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Two possible candidate enzymes from *Ulva lactuca*-associated epiphytic bacteria obtained through PCR and functional evaluation

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Abstract

Epiphytic bacteria from marine macroalgae synthesize enzymes of industrial and biotechnological interest. In this study, we obtained two DNA candidate fragments for lipid-degrading enzymes from the total DNA of *Ulva lactuca*-associated epiphytic bacteria. First, we evaluated a method for total bacterial DNA isolation from the surface of *U. lactuca* thalli. Then, we designed sets of primers and used them directly for PCR amplification. The resulting PCR products were sequence-analyzed and used for expression and functional evaluation with the *Escherichia coli* pBAD-TOPO system. We obtained high molecular weight and good quality total bacterial DNA that served as a template to identify a fragment corresponding to an Acetyl-CoA C-Acetyltransferase (or Thiolase), and a candidate fragment for a versatile "true" lipase. We expressed the possible "true" lipase gene fragment heterologously in *Escherichia coli* and obtained proof of hydrolytic activity on Tributyrin, Tween-20, and Olive-oil media. This study resulted in new knowledge on *U. lactuca*-associated epiphytic bacteria as possible brand-new sources of enzymes such as thiolases and "true" lipases. However, future studies are required to describe the characteristics and important applications of these candidate enzymes.

Keywords: Epiphytic bacteria; lipases; PCR; thiolases; *Ulva lactuca*.

Introduction

The surfaces of marine macroalgae represent promising candidate sources of novel biocatalysts [1]. These surfaces are exposed to different conditions of temperature and salinity and are also important nutrient-rich environments

that enable bacterial colonization and constitute sustainable microhabitats [2]. Bacteria living on macroalgal surfaces (also called epiphytes) promote host development and health, providing CO_2 and minerals. Furthermore, these epiphytes produce specific regulatory factors and antagonistic compounds that promote and enhance algal growth, defense, resilience, and facilitate stress response [3, 4]. On the other hand, the epiphytic bacteria benefit from the organic matter produced by the macroalgae and synthesize a vast variety of enzymes to assimilate these compounds [5-7]. For instance, macroalgae from the species of *Ulva lactuca* have an important content of lipids (8 – 3 % dry weight), especially palmitic and oleic acids [8, 9]; therefore, associated epiphytic bacteria may need to synthesize enzymes such as esterases, lipases, and thiolases [10], among wide variety of other enzymes [11].

Lipases are enzymes that act on triglycerides ester bonds to liberate fatty acids and glycerol, thus participating in lipid degradation and biosynthesis pathways [12]. Likewise, lipases have an enormous catalytic versatility including lipid hydrolysis, trans- and inter-esterification, fat and oil acidolysis, aminolysis, and alcoholysis [13, 14]. Due to these features, lipases have a variety of applications in the food, pharmaceutical, and cosmetic industries, as well as in the production of agrochemicals, biofuels, and detergents, among others [15, 16]. Lipases are divided into two main groups: esterases (EC.3.1.1.1) that prefer water-soluble short-chain fatty acids, and "true" lipases or triacylglycerol hydrolases (EC.3.1.1.3) that prefer low water-soluble long-chain fatty acids [17, 18]. All lipases share a high conserved active site (Ser-Asp-His) and a consensus region motif (Gly-Xaa-Ser-Xaa-Gly) [19].

Thiolases (EC.2.3.1.16), also called Acetyl-CoA C-Acetyltransferases, are transferases that catalyze the reversible cleavage of fatty acids into acyl-CoA and acetyl-CoA throughout the transference and condensation of acyl groups [20, 21]. Further, thiolases are involved in lipid transport and assimilation, β-oxidation, as well as in fatty acid, steroid, and polyketide biosynthesis [22]. These enzymes also have several applications in the production of organic solvents and biofuels, synthesis and degradation of antibiotics, and bioremediation processes [23-25].

Metagenomics studies have revealed the genetic, metabolic, and functional potential of non-cultivable microorganisms [26, 27]. Besides, access to the total DNA of an environmental sample, PCR-based analysis, and functional evaluation have allowed the discovery of lipolytic genes and enzymes from marine environments [28-30]. For instance, LipG, EstA, EML1, and EstHE1 lipases, displaying high salt tolerance, thermostability, activity within a broad pH range, and stability in a high concentration of divalent ions and organic

solvents, have been recovered from the bacterial metagenomes of intertidal flats, coastal environments, deep-sea sediments, and organism-associated bacteria [31-34]. LipA, an alkaline "true" lipase, was described from the metagenome of a marine sponge [35]. Likewise, GmEst_7 and Lip5 quorum sensing lipases were discovered in brown algae epiphytic bacteria [36, 37]. Further, other studies have reported lipases from the metagenome of the green macroalgae *Ulva australis*, such as the *abg3* gene that encodes a β-lactamase-like lipase that displays lipolytic activity and confers antibacterial properties, as shown in a heterologous expression study [38].

Nonetheless, despite "true" lipases and thiolases being important enzymes in several biotechnology and industrial applications, there is limited information about these enzymes in green macroalgae-associated bacteria; therefore, the question arises whether epiphytic bacteria from *U. lactuca* produce "true" lipases and thiolases. In consequence, here we present a suitable method for total bacterial DNA isolation from *U. lactuca* surfaces and a PCR-based identification of these enzymes and closely related enzymes. We found a candidate DNA sequence for a thiolase, and a DNA fragment that displayed "true" lipase activity when was functionally expressed. Our results showed that *U. lactuca*-associated epiphytic bacteria are potential sources of biocatalysts of marine origin.

Material and Methods

Strains, plasmids and media

Lipase-producing strains of *Burkholderia cepacia* and *Pseudomonas aeruginosa* were used as positive controls, and *Escherichia coli* DH5α was used as the negative control. These strains are part of the "Banco de Cepas y Genes del Instituto de Biotecnología de la Universidad Nacional de Colombia". *E. coli* TOP10 and plasmid pBAD-TOPO (Invitrogen) were used for cloning/expression assays. Luria Bertani agar (10 g/L tryptone, 10 g/L NaCl, 5 g/L yeast extract, 15 g/L bacteriological agar) supplemented with tributyrin 1 % v/v and 0.2 ml/L triton X-100 (emulsifier) was used for lipase screening. To confirm lipolytic activity, Tween 20 (10 g/L peptone, 5 g/L NaCl, 15 g/L bacteriological agar, 1.1 g/L CaCl2 and 10 ml/L Tween 20) and Rhodamine-Olive Oil-Agar (8 g/L nutrient broth, 4 g/L NaCl, 15 g/L bacteriological agar, 10 ml/L Olive Oil and 10 ml/L Rhodamine B) were also used. Some culture media contained 0.1 mg/L ampicillin antibiotic.

Sample collection and total DNA isolation

Thalli of green macroalgae *U. lactuca* were collected at 'La Punta de la Loma' rocky coast in Santa Marta-Colombia (11°07'00.9" N, 74°14'01.3" W). The samples were washed several times with sterile seawater and then transferred into sterile refrigerated plastic bags and stored at -80 °C until processing. Afterward, total bacterial DNA from the macroalgal surface was isolated using the ZR Soil Microbe DNA Kit (Zymo Research). Between 0.6 g and 1.0 g of the thallus base section was placed into tubes containing silica-beads and lysis buffer [39]. On a Bead-Beater (Disruptor Genie™, United States) tube holder, physical disruption of bacterial cells was performed at maximum speed for 1.5-min pulses, twice, with a 5-min rest on ice between pulses [40, 41]. Then, tubes were centrifuged at maximum speed and the clear supernatant was transferred and further processed according to the manufacturer's protocol. In the end, total DNA was eluted in 20 μl buffer and then stored at -20 °C. In addition, total DNA was analyzed by electrophoresis on agarose gel, and DNA yield (ng/µl) and quality (Abs at 260/280 nm) were quantified with a Nanodrop 2000C.

Primer design

The *Abg3* gene (from *U. australis*) was used to design primers for homologous sequences. The region between 21704 – 22681 bp of the UaAb1 clone fosmid (HQ162719) corresponding to the open reading frame (ORF) [42], was targeted for primer design through the Primer-BLAST (NCBI) tool. In order to include the whole sequence, we picked the most suitable set of primers (labeled *UaLip*) to be synthesized. In parallel, a set of degenerate primers was designed using a consensus region of nine representative and highly related "true" lipase sequences from different bacteria [43]. First, the alignments were carried using CLUSTAL OMEGA and the consensus region was determined through the Block-Maker tool. Then, the conserved blocks were directly fed to the CODEHOP (Consensus-Degenerate Hybrid Oligonucleotide Primers) program [44, 45]. Default values were used except for degeneracy (256), degeneracy strictness (2.5), and codon usage (bacteria and plastids -gbbct-). The primers (labeled *LipFam1*) were picked in order to include the whole consensus sequence, and were tested for specificity and universality using BLASTx. Lastly, these sets of primers were characterized via Multiple Primer Analyzer (Thermo Fisher Scientific) and Oligo-analyzer (Integrated DNA Technologies) tools and then contrasted with reported optimal values for PCR [46-48]. Oligo sequences are listed in **Table 1.**

Table 1. Set of primers used in this study. [a] N: A, C, G or T; Y: C or T; D: A, G or T. [b] Degenerate sites are labelled in bold and underlined.

Primers	Sequence (5' → 3')[a, b]
*LipFam1*F	GGTACCCCATCGTCCTG**GYNCAYGGNYT**
*LipFam1*R	CCAGTGGTAGTCGGTCCTG**ATNACNTDNCC**
*UaLip*F	AGCGATAATGAAACGGGCCA
*UaLip*R	TCCGTTGATGATGCCGCTTA

Amplification of the target genes by PCR

Hot-Start PCR mixes contained: 1 X Taq Buffer, 0.5 mM dNTPs, 0.5 μM of each primer, 2.0 mM MgCl2, 2 % DMSO (Dimethyl-Sulfoxide), 0.5 mg/μl BSA (Bovine Serum Albumin) and 1 U/μl Taq Polymerase. Total bacterial DNA from *U. lactuca* surface was used as template (50 – 100 ng) for *UaLip* and *LipFam1* primers testing. Amplification using *UaLip* primers was performed following these conditions: 95 °C for 5 min, 30 cycles of 94 °C for 30 s, 61 °C (optimal) for 30 s and 72 °C for 1 min, plus a final extension step at 72 °C for 10 min. Amplification using *LipFam1* primers was carried under the same conditions with a slight modification on annealing temperature: 53 °C (optimal) for 1 min. Then, PCR products were visualized on a 2 % agarose gel and purified using gel extraction with Gene JET Gel Extraction Kit (Thermo Fisher Scientific). Additionally, genomic DNA from "true" lipase producing strains was used as positive control to test *LipFam1* primers.

Expression of a possible "true" lipase gene fragment

Some purified DNA fragments obtained from total DNA of *U. lactuca* surface using *LipFam1* primers, were cloned and expressed through pBAD TOPO system, following the manufacturer's instructions. The cloning reaction was performed at a vector: insert molar ratio of 3:1 and then, chemically

competent cells were transformed by heat-shock and spread on LB agar plates containing tributyrin 1 % v/v and ampicillin. Cells transformed with empty vectors were used as the negative control. Positive clones were randomly selected and confirmed on Tween 20 and Olive-oil agar plates. Activity on tributyrin-LB agar was observed by the formation of a clear zone around the colony [49-51]. Enzymatic activity on Tween 20 was identified through the formation of a white precipitate below the colonies [52-54]; and on Olive-oil agar by the irradiation of fluorescence under UV light [55].

Sequence Analysis

All the cleaned and purified PCR products were SANGER sequenced at the 'Instituto de Genética de la Universidad Nacional de Colombia'. The sequences obtained were analyzed and edited through the BioEdit program, and the resulting high-quality FASTA files were contrasted against RefSeq Nucleotide database using BLASTn. Functional protein domains were searched using BLASTx against non-redundant UniProtKB/Swiss-Prot Protein database.

Nucleotide Sequence Accession Numbers

The accession numbers of the nucleotide sequences used in degenerate primers design were: AAA50466, CAA32193, AAB01071, AEK97793, AAC05510, CAA49812, GAC32742, WP_016349285, and AGE44121.

Results

Total DNA isolation

About 0.8 g – 0.9 g of *U. lactuca* thalli were optimal to obtain high molecular weight bacterial DNA ($\geq$ 10 Kb) (**Fig. 1**). The yield of total DNA was approximately 0.01 μg/g – 0.1 μg/g per algal sample (from 11.17 $\pm$ 0.27 to 75.62 $\pm$ 0.23 ng/μl). DNA purity was homogeneous after each extraction (from 1.6 $\pm$ 0.09 to 1.8 $\pm$ 0.05 260/280 nm). Despite observed degradation, total DNA was appropriate for PCR amplification.

Identification of a Thiolase DNA fragment

After PCR reactions with epiphytic bacterial total DNA, a $\sim$ 1.0 kb fragment (8 – 12 ng/μl) (**Fig. 2A**) was effectively obtained using the *UaLip* primers. After sequencing and editing, our BLASTn search revealed that a 519 pb high quality sequence had the most similarity to an Acetyl-CoA C-Acetyltransferase (or Thiolase) from *Erythrobacter litoralis* (75 % of identity, E-value = 2e-35). After the analysis, an open reading frame (90…446 pb)
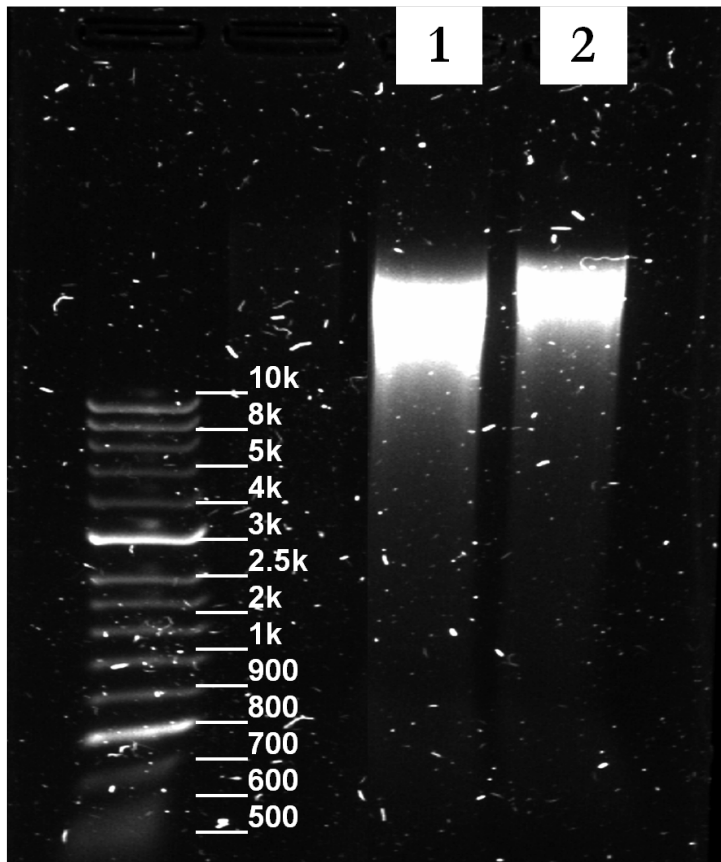
Figure 1. Total bacterial DNA from samples of the thallus of *Ulva lactuca*. The agarose gel shown was run with 1Kb Opti-DNA Marker (abm®).

was detected and corresponded to a 118 aa product. Thiolase (cd00751), Acetyl-CoA acetyltransferase (PRK06025) and PaaJ (COG0183) domains were identified. Additionally, the obtained nucleotide sequence was uploaded to the Genbank with accession number MK418067.

Identification of a "True" lipase DNA fragment

On the other hand, PCR amplification with the degenerate primers *LipFam1* on the total bacterial DNA from *U. lactuca* surface and on the *B. cepacia* and *P. aeruginosa* genomic DNA, resulted in $\sim$ 2.0 kb fragments (14 ng/μl – 20 ng/μl) (Fig. 2B). However, no data was recovered after sequencing due to the low quality and short length of the reads. Despite this, some PCR products were effectively cloned and expressed in *E. coli* TOP10 (efficiency 1 – 5 x 10^6 UFC/μg DNA). The clones tested on tributyrin-LB showed a clear zone around the colonies (**Fig. 3A** and Fig. 3C) while on tween-20 agar, a white precipitate was observed below the colonies and widespread all over the plate (Fig. 3B). Likewise, clones tested on olive-oil agar (**Fig. 4A**) showed a strong visible fluorescence all over the colony after exposure to UV-light
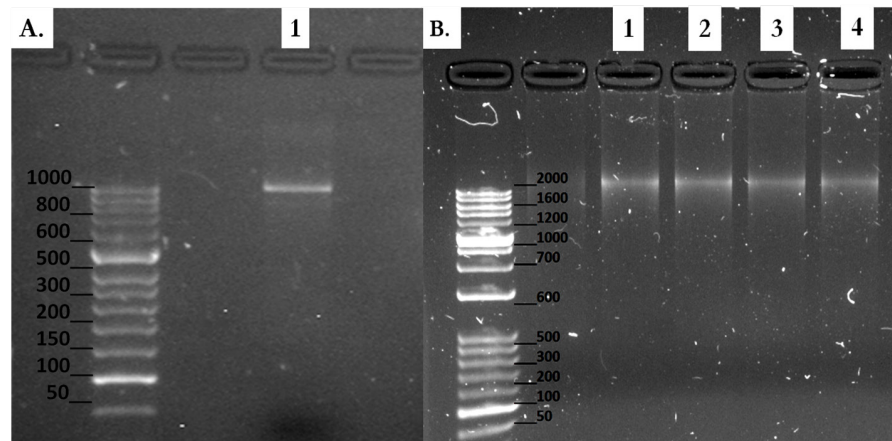
Figure 2. Purified DNA fragments obtained from PCR reactions. **A)** A ∼ 1.0 kb fragment (1) obtained using the *UaLip* primers on total bacterial DNA from the surface of *U. lactuca*. **B)** A ∼ 2.0 kb fragment obtained using the *LipFam1* primers on the total bacterial DNA (1 and 2), and on the genomic DNA of the positive controls *B. cepacia* (3) and *P. aeruginosa* (4). Gel A was run with GeneRuler™ 50 pb DNA Ladder (Thermo Scientific) while gel B with Hyper-Ladder™ 50 pb (Bioline).

(Fig. 4B and C). Similarly, positive controls used to compare the "true" lipase activity showed fluorescence all around the colony (Fig. 4D and E) while negative control ("true" lipase non-producing strain) showed no fluorescence (Fig. 4F) after exposure.
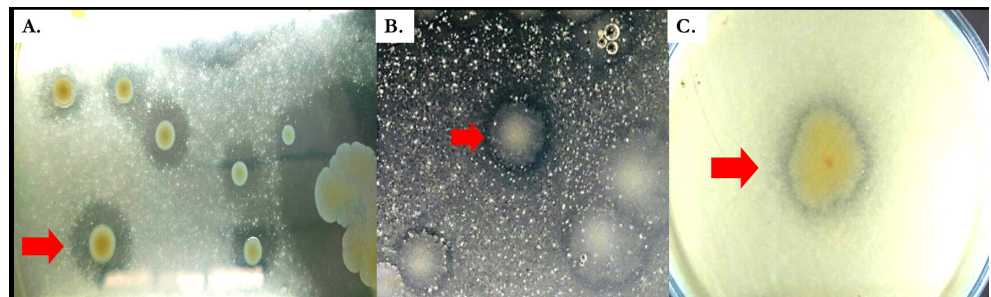

Figure 3. Lipase activities (red arrows) of the expressed *LipFam1* PCR products obtained from the total DNA of sample from the surface of *U. lactuca*. The *LipFam1* clone-libraries were tested on: **A)** Tributyrin agar, showing wide clear zones around the colonies as a result of the loss of emulsion due to the release of the soluble fatty acids and glycerol from the tributyrin; and **B)** on Tween 20 agar displaying a white precipitate below the colonies as a result of the salt-formation between the free fatty acids and the Ca^{2+} in the medium after the hydrolysis of the tween 20. **C)** Lipase activity of a single colony (clone 9) on Tributyrin agar.
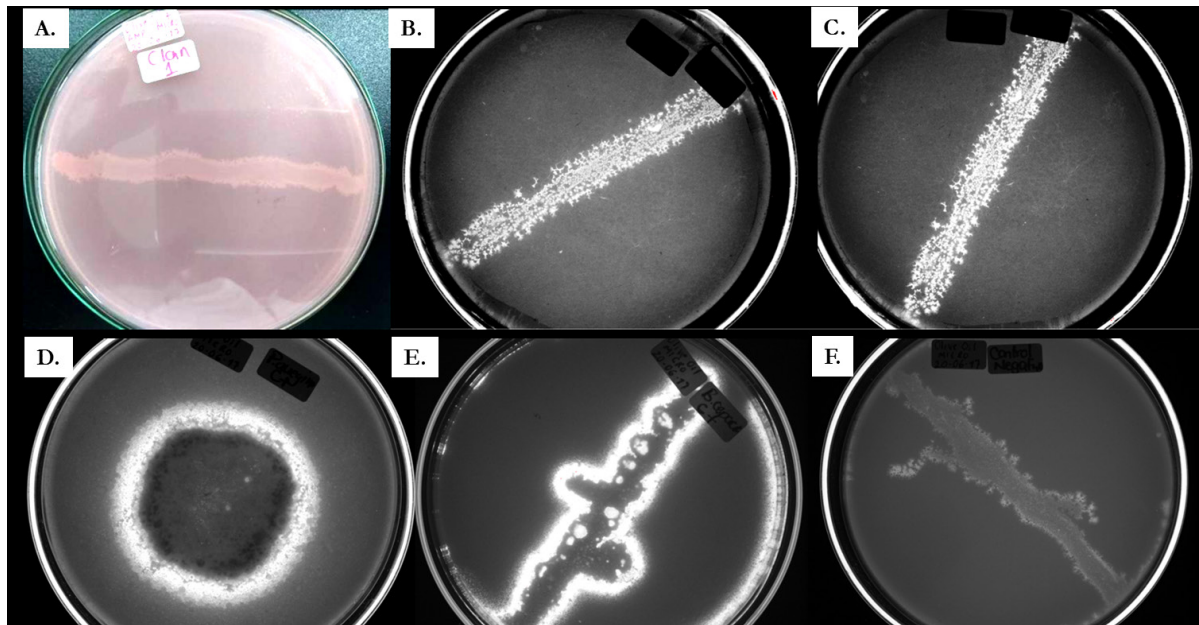
Figure 4. True-lipase activity evaluations on olive-oil agar. **A)** Clone 1 before exposure to UV-light, **B)** and **C)** Clones 1 and 9, respectively, after exposure to UV-light. **D)** *P. aeruginosa* positive control (inoculated by puncture). **E)** *B. cepacia* positive control (inoculated by spreading). **F)** *E. coli* DH5α negative control. The activity was observed by the irradiation of fluorescence as a result of the interaction between the free fatty acids from the olive oil and the Rhodamine B dye in the medium. The negative control did not show any fluorescence after the exposure.

Discussion

Total DNA isolation

The surfaces of macroalgae harbor communities of epiphytic bacteria [2] with broad regulatory roles on macroalgal growth, development, nutrient supply, protection, adaptation, and stress resilience [56]. Likewise, these bacteria may benefit from organic compounds secreted by the algal host. These compounds can be degraded by enzymes that are synthesized by epiphytic bacteria [5]. For this reason, algal surfaces are an important source of a variety of enzymes [36, 57]. Different methodologies have been used for screening enzymes of bacterial origin. This study followed the Burke's standardized method [39, 42] with some suitable modifications for obtaining DNA isolated of epiphytic bacteria. Total DNA was used for PCR-based analysis and subsequent functional evaluation [58]. To isolate bacterial DNA, we selected the thallus base section of *U. lactuca* due to the high density of bacteria

present there (around to 10^7 cells/cm^2), which decreases closer to the distal tips (around to 10^2 cells/cm^2) [2, 59]. Moreover, we attempted physical cell disruption to release the bacteria from the thallus surface as suggested by Longford *et al.* [60]. Various studies have shown that this technique allows the isolation of DNA from bacteria with complex cell walls (thicker peptidoglycan layers) such as Actinobacteria and Firmicutes, which occur at low densities [61] at the surface of *Ulva* species [62, 63]. Additionally, the methodology used in this study allows DNA clean-up [64, 65] throughout the filtration of possible inhibitors such as phenolic and humic compounds that bind to amine groups from the DNA and that negatively affect the PCR reactions [66]. Therefore, the total bacterial DNA isolated in this study served as a template for PCR-based analysis; and additives such as Bovine Serum Albumin (BSA) and Dimethyl-sulfoxide (DMSO) were necessary to enhance PCR reactions [67-69].

Identification of a Thiolase

Although no data was recovered for the *abg3* gene reported by Burke [42], we identified a 1.0 kb fragment corresponding to a "thiolase gene". Thiolases are a group of enzymes that have important uses on cellular engineering for industrial processes [70]. These enzymes appear to be functionally closely related to lipases and esterases since they are involved in the same lipid-degrading pathways and show genetic proximity to thioesterases [71]. In consequence, we gather that the presence of this thiolase on the total bacterial DNA from the *U. lactuca* surfaces could strongly entail lipolytic functions in the epiphytic bacterial communities. Thiolases could be involved in biotic degradation of some lipids on the algal host surface, considering that these enzymes participate in the cleavage of mid and long-length fatty acids in β-oxidation catabolism [25, 72]. Thiolases also catalyze reactions involved in lipid transport [73], biosynthetic metabolism [74], host stress-response [75], and bacterial virulence [76], which necessarily include the use of lipid backbones as different authors have pointed.

It is well known that thiolases are widely distributed among bacteria and eukaryotes and it has been also observed that they possess a promiscuous functionality by catalyzing different reactions [77]. Therefore, thiolases have different roles in cell engineering especially for the polyhydroxyalkanoates (PHAs) [78-80], organic acids and solvents [81, 82], and biofuel overproduction, as well as for wax ester fermentation [83] and bioremediation [84]. In consequence, further studies on thiolases produced by the *U. lactuca* epiphytic communities are required to understand their features and thereby explore their potential applications.

Identification of a "True" Lipase

In this study we proposed the *LipFam1* primers for the PCR-based identification of "true" lipases; however, no data of its sequence was recovered. A problem in genomics is to functionally classify DNA sequences derived from environmental sampling. Sometimes the query sequence does not have a close relative in the database; this could be the case of the fragment obtained with *LipFam1* primers [85].

On the other hand, the bacteria transformed with the PCR products involving the *LipFam1* primers showed hydrolytic activity on fatty acids of different chain lengths such as tributyrin (C: 4), tween 20 (C: 12), and olive oil (C: 18). In tributyrin media, the clear halos around the colonies were the result of the loss of emulsion that indicates the release of soluble glycerol and butyric acid after hydrolysis [12, 86]. The white precipitate was the evidence of the salt formed between the anionic lauric acid from the tween 20 and the Ca^{2+} ions in the medium after the hydrolase activity [52, 87]. Furthermore, the fluorescent halos were visible upon UV irradiation due to the interaction between the Rhodamine B dye and the free long-chain fatty acids (such as oleic, linoleic and palmitic) after the hydrolysis of olive oil [88-90]. In consequence, here we infer that this fragment could correspond to a versatile true lipase since it appears to hydrolyze different length-chain fatty acids, in contrast to common true lipases which only are capable of hydrolyzing ester bonds from long-chain fatty acids (> C: 12) in triacylglycerides [17]. Various lipases show a broad substrate specificity and regiospecificity [18] because of a flexible active site that appears to change its conformation with the presence and binding of different substrates [91, 92].

Our work is potentially the first report of a versatile "true" lipase identified on the total DNA of the epiphytic bacteria from *U. lactuca*. Marine "true" lipases have been only identified on the free-living bacteria such as *Oceanobacillus* sp. [93] and *Pseudomonas* sp. [94], the metagenomes of the marine sponges *Ircinia* sp. (LipA) [35] and *Haliclona simulans* (Lpc53E1) [95], and recently, in the epiphytic bacterium *Shewanella algae* from the brown macroalgae *Ascophyllum nodosum* [96]. Although, knowledge of lipases from green macroalgae epiphytic bacteria is limited [57, 97], the efforts to describe and characterize marine lipases from this source have actually shown that cultivable epiphytic bacteria from *U. lactuca* are capable of producing lipases and other hydrolytic enzymes [1].

Future studies on this topic could reveal the features and properties of these "true" lipases from green macroalgae-associated bacteria. Lipases from marine sources have shown attractive characteristics such as thermostability

[98], high-salt tolerance [99], cold-adaptation [100], extreme pH tolerance [101], organic solvents tolerance [102], and enantioselectivity [103]. These important properties are largely required in industrial and biotechnological applications such as plastic degradation [104] and biodiesel synthesis [105], as well as anti-biofilm and biofouling additives [106].

Conclusions

According to the importance of the marine biocatalysts for industrial and biotechnological purposes, here we were able to describe a suitable method to obtain two possible candidate enzymes. These candidates appear to be synthesized by the *Ulva lactuca*-associated epiphytic bacteria and could play important roles in lipid metabolism and lipid degradation.

One candidate was sequence-identified as a thiolase and the other was functionally-described as a versatile "true" lipase. The presence of these candidates can be related to the lipolytic functions in the *U. lactuca*-associated bacterial communities, and therefore, could represent a potential source of these enzymes and their related.

Notwithstanding this functional evidence, only nucleotide and peptide sequence analyses of these fragments will confirm our assumptions, and further structural and functional characterizations will reveal the features and properties of these candidates. Finally, these approaches will allow the search of potential candidate enzymes from un-exploited sources, such as green algae-associated epiphytic bacteria.

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Conflict of interest

The authors certify that they have no affiliations or involvement in any organization or entity with any financial interest. Also, the authors declare that they have no conflict of interest.

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Dos posibles enzimas candidatas de bacterias epifíticas asociadas a *Ulva lactuca*, obtenidas a través de PCR y evaluación funcional

Resumen: Las bacterias epifíticas de macroalgas marinas sintetizan enzimas de interés industrial y biotecnológico. En este estudio, se obtuvieron dos fragmentos de DNA candidatos para enzimas degradadoras de lípidos del total de DNA de bacterias epifíticas asociadas a *Ulva lactuca*. En primer lugar, se evaluó un método para el aislamiento del DNA bacteriano total de la superficie del talo de *U. lactuca*. Posteriormente, se diseñaron conjuntos de primers y se usaron directamente para amplificación por PCR. Los productos PCR resultantes se analizaron por secuenciación y se utilizaron para expresión y evaluación funcional con el sistema *Escherichia coli* pBAD-TOPO. Se obtuvo DNA bacterial total de alto peso molecular y buena calidad, que sirvió como plantilla para identificar un fragmento correspondiente a una Acetil-CoA C-Acetiltransferasa (o Tiolasa) y un fragmento candidato para una "verdadera" lipasa versátil. El fragmento de gen de la posible "verdadera" lipasa se expresó heterólogamente en *Escherichia coli* y se obtuvo prueba de actividad hidrolítica en medios de Tributirina, Tween-20 y aceite de oliva. De este estudio resultó nuevo conocimiento sobre bacterias epifíticas asociadas a *U. lactuca* como posibles nuevas fuentes de enzimas, tales como tiolasas y "verdaderas" lipasas. Sin embargo, se requieren estudios en el futuro que describan las características y aplicaciones importantes de estas enzimas candidatas.

Palabras clave: Bacterias epifíticas; lipasas; PCR; tiolasas; *Ulva lactuca*.

Duas possíveis enzimas candidatas de bactérias epifíticas associadas a *Ulva lactuca*, obtidas através de PCR e avaliação funcional

Resumo: As bactérias epifíticas de macroalgas marinhas sintetizam enzimas de interesse industrial e biotecnológico. Neste estudo, se obtiveram dois fragmentos candidatos de DNA para enzimas degradantes de lípidos a partir de um DNA total de bactéria epifítica associada a *Ulva lactuca*. Primeiramente, foi avaliado um método para o isolamento de DNA bacteriano total da superfície do talo de *U. lactuca*. Posteriormente, se desenharam conjuntos de primers e se usaram diretamente para amplificação por PCR. Os produtos de PCR resultantes foram analisados por sequenciação e se utilizaram para expressão e avaliação funcional com o sistema *Escherichia coli* pBAD-TOPO. Obtive-se DNA bacteriano total de alto peso molecular e boa qualidade, que serviu como modelo para identificar um fragmento correspondente a uma Acetil-CoA C-Acetiltransferase (ou Tiolase) e um fragmento candidato para uma "verdadeira" lipase versátil. O fragmento do gen da possível "verdadeira" lipase se expressou heterologamente em *Escherichia coli* e se obteve prova de atividade hidrolítica em meios de Tributirina, Tween-20 e aceite de oliva. Este estudo propiciou novos conhecimentos sobre bactérias epifíticas associadas a *U. lactuca* como possíveis novas fontes de enzimas, tais como tiolases e "verdadeiras" lipases. Entretanto, novos estudos são requeridos no futuro que descrevam as características e aplicações importantes de estas enzimas candidatas.

Palavras-chave: Bactérias epifíticas; lipases; PCR; tiolases; *Uva lactuca*.

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