-associated bacteria) play a key role in algal defense (by producing antimicrobial and antifouling compounds), spore germination and algal morphology (Hehemann et al. 2014). Previous studies have assessed the antifouling traits in epiphytic bacteria of *Ulva lactuca* and *U. australis*, which are capable of preventing the settlement of invertebrate larvae and germination of algal spores. The results and conclusions showed that microbial colonization of macroalgae surfaces is a dynamic process with differences in settlement strategies exhibited by epiphytic bacteria (Egan et al. 2000, Rao et al. 2006).

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Compared to terrestrial plants, macroalgae contain a high content of water (90% fresh weight), carbohydrates (25–50% dry weight), proteins (7–15% dry weight) and lipids (1–5% dry weight) (Sudhakar et al. 2018). In addition, algal monosaccharides (rhamnose, xylose, glucose, mannose and galactose) and complex polysaccharides (ulvans from green algae; alginates, fucans and laminarin from brown algae; and carragenans and porphyrin from red algae) are a source of carbon and energy for epiphytic bacteria (Hehemann et al. 2012, Egan et al. 2013, Singh & Reddy 2014, Hamed et al. 2018). These compounds can be degraded by enzymes that are synthesized by epiphytic bacterial groups (de Nys & Steinberg 2002, Kennedy et al. 2008, Ramanan et al. 2016).

Marine environments are sources of enzymatic biocatalysts, and microorganisms associated with macroalgal surfaces have proven to be producers of enzymes required to metabolize macroalgae-synthesized compounds (Egan et al. 2000, Trincone 2010, Bakunina et al. 2012a, Rampelotto 2013). Examples are bacteria of the genera *Pseudomonas* and *Vibrio* isolated from marine samples that are able to produce extracellular agarases, carrageenases and β-galactosidases (Ziayoddin et al. 2010, Lee et al. 2014). Epiphytic bacteria isolated from *Ulva* samples, specifically *Bacillus aquimaris* and *Cellulosimicrobium* sp., synthesize extracellular alkaline cellulases as well as amylases, with highest activity in the presence of sodium chloride (NaCl) (Trivedi et al. 2011b, Al-Naamani et al. 2015). These enzymes are used in applications in pharmacological and food industries, textile production, cosmetics and other fields including (1) environmental biotechnology and (2) biofuel and bioenergy resource production (Antranikian et al. 2005, Leary et al. 2009, Bakunina et al. 2012b, Hehemann et al. 2014).

The cultivable fraction of bacterial communities associated with macroalgae is a potential source of novel marine enzymes with biotechnological potential. This culture-based approach also allows the identification and selection of isolates showing enzymatic activities and the production of bioactive compounds (Hehemann et al. 2014, Martin et al. 2015).

The carbohydrates, proteins and lipids on macroalgal surfaces are likely to be a source of carbon and energy for epiphytic bacteria. We thus surmised that bacteria associated with the marine macroalga *U. lactuca* would be a source of enzymes (amylases, lipases, cellulases and agarases) and siderophores. We isolated bacterial strains from *U. lactuca* growing in the Santa Marta region (Colombian Caribbean Sea). We taxonomically identified some of these isolates and showed that *U. lactuca* provides a biotic environment for the discovery of biocatalysts and bioactive compounds of marine origin.

MATERIALS AND METHODS

Isolation and culture

Sea lettuce *Ulva lactuca* was collected on the rocky littoral site La Punta de la Loma (Santa Marta, Colombian Caribbean) (11° 07′ 00.9″ N, 74° 14′ 01.3″ W). La Punta de la Loma is an exposed rocky platform that allows the establishment of a diverse algal community. The region has 2 main climatic seasons, the dry season, from December to April, and the rainy season, from May to November. La Punta de la Loma has more than 55 macroalgal species, and the most abundant include the brown alga *Padina gymnospora*, the green algae *Caulerpa sertularioides* and *U. lactuca* and the red algae *Acantophora spicifera, Laurencia papillosa* and *Gracilaria mamillaris*. In addition, the macroalgal community from La Punta de la Loma is more diverse in the rainy season than in the dry, although species such as *U. lactuca* are common in both seasons (García & Díaz-Pulido 2006).

Samples were collected during 2 yr (2015–2016) in February and July, under the Framework Permission (Resolution No. 0255 of March 14, 2014). Macroalgae were stored inside sterile plastic bags containing seawater and transported on ice until processing. In the laboratory, macroalgae were washed several times with sterile seawater to remove loosely associated microorganisms, and part of each macroalgal thallus was transferred to conical flasks with marine broth (Zobell, HiMedia Laboratories) and placed in a rotatory shaker at 180 rpm and 30°C for about 24 to 48 h. After incubation, a loop full of each sample mixture was streaked onto nutrient agar with NaCl (1% w/v) and incubated another 24 h.

Subsamples of macroalgal thalli were also transferred to petri dishes with marine agar and placed in an incubator at 30°C for 24 to 48 h. In both isolation strategies, bacterial colonies were separated by optical appearance and subcultured several times until pure bacterial cultures were obtained. The isolates (207 strains) were deposited in the bank of strains and genes of the Instituto de Biotecnología of the Universidad Nacional de Colombia. Strains with larger degradation zones (zones of clearance) were also deposited in the International Collection of Microorganisms of the Pontificia Universidad Javeriana (CMPUJ), with the codes CMPUJ 454–456.

Screening of amylolytic enzyme production

To identify the amylolytic activity of the bacterial strains, each isolate was cultured in nutrient starch agar plates (containing 2% starch, 0.5% peptone, 0.3% beef extract, 1% NaCl and 1.5% agar) and incubated at 30°C for 24 to 48 h (Tiwari et al. 2014). After incubation, amylase production was determined by a zone of clearance surrounding the strain, after Lugol's iodine solution (1% iodine in 2% potassium iodide w/v) was added to the plate. The diameters of the digestion zones were measured in each isolated strain. A strain of *Bacillus subtilis* was used as a positive control.

Screening of cellulase producers

Marine bacterial cultures isolated from macroalgae surfaces were grown in carboxymethycellulose (CMC) agar (0.2% sodium nitrate, 0.1% dipotassium phosphate, 0.05% magnesium sulfate [$MgSO_4$], 0.05% potassium chloride [KCl], 0.2% CMC sodium salt, 0.02% peptone and 1.7% agar). Plates incubated at 30°C for 48 h were flooded with Lugol's iodine. A strain of *B. subtilis* was the positive control for this activity. The plates that showed cellulase production were those with clear zones around the colonies and dark coloration in the non-hydrolyzed part of the medium (Kasana et al. 2008).

Screening of lipolytic enzyme production

Bacterial isolates were screened on nutrient agar supplemented with 1% tributyrin and 1% NaCl. After the sterilization process, agar nutrient, tributyrin and Triton X-100 (emulsifier) were sonicated in distilled water for 30 min. Isolate colonies were streaked on tributyrin plates and incubated at 30°C for 48 h (Kiran et al. 2014). Colonies with activity showed a clear halo formed by the hydrolysis of tributyrin. A strain of *Pseudomonas aeruginosa* was used as a positive control for the enzymatic activity.

Screening of agarase producers

Isolated colonies were grown at 30°C for 48 h in plates containing modified Zobell medium (2.47% NaCl, 0.63% $MgSO_4$, 0.07% KCl, 0.5% tryptone, 0.1% yeast extract, 5% alginate salts, 40 mM magnesium chloride and 1.5% agar) (Martin et al. 2015). After 48 h to 1 wk, strains that showed hydrolytic activity (a hole in the jellified medium) were considered as positives for this activity. A strain of *Vibrio azureus* was used as the control.

Siderophore detection

To detect the production of siderophores in the bacterial isolates, the overlaid chrome azurol sulfonate (CAS) assay was used, as modified by Pérez-Miranda et al. (2007). The composition per liter of the overlying medium was 60.5 mg CAS, 72.9 mg hexadecyltrimetyl ammonium bromide and 1 mM ferric trichloride hexahydrate in 10 mM HCl. Agarose (0.9% w/v) was the gelling agent. Siderophore detection was achieved after a volume of 10 ml of this medium was poured over the agar plates containing cultivated microorganisms. After 4 h, a change in color was observed surrounding the siderophore-producing microorganisms: from blue to purple (for siderophore production of the catechol type), from blue to orange (for microorganisms that produce hydroxamates) and from blue to light yellow (for bacteria that produce siderophores of the carboxylate type) (Pérez-Miranda et al. 2007). For this assay, a strain of *P. aeruginosa* was used as a positive control.

Bacterial identification

Several isolated bacteria (52 strains) were selected on the basis of enzymatic activities and siderophore production for further taxonomic identification. For each, strains were cultivated in marine broth (Zobell) at 30°C for 24 h. Genomic DNA was manually extracted using protocol standardized by Kim et al. (2007) with small modifications.

The 16S ribosomal RNA (16S rRNA) gene of the strains was amplified by PCR using the primers 27F and 1492R (Lane 1991). Sanger sequencing was used to identify the sequences of the amplified rRNA genes. Low-quality bases were manually trimmed, and the sequences were then analyzed using the BLAST program of the NCBI and the NIH genetic sequence database (GenBank). Closest neighbors were found by alignments with verified species through the EzBioCloud platform (Chun et al. 2007). Multiple sequence alignment was performed using Clustal_W. The evolutionary relatedness of strains was inferred with the p-distance method, in addition to a bootstrap test in 1000 replicates for each cluster.

The phylogenetic tree was drawn using the MEGA 5.0 program available at www.megasoftware.net/. Nucleotide sequences were deposited in GenBank under accession numbers KY421550–KY421591 and MF000975–MF000984.

Statistical analysis

Experiments were performed in triplicate. All of the data were checked for normality by Shapiro-Wilks test and homogeneity of variances by the Bartlett test. Because these conditions were not satisfied, the Kruskal-Wallis test was performed to identify differences between years of sampling. A principal component analysis (PCA) was used to show possible associations between variables. All of the tests were performed using R statistical software (R Development Core Team 2011).

RESULTS

A total of 207 bacteria were isolated from *Ulva lactuca*. All strains were tested for hydrolytic activities (amylolytic, lipolytic, cellulolytic and agarolytic) as well as for siderophore production. We found 20 isolates (10%) with enzymatic activity in one of the tested substrates besides siderophores; as many as 82 strains (40%) produced 2 of the 4 enzymes and siderophores, while 19 isolates (9%) synthesized siderophores and had activity on 3 substrates. The zones of clearance showed the extent of activity, and a diameter >1.5 cm was considered significant (Tiwari et al. 2014).

Significant differences between samplings were identified in enzyme activities and siderophore production (Kruskal-Wallis $p < 0.05$). In the PCA, the first 3 axes explained 75% of the variation. Fig. 1 shows a grouping of the data corresponding to the year 2015 and another group for 2016. Cellulolytic activity, amylolytic activity and siderophore production also showed grouping (Fig. 1).

Amylase production

Bacterial strains producing variable amylolytic zones on starch agar plates were isolated from the macroalgal surface. After staining with iodine solution, the zones of clearance by isolates showed the amylolytic activity (Fig. 2a). Clearance zones >1.5 cm (a criterion of substantial activity as in Tiwari et al. [2014]) were produced by 31 strains (32% of the isolates with amylolytic activity).

Cellulase production

The epiphytic bacteria were evaluated for cellulolytic activity. In total, 78 strains (66% of the isolates with activity) produced a clearance zone >1.5 cm on CMC agar plates and after flooding with Lugol's iodine (Fig. 2b).

Lipase production

All isolated bacteria were screened for lipolytic activity on tributyrin agar (Fig. 2c). In total, 4 strains (15% of those with activity) produced a clear zonation >1.5 cm, and one of these caused a clear halo zone of 2 ± 0.3 cm (mean ± SD) on the substrate (Fig. 2d).

Agarase production

Bacteria isolated from the surface of macroalgae *U. lactuca* were screened for agar-decomposing enzymes. In 25 strains (12% of all 207 isolates), agarase

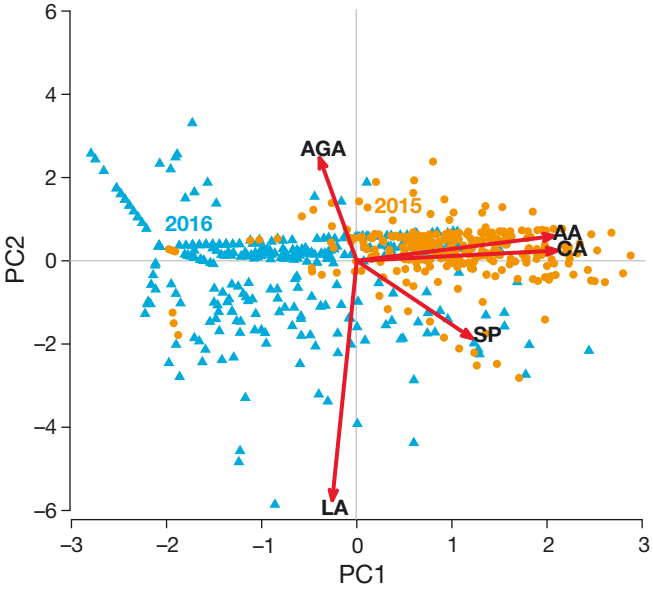

Fig. 1. Principal component analysis with data of enzymatic activities and siderophore production in 2 yr of sampling; 2015 data are represented by yellow ocher circles, and 2016 data are represented by blue triangles. PC1: principal component 1; PC2: principal component 2; CA: cellulolytic activity; AA: amylolytic activity; LA: lipolytic activity; AGA: agarolytic activity; SP: siderophore production

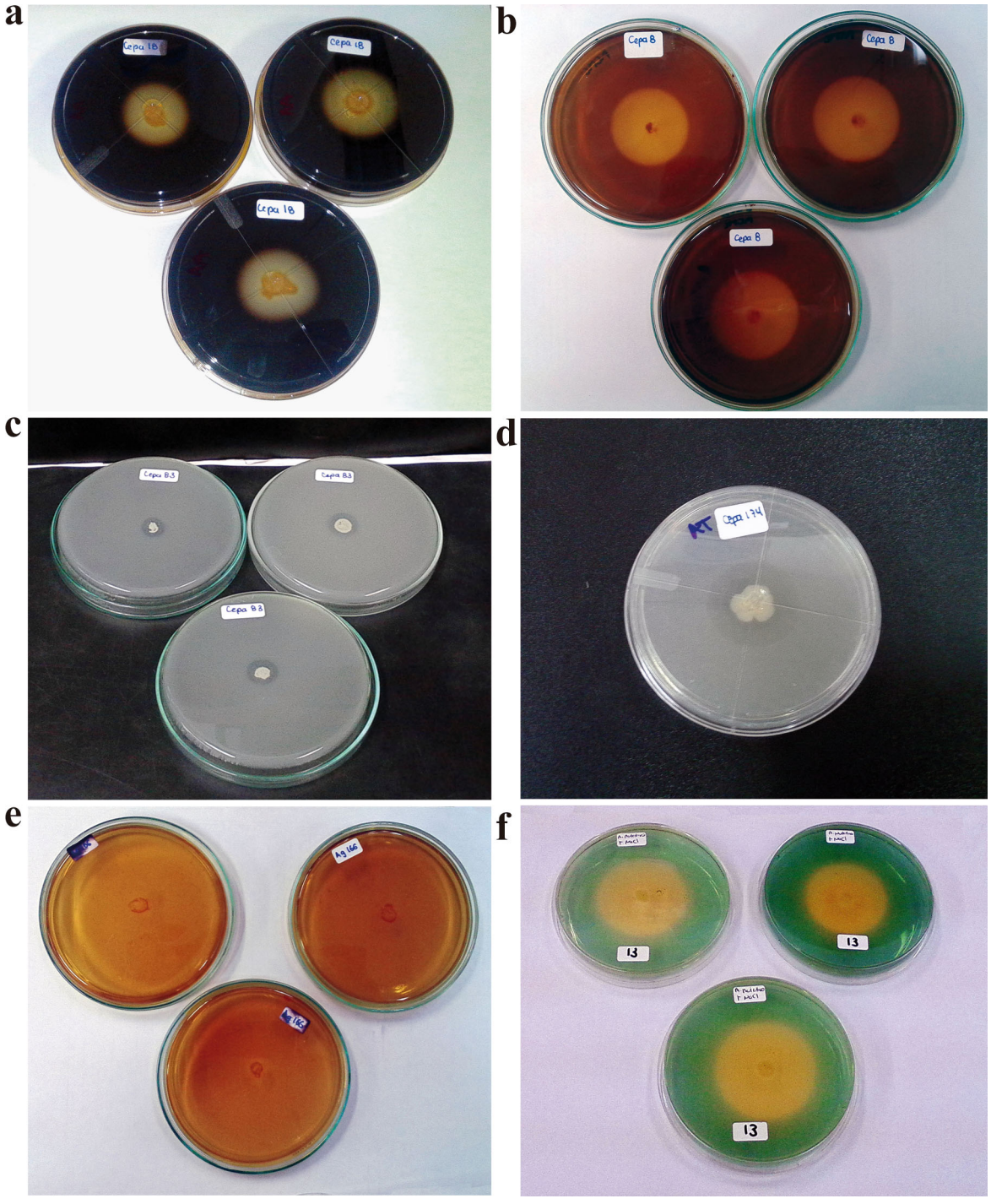

Fig. 2. Enzymatic activity in culture media using different substrates. (a) Amylolytic activity on starch agar. (b) Cellulolytic zone in carboxymethycellulose agar plates. (c,d) Hydrolysis zone on tributyrin plate. (e) Agarolytic activity as indicated by a hole in the jellified medium. (f) Siderophore production

production was identified (Fig. 2e). Holes on the agar surface around the bacteria colonies indicated the ability of the strains to degrade agar (Wang et al. 2006).

Siderophore detection

The siderophore-producing epiphytic bacteria produced changes in color of the overlying medium. In

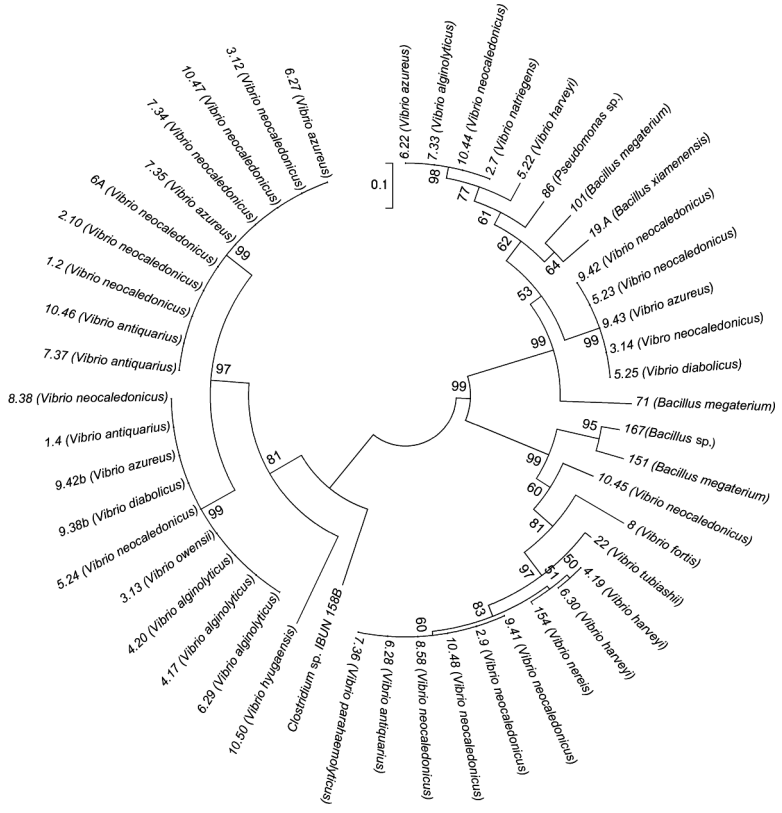

Fig. 3. Phylogenetic tree of isolates using 16S ribosomal DNA sequences. Identification number of the strains appears before the name. The evolutionary history was inferred using the neighbor-joining method, and the sequence of *Clostridium* IBUN 158B was used as the outgroup. A bootstrap test was performed on the clusters in 1000 replicates. The evolutionary distances were computed using the p-distance method. Bootstrap values are displayed as percentages on their relative branches. The length of the nucleotide sequences ranged from 359 to 838 base pairs

Table 1. Taxonomic identification of the isolates based on the analysis of partial sequences of the 16S ribosomal RNA gene. The length of the nucleotide sequences ranged from 359 to 838 base pairs

Strains	Taxon	Similarity (%)	Sampling date
5	*Vibrio antiquarius*	99.36–97.83	Jul 2015
19	*V. neocaledonicus*	97–100	Jul 2015, Jul 2016
4	*V. harveyi*	97–100	Jul 2015, Jul 2016
5	*V. azureus*	99.7–100	Jul 2015
4	*V. alginolyticus*	98.25–100	Jul 2015, Jul 2016
1	*V. natriegens*	100	Jul 2015
1	*V. nereis*	99.57	Feb 2016
1	*V. fortis*	97.77	Jul 2015
1	*V. tubiashii*	99.84	Jul 2015
1	*V. hyugaensis*	98	Jul 2016
1	*V. diabolicus*	100	Jul 2015
1	*V. owensii*	99.55	Jul 2016
1	*V. diabolicus*	100	Jul 2015
1	*V. parahaemolyticus*	99.84	Jul 2015
1	*Pseudomonas* sp.	97	Jul 2015
3	*Bacillus megaterium*	99.74–100	Jul 2015, Jul 2016
1	*Bacillus* sp.	97	Feb 2016
1	*B. xiamenensis*	100	Jul 2015

total, 121 strains (58% of all 207 isolates) changed the medium from blue to a light yellow color (Fig. 2f). This analysis suggested that the epiphytic bacteria produced siderophores of the carboxylate type (Pérez-Miranda et al. 2007).

Bacterial identification

A subset of the strains isolated from *U. lactuca* that produced enzymes and siderophores was selected for DNA extraction and analysis (see Table A1 in the Appendix). All the isolates were matched according to genus or species levels, based on degree of sequence similarity (Table 1), and were assigned to taxonomic groups, which are presented in a phylogenetic tree (Fig. 3).

Among the 52 identified bacterial strains (Table 1), 2 phyla were represented: *Proteobacteria* and *Firmicutes*. Bacterial isolates related to *Firmicutes* were affiliated to the genus *Bacillus* (5 isolates). In the case of *Proteobacteria*-like isolates, most of them were all assigned to the genus *Vibrio* (46 isolates), while 1 isolate was assigned to the genus *Pseudomonas* (Fig. 3).

DISCUSSION

Epiphytic bacteria are dependent on the organic carbon sources produced by the host. The surfaces of *Ulva lactuca* contain carbohydrates (44%), lipids (5%) and proteins (16%) (El-Naggar et al. 2014); additionally, these macroalgae have a high content of cellulose as the main component of their cell walls (Lachnit et al. 2011, Choi et al. 2012). To assimilate these compounds, enzymes such as cellulases, beta-glucosidases and amylases are found in the bacteria living in such habitats (Martin et al. 2014, 2016). In this study, we identified 121 isolates (58% of the total) with enzymatic activity on the tested sub-

strates. Some isolates produced particularly large halo zones compared to other strains that have been previously tested (Table 2).

One of the most striking results in this study was the high number of bioactive vibrios isolated from *U. lactuca*. The abundance of *Vibrio* sp. in seawater is in the range of 10^3 to 10^4 cells ml^{-1}; in marine hosts such as macroalgae, mussels, tunicates and sponges, species of the genus *Vibrio* are among the most abundant culturable constituents of macroalgal communities (Takemura et al. 2014). Vibrios can metabolize algal polysaccharides such as alginate, mannan, cellulose, pectin and laminarin (Duan et al. 1995). Other biological activities that facilitate their association with the macroalgal host include direct antagonism towards potential competitors on the macroalgal surface environment (Dobretsov & Qian 2002, Kanagasabhapathy et al. 2008), effects on the morphogenic development of macroalgae (Nakanishi et al. 1999, Singh & Reddy 2014) and participation in the spore germination of some *Ulva* species (Tait et al. 2005).

Another interesting result was the statistically significant differences between sampling times in enzyme and siderophore production. A possible reason was the occurrence of the 2015–2016 El Niño, which affected the study area. During this climate phenomenon, rainfall dropped by 30 to 40% and sea temperatures rose by up to 2.5°C (Institute of Hydrology, Meteorology and Environmental Studies, IDEAM). These conditions could have influenced the bioactive epiphytic bacteria (Ducklow et al. 1995).

In the enzyme tests in this study, 32% of the isolates that showed amylolytic activity had clearance zones >1.5 cm (indicating substantial activity). Production of amylases by epiphytic bacteria is required to metabolize starch, which in macroalgae of the genus *Ulva* can be 20% of total biomass (Choi et al. 2012, Tsuji et al. 2014). In the present study, the epiphytic bacteria with the greatest enzymatic activity were species of *Bacillus* (Table 2), consistent with previous studies (Al-Naamani et al. 2015, Zhou et al. 2015, Homaei et al. 2016).

We found that many strains (66% of the isolates with cellulolytic activity) produced large clearance zones indicative of cellulolytic activity. Epiphytic bacteria can incorporate α-cellulose from macroalgae cell walls through cellulases and include the genera *Cytophaga*, *Cellulomonas*, *Vibrio*, *Clostridium*, *Nocardia*, *Pseudoalteromonas* and *Streptomyces* (Zhang & Kim 2010, Trivedi et al. 2011a,b). In this study, most of the isolates with cellulolytic activity were assigned to *Vibrio* sp., and some of them showed diameters of zones of clearance greater than the halos displayed by strains reported elsewhere (Table 2).

Concerning lipases, 4 isolates (15%) showed a clearance zone >1.5 cm and one of them exhibited a clear halo zone of 2 ± 0.3 cm. Functional metagenomics analysis has been used to identify lipases synthesized by epiphytic bacteria from *Ulva*, and the results highlighted the importance of these enzymes in the interactions between macroalgae and epiphytic bacteria (Fang & Zhang 2011, Yung et al. 2011, Krohn-Molt et al. 2013, Peng et al. 2014). However, there were no previous records of lipases obtained from cultivable bacteria isolated from *U. lactuca*. Our results revealed that epiphytic bacteria isolated from macroalgae surfaces are a potential target in the search for marine lipases.

We found that 25 strains (12% of the total number of isolates) produced agar-decomposing enzymes. Agarases have been found in marine mollusks and in bacteria of the genera *Vibrio* and *Bacillus* (Fu & Kim 2010, Hehemann et al. 2012, Arnosti et al. 2014). The genera *Cytophaga*, *Actinomyces*, *Alteromonas*, *Pseudomonas* and *Pseudoalteromonas* are associated with hosts and may also contain agar-attacking enzymes (Wang et al. 2006, Zhang & Kim 2010, Martin et al. 2014, Gurpilhares et al. 2016). Our results revealed that bacterial species isolated from *U. lactuca* such as *V. neocaledonicus* and *V. azureus* are producers of agarases.

In addition to enzymatic activity, we evaluated siderophore production in strains isolated from *U. lactuca* thallus samples. These compounds are produced to sequester iron through functional groups with a strong affinity to ferric ion (Pérez-Miranda et al. 2007). We found that 121 strains (58% of the total) produced siderophores; this production was also reported by studies of isolated bacteria from diverse marine and terrestrial environments (Table 2). However, there have been no previous records of siderophores detected in epiphytic bacteria from *U. lactuca*, and our results show that these bacterial groups are also sources of interest for marine siderophores.

The number of studies searching for enzymes in marine environments is continuing to increase each year. This is because enzymes such as amylases, cellulases, lipases and agarases are biological catalysts with potential applications in pharmaceutical, cosmetic, algal polysaccharide degradation, food, agrochemical and biofuel industries (Bell et al. 2002, Trincone 2010, Verma et al. 2012, Arnosti et al. 2014, Prabhawathi et al. 2014). Siderophores allow the development of food supplements and are used for

Table 2. Characteristics of cultivable bacteria tested for enzymatic activities (amylolytic, cellulolytic, lipolytic and agarolytic) and production of siderophores. **Bold** font highlights the species isolated in this study and blanks indicate that all the isolates were obtained from macroalgal surfaces

Activity Strain	Source	Halo zone characteristic	Reference
Amylolytic activity			
Bacillus sp. MRS6	Solid wastes	0.8 cm	Sahoo et al. (2016)
B. cereus SB2	Soil	1.2 cm	Raplong et al. (2012)
B. subtilis	Rhizospheric soil	2.3 cm	Vijayalakshmi et al. (2012)
B. megaterium	**Macroalgal surfaces**	**2.8 cm**	**This study**
Vibrio harveyi		**2.2 cm**	
V. neocaledonicus		**2.1 cm**	
V. parahaemolyticus		**2.1 cm**	
V. natriegens		**1.9 cm**	
V. diabolicus		**1.8 cm**	
V. antiquarius		**1.8 cm**	
B. megaterium		**1.8 cm**	
***Bacillus* sp.**		**1.8 cm**	
V. alginolyticus		**1.6 cm**	
Cellulolytic activity			
Bacillus sp. Y3	Soil	0.9 cm	Lugani et al. (2015)
Bacillus sp.	Soil	2.9 cm	Akaracharanya et al. (2014)
B. subtilis BAB-2742	Compost	3.4 cm	Emmyrafedziawati & Stella (2015)
V. neocaledonicus	**Macroalgal surfaces**	**4.2 cm**	**This study**
V. azureus		**3.5 cm**	
V. parahaemolyticus		**3.4 cm**	
V. antiquarius		**3.1 cm**	
V. natriegens		**2.3 cm**	
V. alginolyticus		**2.9 cm**	
***Bacillus* sp.**		**2.5 cm**	
B. megaterium		**2.2 cm**	
V. diabolicus		**1.8 cm**	
Lipolytic activity			
Pseudomonas sp.	Oil factories	1.5 cm	Noormohamadi et al. (2013)
B. cereus MSU AS	Gut of a marine Fish	1.7 cm	Ananthi et al. (2014)
V. harveyi	**Macroalgal surfaces**	**2.0 cm**	**This study**
V. tubiashii		**1.5 cm**	
Agarolytic activity			
Vibrio sp. F-6	Coastal water	Pits around colonies	Fu et al. (2008)
Micrococcus sp. GNUM-08124	Macroalgal Surfaces	Pits around colonies	Choi et al. (2011)
Flammeovirga sp.	Coastal Sediments	Pits around colonies	Han et al. (2012)
V. neocaledonicus	**Macroalgal surfaces**	**Pits around colonies**	**This study**
V. azureus		**Pits around colonies**	
Siderophore production			
V. harveyi	Coastal water	Orange halos around colonies	Murugappan et al. (2011)
P. aeruginosa FP6	Rhizospheric soils	Orange halos around colonies	Sasirekha & Shivakumar (2016)
***Pseudomonas* sp.**	**Macroalgal surfaces**	**Yellow halos around colonies**	**This study**
***Vibrio* sp.**			
***Bacillus* sp.**		**Yellow halos around colonies**	

biosensor and artificial biofilm construction in biotechnological applications (Guan et al. 2001, de Carvalho & Fernandes 2010).

In conclusion, macroalgae-associated microorganisms are known to produce diverse hydrolytic enzymes and bioactive compounds during their interactions with the host and with the marine environment. In this study, we isolated 207 bacteria from the surface of the macroalga *U. lactuca* and found that many of them are promising sources of amylases, cellulases, lipases, agarases and siderophores of marine origin. Strains with the activities evaluated were

assigned to the genera *Vibrio*, *Bacillus* and *Pseudomonas*. The results obtained in this study are the first step in isolating and characterizing the enzymes and bioactive compounds synthesized by bacteria from the cultivable microbiota associated with green macroalgae located in Santa Marta (Colombian Caribbean). Our results indicate that *U. lactuca* offers a promising biotic environment in the search for enzymes and bioactive compounds of marine origin and potential biotechnological application.

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Appendix: Table A1. Zone of clearance (cm) on the substrates evaluated for the isolates identified. Enzymatic activity and siderophore. (+): observed, (–): not observed

Accession number	Close relative in Genbank	Enzymatic activity and siderophore production					Diameter of zone of clearance (cm)		
		Amylases (AA)	Cellulases (CA)	Lipases (AL)	Agarases (AGA)	Siderophores (SP)	AA	CA	LA
KY421553	*Vibrio neocaledonicus*	+	+	–	–	+	1.5	1.1	–
KY421586	*Vibrio antiquarius*	+	+	–	–	+	1.2	3.2	–
KY421552	*Vibrio natriegens*	+	+	–	–	+	1.9	2.3	–
KY421559	*Vibrio nereis*	+	+	+	–	+	0.9	1.2	0.7
MF000977	*Vibrio diabolicus*	+	+	–	–	+	1.8	1.4	–
KY421555	*Vibrio neocaledonicus*	+	+	–	–	+	1.4	1.3	–
KY412567	*Vibrio tubiashii*	+	+	+	–	+	1.3	1.5	1.5
KY421557	*Vibrio neocaledonicus*	+	+	–	+	+	1.1	1.6	–
KY412562	*Vibrio neocaledonicus*	+	+	+	+	+	1.1	2.1	0.6
KY412575	*Vibrio neocaledonicus*	+	+	+	+	+	1.6	3	1.3
KY412583	*Vibrio neocaledonicus*	+	+	–	–	+	1.1	2.1	–
MF000980	*Vibrio owensii*	+	+	–	–	+	1.6	1.4	–
KY421584	*Vibrio neocaledonicus*	+	+	–	–	+	1.6	1.8	–
KY412576	*Vibrio neocaledonicus*	+	+	–	–	+	1.5	2.8	–
MF000984	*Vibrio alginolyticus*	+	+	–	–	+	1.7	1.7	–
KY421556	*Vibrio harveyi*	+	+	+	–	+	1.9	2.9	0.7
MF000981	*Vibrio alginolyticus*	+	+	–	–	+	1.6	2.9	–
KY421561	*Vibrio azureus*	+	+	–	+	+	1.5	1.1	–
KY421578	*Vibrio neocaledonicus*	+	+	–	–	+	2	1.9	–
KY421566	*Vibrio harveyi*	+	+	+	–	+	1.5	1.7	2
MF000979	*Vibrio neocaledonicus*	+	+	–	–	+	1.3	2.8	–
MF000983	*Vibrio diabolicus*	+	+	–	–	+	1.8	1.4	–
KY421587	*Vibrio azureus*	+	+	–	–	+	1.1	3.5	–
KY421588	*Vibrio antiquarius*	+	+	–	–	+	1.4	2.8	–
MF000982	*Vibrio alginolyticus*	+	+	–	–	+	1.4	2.8	–
MF000978	*Vibrio harveyi*	+	+	–	–	+	2.2	2.5	–
KY421563	*Vibrio antiquarius*	+	+	–	–	+	1.5	1.2	–
KY421590	*Vibrio alginolyticus*	+	+	–	–	+	1.4	2.2	–
KY421574	*Vibrio neocaledonicus*	+	+	–	–	+	2.1	3.1	–
KY421564	*Vibrio azureus*	+	+	–	+	+	1.2	3.0	–
KY421591	*Vibrio parahaemolyticus*	+	+	–	–	+	2.1	3.4	–
KY421550	*Vibrio antiquarius*	+	+	–	–	+	1.7	3.3	–
KY421579	*Vibrio diabolicus*	+	+	–	–	+	1.4	1.8	–
KY421565	*Pseudomonas* sp.	+	+	+	–	+	1.4	1.3	0.9
KY421580	*Vibrio neocaledonicus*	+	+	+	–	+	1.4	2.9	–
KY421570	*Vibrio neocaledonicus*	+	+	+	–	+	1.6	2.4	–
KY421577	*Vibrio neocaledonicus*	+	+	+	–	+	1.8	2.4	–
MF000976	*Vibrio azureus*	+	+	+	–	+	1.2	1.3	–
KY421581	*Vibrio azureus*	+	+	+	–	+	1.7	2.4	–
KY421569	*Vibrio neocaledonicus*	+	+	+	–	+	0.7	4.2	–
KY421585	*Vibrio neocaledonicus*	+	+	+	–	+	1.6	3.0	–
KY421551	*Vibrio antiquarius*	+	+	+	–	+	1.8	1.8	–
KY421582	*Vibrio neocaledonicus*	+	+	+	–	+	1.5	2.7	–
KY421568	*Vibrio neocaledonicus*	+	+	+	–	+	1.4	3.2	–
MF000975	*Vibrio hyugaensis*	+	+	+	–	+	1.3	2.0	–
KY421558	*Bacillus megaterium*	–	+	+	–	+	–	1.5	0.2
KY421572	*Bacillus megaterium*	+	+	+	–	+	1.4	1.7	0.5
KY421573	*Bacillus megaterium*	+	+	+	–	+	1.8	2.2	1.2
KY421560	*Bacillus* sp.	+	+	+	–	+	1.8	2.5	0.4
KY421571	*Bacillus xiamenensis*	–	+	+	–	+	–	1.3	0.7
KY421554	*Vibrio neocaledonicus*	+	+	–	–	+	1.0	1.6	–
KY421589	*Vibrio azureus*	+	+	–	+	+	1.1	3.5	–

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