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Production and characterization of lipase and glucolipids by *Halomonas zhanjiangensis* and study of their antimicrobial potential

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ABSTRACT

Extremophilic microorganisms, with their remarkable adaptability to harsh environments, hold immense potential for biotechnological and industrial applications. This study represents a pioneering investigation into the production and characterization of lipase and biosurfactants (BSs) by Halomonas zhanjiangensis. The GC-MS analysis of fatty acid profiles indicates that methyl 2-hyroxydodecanoate is the major component with 59.130%, followed by methyl 14-methylhexadecanoate (18.98%) and vaccenic acid (10.902%). The carbon sources and the composition of the culture medium were tested to optimize the lipase and biosurfactant productions. The strain Mn8 was able to produce one lipase with a molecular mass of 64 kDa. The optimum enzyme production was obtained at pH 5, pH 8, and 50 °C. The maximum BSs production obtained in the presence of LB auditioned with 1% sunflower oil. The FTIR analysis indicates that the BSs belong to the glucolipid type. The chemical composition of the BSs by GCMS shows that the major fatty acid is 2-Hendecanone C11 with 67.37%, followed by 2-Nonanone with 21.28%. The glycolipid possesses high bioemulsifying activities. In conclusion, based on the activity of the lipase at extreme conditions, associated with the bioemulsifying potential, we conclude that the novel strain Mn8 the development of industrial formulation.

KEYWORDS

Lipase; Halomonas zhanjiangensis; Glycolipid; Bioemulsifying activities

ARTICLE HISTORY

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Introduction

Extremophiles, microorganisms capable of surviving in extreme conditions due to their adaptive mechanisms, hold significant potential for biotechnological and industrial applications. Derived from extremophiles, hydrolytic enzymes, including microbial lipases (EC 3.11.3) extracted by fungal, yeast, and bacterial species, exhibit diverse functionalities. Bacterial lipolytic enzymes that belong to different classes, including carboxylesterases (EC 3.1.1.1), which hydrolysis small ester; lipases (EC 3.1.1.3), which hydrolysis long chain triglycerides and phospholipases [1-4]. Lipases have many industrial applications and benefits, such as foods, detergents, textiles, biodiesel, pharmaceuticals, and medical fields [5-7]. Industrial applications of enzymes presented between 5000 and 5500 million in 2016; lipase constitutes 10% of the global enzymes market [8].

Due to the stability of enzymes from extremophiles, in the presence of detergents, and organic solvents as well as at extreme pH, they exhibit a successful alternative to replace the currently insufficient commercialized enzymes [9]. Lipase enzymes from extremophiles can be used to design new enzyme properties, performing benefit activities in extreme conditions [10]. Microbial lipases are mostly extracellular; the extremophile ones are the most economical and stable. Moreover, their production was strictly related to culture medium composition, temperature, pH, and oxygen. Recently, lipase can enhanced the bioremediation process of the agricole wastes [11]. Some research highlighted the lipase production from halophilic bacteria such as *Halomonas sp*., Virgibacillus, Halobacillus, Chromohalobacter [10]. Various physicochemical parameters were optimized for maximum production. However, as per our knowledge, this is the first report of lipase and biosurfactant production and characterization by *Halomonas zhanjiangensis*.

Surfactants are important chemical molecules widely used in various industrial applications such as detergent, food, cosmetic, agricultural, pharmaceutical, textile, and paper [12,13].

However, the majority of these synthetic surfactants are chemically synthesized from petroleum derivated and are non-biodegradable in nature due to their persistence and eco-toxic nature [14]. Hence, biosurfactants can be the best alternative [15]. Therefore, in recent years, researchers have directed to biosurfactants (BSs) or biological surfactants produced by microorganisms. The BSs possess the same functions of face active but they are biodegradable and non-toxic. Based on the chemical structure, the BSs can be classified as glycolipids, polypeptides, lipoproteins, phospholipids, fatty acids, neutral lipids, particulate and polymeric surfactants [16]. Among these, glycolipids and lipopeptides produced by Bacillus and Pseudomonas species, are notable. Glycolipid, the most common type, consist of a

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hydrophobic moiety (long chain fatty acid) in combination with a hydrophilic moiety of carbohydrates. Besides, BSs show more interest than chemical surfactants due to their biodegradability, non-toxic nature, and enhanced effectiveness in extreme conditions (pH, temperature, and salinity) [13]. Optimizing the production of biosurfactants involves manipulating various factors, including culture medium, conditions, and mode of culture, may present an interest, due to the low product yields. This study aimed at the optimization of lipases and biosurfactants production and characterization by a new strain Mn8 of *Halomonas zhanjiangensis*.

Material and Methods

Bacterial isolation, 16S rDNA amplification, sequencing and phylogenetic analysis

The halophilic bacterium isolate strain Mn8 used in this work was isolated on Tryptic Soy Agar medium (TSA) medium, from the rhizosphere of Mesembryanthemum crystallinum located in South Tunisia, region Gabes. For that, the root tissues were washed with distilled water and disinfected with Sodium hypochlorite at 0.1%, then washed several times before left to dry at room temperature. Small pieces of disinfected root tissues were transferred aseptically to the appropriate bacterial medium for culture at 30 °C for 72 hr. A single bacterial colony is taken and transferred to new petri dishes, for purification.

The morphological and biochemical characterizations were undertaken according to the standard biochemical tests as recorded in Bergey's Manual of Systematic Bacteriology. The DNA of bacteria was extracted using standard protocol. The 16S rDNA was PCR amplified using a universal primer pair, Fd1 (5'AGAGTTTGATCCTGGCTCAG3') and S17 (5'GTTACCTTGTTACGACTT3'). The PCR reaction was performed in 25 µl volume containing 1X buffer, 0.2 µmol L-1 of each primer, 0.2mM dNTPs, 0.2 U Taq DNA polymerase (Sigma), and 25 ng of template DNA. The PCR conditions consisted of initial denaturation at 94 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 30s, an extension at 72 °C for 45s, and a final extension at 72 °C for 7 min [17]. The PCR products were cleaned and size selected, and the libraries eluted were sequenced on an Illumina MiSeq platform, Illumina [18].

The obtained ADNr16S is deposited on the GenBank, with the Access number of (OK326787). Blast pairwise global sequence alignments (EzTaxon database) [19], was used to align the sequence. The most closet strains related to strain Mn8 were aligned by Clustal W [20]. The neighbor-joining method was used to construct the phylogenetic tree and evaluated by bootstrap analysis of 1000 data sets using MEGAX [21]. The culture was maintained on nutrient agar slants at 4 °C until use.

Fatty acid analysis from cell membrane of strain Mn8

After culture on Luria Bertani medium (LB) at 30 °C, the bacterial colonies were harvested from the agar surface in a sterile tube. The whole fatty acids from the cell membranes of strain Mn8 were first saponified and methylated, and then the extraction was done according to the method of MIDI, Microbial identification system (version 6.0) as described by Sasser [22]. The analysis of the fatty acid methyl esters was done by GC (689N, network GC system Hewlett, Packard), and the TSBA6 database of the identification system was used as described by Chen et al. [23].

Optimization of lipase production

Effect of medium culture composition

Four compositions of culture medium were used to detect the lipase production by the strain Mn8, as follow: M1 containing: Tryptone Glucose Yeast Extract medium (TGY) and 1% of oil olive), M2: composed of TGY and 1% Tween 80, containing TGY and 0.01% Glucose and 1% oil olive, and M4: TGY added with 0.5% tween 80 and 0.5% oil olive.

Effect of carbon sources

To evaluate the effect of carbon sources on lipase production by the strain Mn8, TGY medium was supplemented separately with carbon sources used at 1% (oil olive, tween 80, sunflower oil, and corn oil). Bacteria were grown on a shaker at 30 °C for 48 hr. The bacterial culture was harvested by centrifugation for 8000rpm /10min at 4 °C. The cell free supernatant was recuperated for further use [24].

Lipase activity detection

Qualitative detection of lipase

The qualitative lipase detection was undertaken by using the Spot method: Each surface medium was inoculated by one bacterial colony, the petri dishes were incubated for 24 hr to 72 hr at 30 °C, and the observation of a halo clear around the bacterial colony indicates the lipolytic activity [25].

Quantitative detection of lipase

Titration method is used to determine the lipase activity as previously described [26]. This method performs the quantity of the lipase production in the sample (cell free supernatant), expressed in U/mL. In brief, the mix reaction prepared was incubated at 50 °C for 1 hr, under agitation at 160 rpm/min. The reaction was stopped by the addition of 2 mL of acetone/ethanol (1:1 v/v). Then, 100µL of phenolphthalein at 0.1% were added to the volume of cell free supernatant (500 µL). The titration was done by using 0.5N of NaOH normalized with oxalic acid at 0.01N. One Unit of lipase activity was expressed as the quantity of enzyme that produces 1 µmoL/min/mL of Fatty acid and calculated by equation (Eq1)

Lipase activity (Units/mL) = Nx (Vs-Vc) x 1000/ min. -------(Eq 1)

Where, N= normality of NaOH.
Vs: volume of NaOH solution in sample culture supernatant reaction Vc: 500µL of sterile medium culture without any bacterial strain (control).

Temperature, pH and salinity effects on lipase

To check the effect of pH and temperature on lipase activity, the mix reaction was prepared in various pH buffers (3; 4; 5; 6; 7; 8; 9; 10 and 11), the enzymatic reaction was maintained for 1 hr at 50 °C. For determining the optimal temperature, the activity was maintained at pH 5 (optimum pH), ranging temperature from 30 °C to 60 °C [27], and to evaluate the salinity on lipase production, NaCl concentrations tested ranged from 0% to 20% (W/V), in the presence of tween 80 as carbon sources at 1%.

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Enzyme concentration and molecular weight determination

The strain Mn8 of *Halomonas zhanjiangensis* was grown in 300mL of the optimal medium M2. After incubation for 48 hr at 28 °C, the bacterial culture was centrifuged at 8000 rpm/20min and the culture supernatant was concentrated by the Acetone precipitation. For that, we mix the cell-free supernatant with acetone at (1:2 v/v). The mix was kept at -20 °C for at least 3 hr. Then, the pellet containing the enzyme was recuperated by centrifugation at 12000 rpm for 30 min. The molecular weight was analyzed by denaturing SDS-PAGE (12%) and the zymography in NATIVE-PAGE (10%), based on the method detailed by Anil et al. [27].

Biosurfactants detection

Oil spreading test

The oil spreading test was done by using 500µL of the vegetable oil deposed on the water surface in petri dishes, then after 500µL of the free cell supernatant was gently put on the oil surface. The diameter of the clearing zone was assessed and measured in mm. Triton X100 was used as a positive control and olive oil as a negative one [17,28].

Emulsification index (E24)

To detect the bioemulsifier action of the cell-free supernatant of the strain Mn8, we have used the emulsification index (E24) method of [29]. Brief the index E24 was estimated by mixing v/v of cell-free supernatant and vegetable oil, in a test tube and mixed with vortex for about 2 min. The emulsification index E24 was determined after 24 hr, it was calculated from the ratio of the height (h) of the emulsion layer to the total height of liquid (mm) [17].

Carbon source effect on BSs production

Tween 80, olive oil, corn oil, and sunflower were added separately to the LB medium, and the bacterial culture was incubated at 30 °C for 72 hr with agitation at 130rpm. After incubation, the culture was centrifuged at 8000 rpm/min at 4 °C for 10 min to recuperate the cell free supernatant and used to measure the OD at 400 nm as described by Essghaier et al. [17].

Biosurfactants characterization

The FTIR spectrum analysis of BSs was as previously reported by Essghaier et al. [17]. By using an FTIR Bruker Equinox 55 spectrometer (Bruker Co, Ettlingen, Germany), using an attenuated total reflection (ATR) accessory with a diamond ATR crystal [30].

Fatty acid analysis of BSs by GC-MS

Analysis of the biosurfactants structure was performed based the same method of Sasser [22], using an Agilent 6890N, network GC system gas (Agilent Technologies, Hewlett Packard), and the TSBA6 database of the identification system was used as previously described by Guerrand [8].

Antimicrobial potential of BSs and lipase

To test the antimicrobial potential of BSs, the strain Mn8 was cultivated on 150mL of LB medium supplemented with the optimum carbon sources (sunflower oil at 1%), for 72 hr at 30 °C. The bacterial culture was centrifuged at 8000 rpm at 4 °C for 30 min to recuperate the cell free supernatant. This latter was acidified by 1N HCl until pH2 and incubated overnight at 4C. To recuperate the pellet of BSs, the solution was centrifuged at 8000 rpm for 30 min at 4 °C [31]. The BSs were dissolved into ethyl acetate. 40µL of BSs solution (2 mg/mL) and lipases were tested by using the agar well diffusion method as described by [17,32]. The anti-biofilm effect of both samples was also detected by using the surface of 96 well flat bottomed microliter plates [17,33].

Statistical Analysis

The Student Newman-Keuls (SNK) tests were used at a threshold of 5% for multiple comparisons of means. All values were expressed as means ± SEM. Comparisons between groups were performed using the generalized linear model (GLM). Means with the same letter are not significantly different.

Results

Taxonomic characterization of bacterial strain

In the present study, a new bacterial isolate Mn8 was obtained after purification of a bacterial colony that appeared around the disinfected pieces of root from the halophyte plant, on growth medium TSB after 72 hr at 30 °C. The bacterial strain was gram negative, catalase-positive oxidase-negative, short rod shape, and motile. The 16SDNA sequence was deposited in GenBank with the accession number of OQ213927. The 16Sgene sequence of the strain Mn8, shows highest similarity of 99.02% with *Halomonas zhanjiangensis* (JSM078169) (Figure 1).

Fatty acid composition of the cell membrane

Results of the fatty acid composition from the cell membrane of strain Mn8 of Halomonas was shown in Table 1 and the chromatogram profiles of fatty acid in Figure 2. The major components were, respectively, the methyl.2-hyroxydodecanoate with an amount of 59.130%, followed by methyl.14-methylhexadecanoate with 18.98% and Vaccenic acid with 10.902%.

The fatty acids profile from the cell membrane of strain Mn 8 of *Halomonas zhanjiangensis* was distinguishable from other Halomonas species by owing C12:0 2OH (59.13%), C13:0 (0.079%), iC16:0 (0.211%) and iC17:0 (18.985%) (Table 2).

Lipase activity

Among the four composition, mediums tested in the present work (M1, M2, M3 and M4), the spot assay method indicates that the strain Mn8 was able to produce lipase activity on three culture mediums M2, M3, and M4. The lipolytic zone diameters varied according to the tested mediums: 8.5 (M2), 13 (M3) and 15 mm (M4). Unlike, no lipase activity was observed in the presence of medium M1 (Figure 3A). On the other hand, well diffusion method shows that the M4 medium composition was the most inducer of lipase production by strain Mm8 giving the superior lipolytic zone of about 39 mm (Figure 3B). Titration AG method indicates that the M2 medium was the most inducer of the lipolytic activity with 5U/mL as compared to 3.4U/mL, in the presence of medium culture M4 (Figure 3C).

In order to optimize the lipolytic activity by strain Mn8, TGY medium composition was used and supplemented with Tween 80, Corn oil, or Sunflower oil, at 1% separately. After incubation for 72 hr, the cell free supernatant was used to determine quantitatively the lipase production by titration assay. The tween 80, as a single Carbone source, presents superior lipase activity production with 21.25U/mL, as compared to other tested Carbone sources (sunflower oil and Corn oil) (Table 3).

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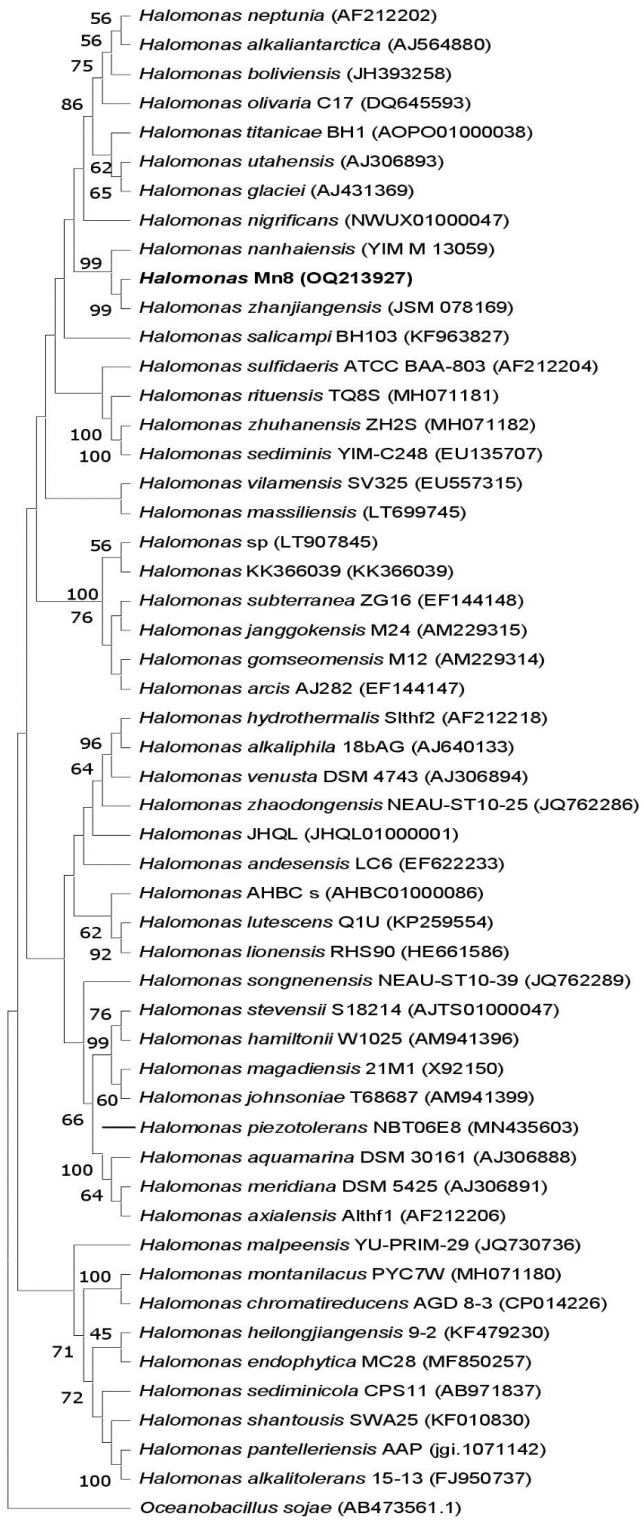

Figure 1. Phylogenetic tree highlighting the position of *Halomonas sp.* Mn8 (OQ213927) among related taxa within the Halomonas genus based on 16S rDNA gene sequences. Evolutionary distances were calculated using the Neighbor-Joining method. The evolutionary distances were computed using the Jukes-Cantor method using MEGA 11. Numbers on the nodes present % bootstrap values based on 500 replicates. The 16S rDNA gene sequence of *Oceanobacillus sojae* (AB473561.1) was arbitrarily chosen as the outgroup to define the root of the tree.

The Different subscript letters indicate significant difference at $p< 0.05$. **M2**: composed of Tryptone Glucose Yeast Extract medium (TGY) TGY and 1% Tween 80, **M3**: containing TGY and 0.01% Glucose and 1% oil olive, and **M4**: TGY added with 0.5% tween 80 and 0.5% oil olive.

The maximum lipase production was obtained at 50 °C with 5.83U/mL followed by 3.75U/mL at 60 °C (Figure 4A). For pH value effect, the highest production was observed at pH5 with 5.83U/mL, followed by pH3 with 4.17U/mL and 4U/mL at pH 8 (Figure 4B). The lipase enzyme show activity in the absence and in the presence of high salinity (NaCl 12.5%), this result proved its halotolerant nature (Figure 4C).

SDS PAGE results indicate that the strain Mn8 of *Halomonas zhanjianzensis* able to produce a single lipase with approximately a molecular weight of 64kDa (Figure 5).

Biosurfactants activity

The cell free supernatant of strain Mn8 shows a superior emulsification activity (Figure 6A), as compared to triton X100. In addition, the oil spreading test also indicates high spreading surface oil obtained by the application of the cell free supernatant (Figure 6B). These findings highlight the biosurfactants properties of the strain Mn8. Table 4 shows the results of the carbon sources effect on BSs production. The maximum BSs activity was observed in the presence of sunflower at 1% as a single carbon source compared to other Carbone sources (Table 4).

FTIR analysis of BSs

The absorbance bands at 3330 cm-1 and 3010 cm-1 were attributed to stretching OH group and NH, respectively, The peak at 2923 and 2854, corresponds to CH3 and CH2 stretching, respectively. The band at 1743 was assigned to stretching C=O in ester groups. Band at 1641cm-1 COO str, band at 1459 CH, and at 1004 correspond to C=C. The presence of these functional groups indicates the chemical structure of the Biosurfactants belonging to group glucolipids (Figure 7).

Fatty acid composition of biosurfactants

Table 5 shows the fatty acid profiles in the Biosurfactants produced by the strain Mn8 of *Halomonas zhanjiangensis*. The composition of biosurfactants by GC-MS indicates that the major lipid is 2-Hendecanone C11, with 67.37% (Table 5).

Antimicrobial activity of lipase and biosurfactants

Results of the antimicrobial assay show that both lipase and biosurfactants gave antimicrobial inhibitory effects on tested bacterial and fungal strains by inhibition of growth culture and biofilm production. The maximum inhibitory action was observed by both molecules on Fusarium. The percentage of growth inhibition of Fusarium was 65.62 (Figure 8A). Fusarium biofilm was most sensitive to the application of BSs and lipase, with a percentage of biofilm inhibition of about 82.12% and 78.57%, respectively (Figure 8B). Only BSs show a high inhibitory effect on the growth of Klebsiella with 72%.

Discussion

Halophilic bacteria present remarkable sources of different molecules having biotechnological interest, such as hydrolase, biocatalysers. In the current study, we aimed to describe a novel bacterial strain producing lipase and biosurfactants. In literature, several halophilic bacterial lipases were reported,

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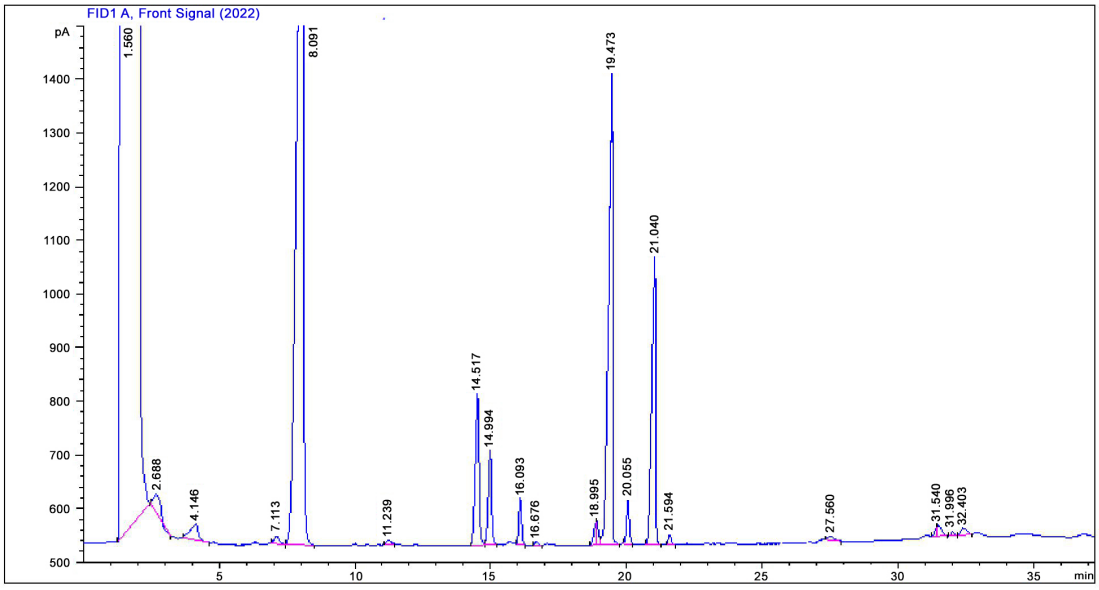

Figure 2. Chromatogram profiles of Fatty acid cell membrane composition of strain Mn8 of *Halomonas zhanjiangensis*.

Table 1. Fatty acid composition from cell membrane of strain Mn8 of *Halomonas zhanjiangensis.*

	Formula	RT	%
Me.2-hyroxydecanoate	2-OHC10:0	4.146	1.473
Lauric acid	C12:0	6.332	0.210
Me.2-hyroxydodecanoate	2-OHC12:0	8.091	**59.130**
Tridacycl acid	C13:0	10.030	0.079
Me.3-hyroxytetradecanoate	3-OHC14:0	11.239	0.363
pentadecyclic acid	C15:0	12.237	0.045
Me.13-methyltetradecanoate	iC15:0	13.946	0.019
Me.12-methyltetradecanoate	a-C15:0	14.517	6.032
Me.14-methylpentadecanoate	iC16:0	15.697	0.211
Me.2-hyroxyhexadecanoate	2-OHC16:0	16.676	0.128
Palmitoleic acid	C16:1n-9	17.266	0.052
Magaric acid	C17:0	18.891	0.658
Me.14-methylhexadecanoate	iC17:0	19.473	**18.985**
Stearic acid	C18:0	20.055	1.389
Vaccenic acid	C18:1n-11	21.040	**10.902**
Nonadecyclic acid	C19:0	21.594	0.274
Me.cis-9,10-methyleneoctadecanoate	C19:0d	23.192	0.018
Arachidic acid	C20:0	23.380	0.033

RT: Retention time, Me: Methyl

Table 2. Comparative fatty acids profile from cell membrane composition of strain Mn8 of *Halomonas zhanjiangensis* and other related *Halomonas species*.

Strains	Fatty acid composition expressed in %							
	C12	C13	C14	C15	C16	C17	C18	C19
Halomonas zhanjiangensis Strain Mn8 [This study]	C12:0 2OH (59.13)	C13:0 (0.079)	C14:0 3OH (0.363)	C15:0 (0.045)	iC16:0 (0.211)	C17:0 (0.658); iC17:0 (18.985)	C18:0 (1.389)	C19:0 (0.274)
Halomonas campisalis [43]	0	0	C14:0 (0 to 1)	C15:0 (0 to 0.3)	C16:0 (13.9 to 32.2)	C17:0 (0-0.2)	C18:0 (0.2 to 0.7)	CyC19:0 0 (1.3 to 34.5)

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Halomonas shantousis [42]	C12:0 (3.6); C12:0 3OH (1.6)	0	C14:0 (0.6)	C16:0 (19.1)	C18;0 (1.3)	
Halomonas xianhensis [42]	C12:0 (2.2); C12:0; 3OH(2.8)	0	C14:0 (0.2)	C16:0 (22.4)	C18;0 (1.3)	
Halomonas muralis [42]	C12:0 (5.6); C12:0; 3OH(9.2)	0	C14:0 (1.9)	C16:0 (23.4)	C18:0 (0.5)	
Halomonas lutea [42]	C12:0 (5.6); C12:0; 3OH(4.8)	0	C14:0 (0.9)	C16:0 (15.9)	C18:0 (2.6)	
Halomonas socia [56]				C16:0 (16.05)	C18:1 (37.59)	Cyc C19:0; (18.29)
Halomonas titanicae [57]				C16:0 (18.4)	C18:1 (36.3)	C19:0 (17.9); CycC19:0 (17.9)

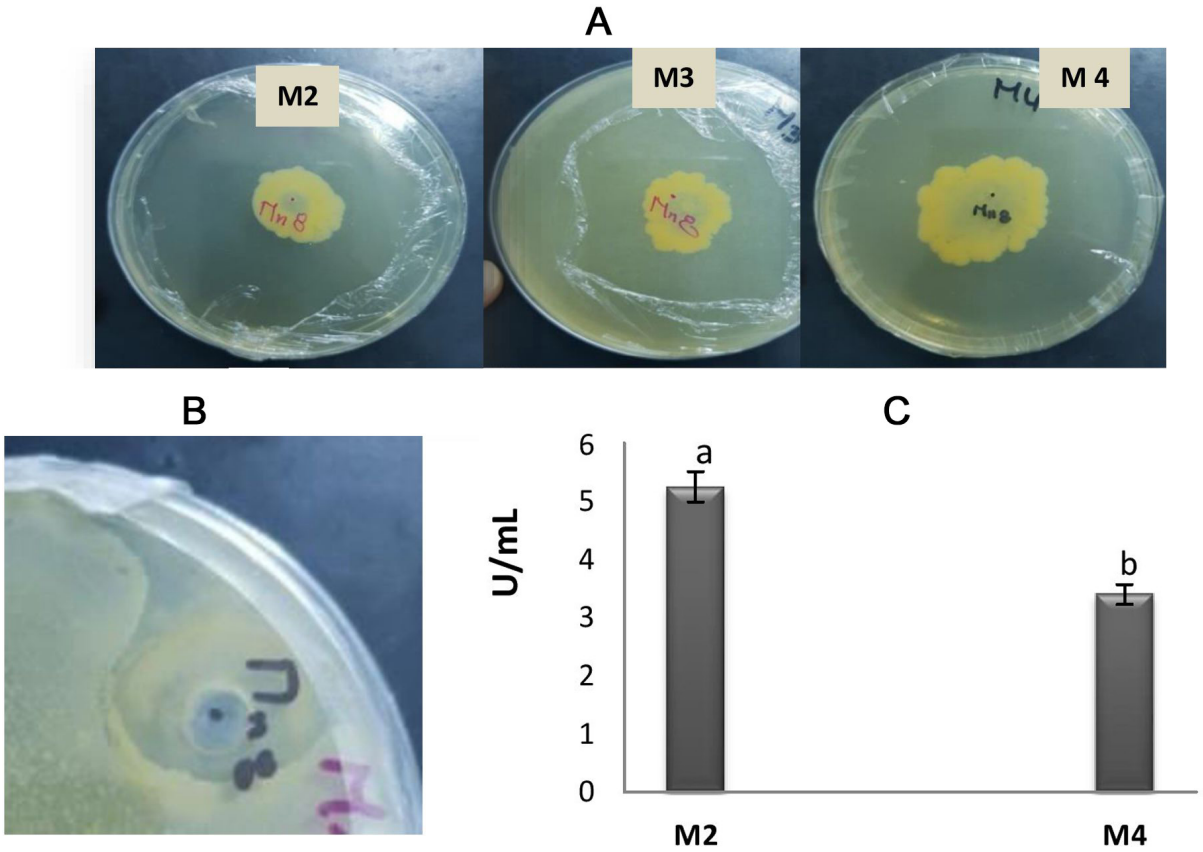

Figure 3. Composition medium effect on lipase activity: (A) Lipase activity detection by spot assay on various culture mediums M2, M3 and M4, (B) Lipolytic activity detection of strain Mn8 by well diffusion method and (C) Lipolytic activity assay by titration method expressed in U/mL.

Table 3. Carbone sources effect on lipase production.

Carbon sources	Lipase titration (U/mL)
Sunflower oil	2.08
Tween 80	**21.25**
Corn oil	18.75

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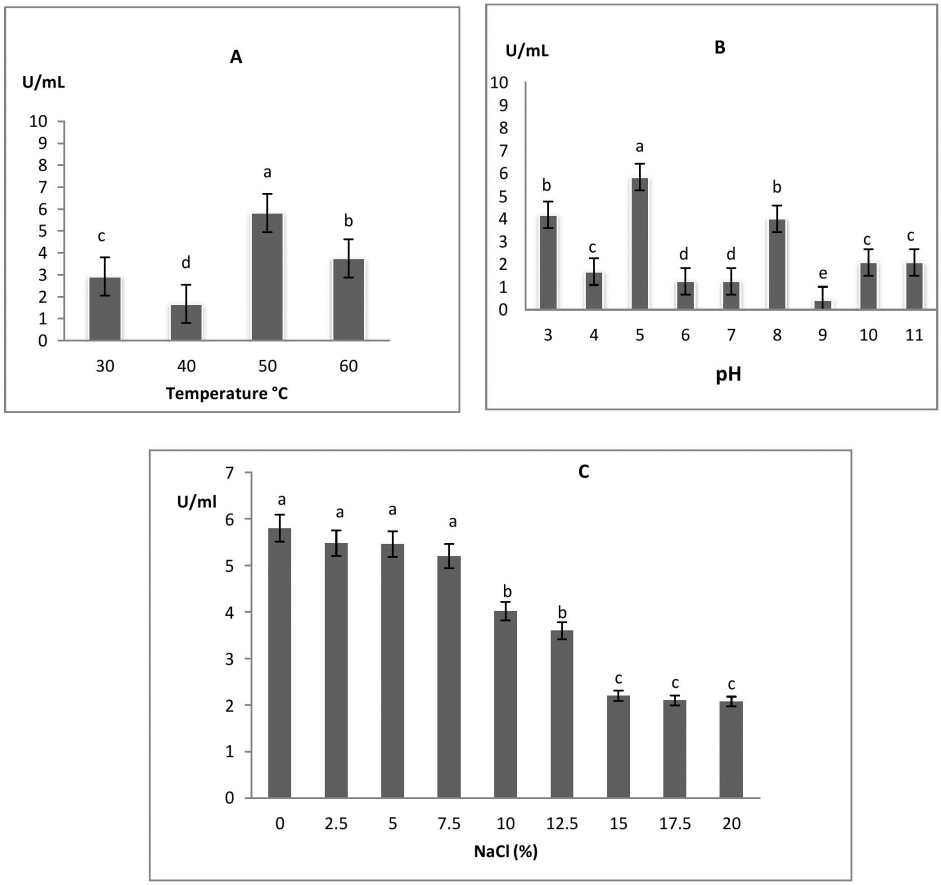

Figure 4. . The optimum condition of biosurfactants production: (A) effect of temperature, (B) effect of pH and (C) effect of salinity. Values expressed in U/mL. Means with different letters present significant difference with $p < 0.05$.

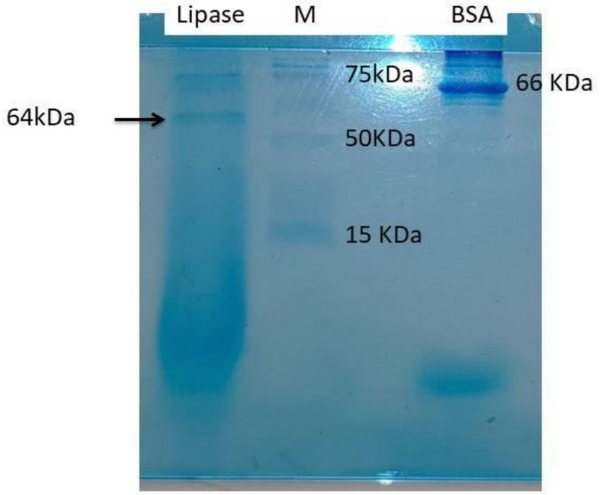

Figure 5. SDS-PAGE results of the lipase produced by strain Mn8 of *Halomonas zhanjiangensis*.

for example, *Halomonas elongata* and *Halomonas sp*. [34, 35]. Despite the promising potential of halophilic enzymes, they are still not commercially available due to knowledge gaps, such as optimum medium compositions and conditions of culture. Several works have been focused on the isolation, identification, and characterization of different strains of producers of promising enzymes [36,37]. Moreover, Halomonas were known for their ecological diversity and large industrial applications based on their relevant biomolecules [38].

This study aimed at the optimization and characterization of lipase and biosurfactant production by *Halomonas zhanjiangensis* species that was first described in 2009. This species was able to produce esterase (C4), esterase lipase (C8) and lipase (C14) [39]. The present results report a novel endophytic isolate able to produce lipase induced by tween 80. According to the work of Balaji et al. [40], who also describe that lipase produced by Bacillus was induced by the presence of 6% olive oil with 0.9% tween 80 in the culture medium.

Most of the lipase enzymes used in laboratory or industrial fermentations are substrate tolerant related to the microbial origins. Microbial lipases are mostly extracellular, and their production is greatly influenced by the culture medium. Recently, lipases play a crucial role in medicine and industry. Lipase is an important component of detergent powders [41]. The present findings describe the first report of lipase production from *Halomonas zhanjiangensis*, and compared to the previous study of [39], we confirm that the fatty acid composition in cell membrane varied with Halomonas sp. Here, the most dominant fatty acid in the cell membrane of strain Mn8 of *H. zhanjiansensis* was C12:0 2OH, unlike other Halomonas strains species studied by Chen et al. [39], who mentioned that this fatty acid does not exceed 1.9%. The value of C17:0 is 0.658% and nearest to those presented by the strain DSM 21076T of *H. zhanjiansensis* (with the amount of 0.7%) [39].

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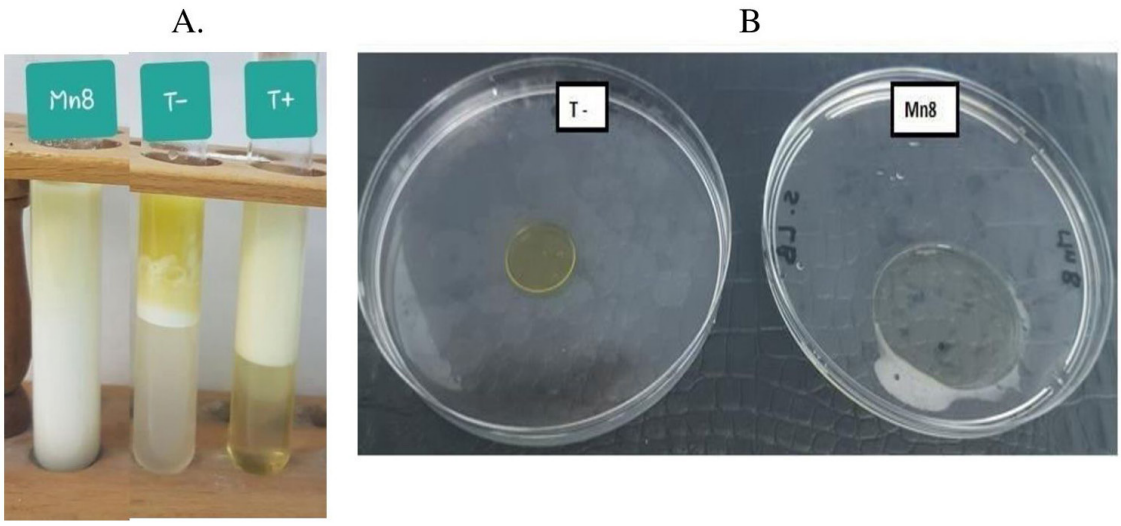

Figure 6. Evaluation of biosurfactants activity (A) Biosurfactants detection by the Emulsification index and (B) The oil spreading test obtained by the application of the cell free supernatant of the strain Mn8 of *Halomonas zhanjiangensis.*

Table 4. Luria Bertani (LB) medium and carbon sources effect on the production of biosurfactants.

Carbon sources	Corn oil	Sunflower oil	Tween 80	Palm oil
Biosurfactants (UA/mL)	0.0152 ± 0	0.0199 ± 0	0.013 ± 0	0.0146 ± 0

Values are mean ± standard deviation of three triplicates

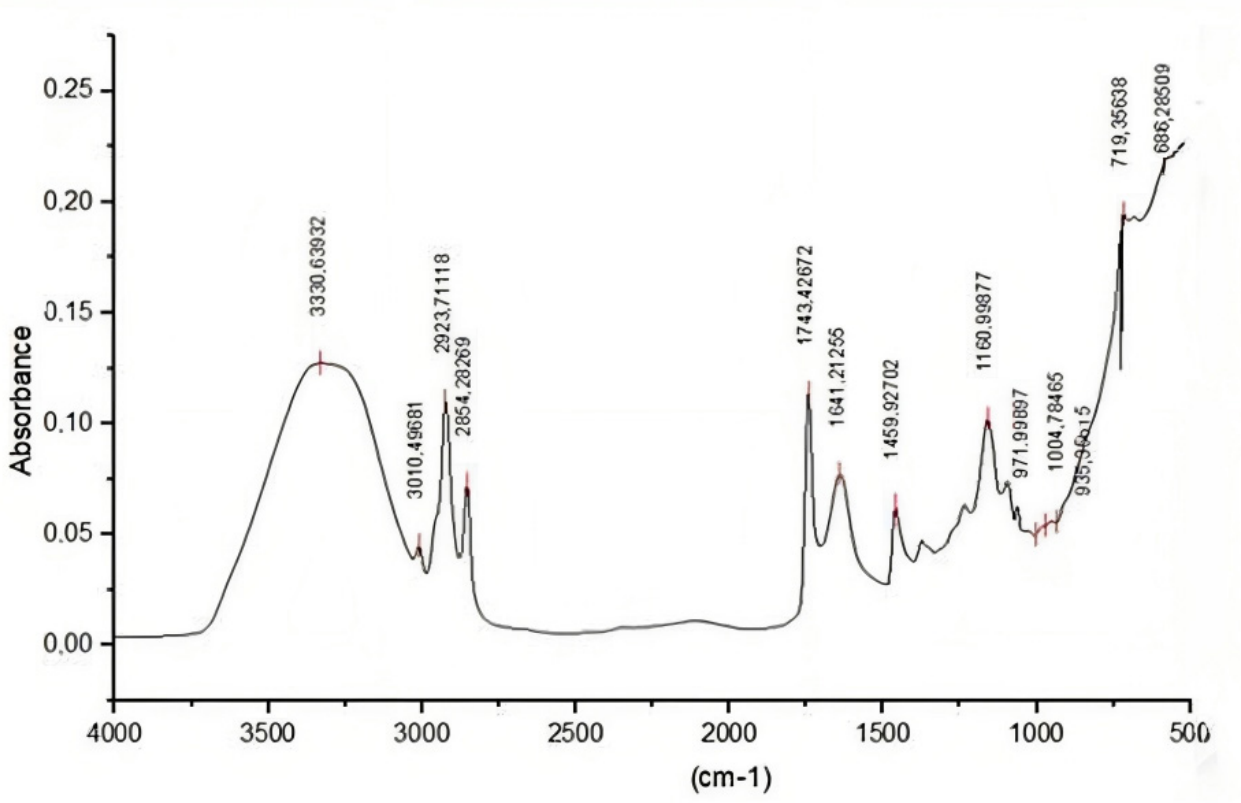

Figure7. FTIR analysis of the biosurfactants produced by the strain Mn8 of *Halomonas zhanjiangensis*.

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Table 5. Fatty acid composition of the biosurfactants produced by strain Mn8 of *Halomonas zhanjiangensis*.

Compound	Carbon Number	RT	KI	Average percentage (%)
Limonen	C10	11.13	11,45,72,414	1.86%
2-Nonanone	C9	13.14	12,71,08,696	21.28%
2-decanone	C10	16.13	10,45,77,061	2.90%
2-Hendecanone	C11	19	13,83,95,062	**67.37%**
Others				
Cyclohexanone	C6	17.63	--	4.34%
cis-4-Hydroxymethyl- 2-methyl-1,3-dioxolane	C5	20.98	--	2.25%
Total of identified compound				100%

RT, Retention Index, and **KI**, Kováts Index calculated on HP-5MS capillary column (30m x 0.25 mm film, thickness 0.25 microns). Predominant and major components are marked in bold

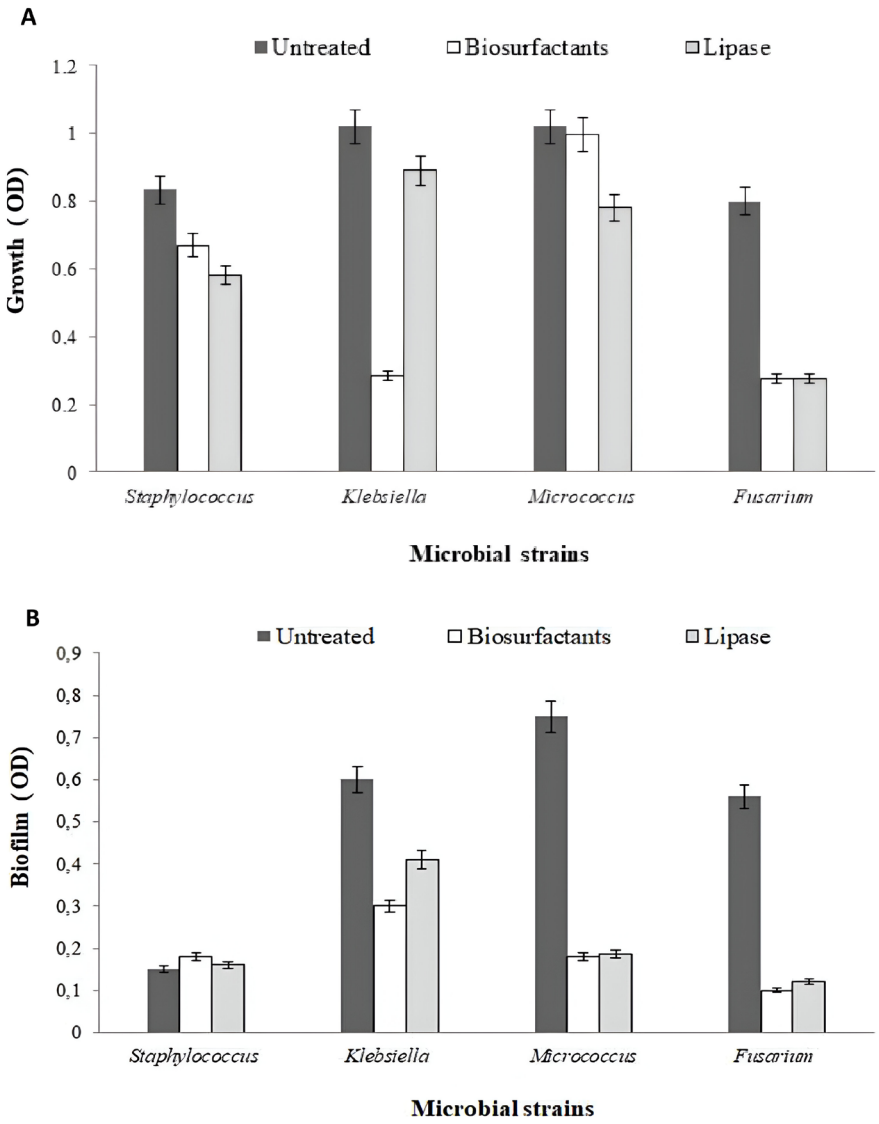

Figure 8. Antimicrobial effect of lipase and glycolipids produced by strain Mn8 of *Halomonas zhanjiangensis* against some clinical strains on growth culture (A) and on Biofilm formation (B).

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These findings highlighted that the fatty acid cell membrane composition of strain Mn8 of *Halomonas zhanjiangensis* was distinguishable from other *Halomonas sp.* by owing C12:0 2OH (59.13%), C13:0 (0.079%), iC16:0 (0.211%) and iC17:0 (18.985%). A similar rate of C15:0 (0.045%) was observed with *Halomonas campisalis*. An identical percentage of C18:0 (1.389) was obtained among other *Halomonas species*, such as *H. campisalis, H. shanpusis, H. xianhensis* [42, 43] (Table 2). The optimal temperature of the lipase of *Halomonas zhanjiangensis* strain Mn8 is found to be 50°C. Several studies mentioned thermo-resistant lipases produced by bacterial strains, for example, *Bacillus sonorensis*, with an optimal temperature of 60 °C [44]. Furthermore, the work of Khaimssa et al., showed that the lipase produced by *Halomonas sp*. exhibited the maximum activity at 60 °C and pH values ranging from 7 to 10 [18].

The present results of the pH effect on lipase activity indicate that the pH optimum was 5. Similar results were described by Kumar et al., using *Bacillus pumilus* with a pH optimum ranging from pH 4 to pH 7 [45]. In contrast, *Chromohalobacter sp*. showed optimum pH 8 and pH 9 optimum for lipase produced by *Bacillus sonorensis* [34]. The SDS PAGE results of lipase from *Halomonas zhanjiangensis* strain Mn8 show a single band with an apparent molecular weight of 64kDa. In fact, the molecular masses of lipase were related to the bacterial strains. For example, the molecular weight of lipases produced by *Chromobacter sp*. was 44 KDa [46], by Bacillus strain A30-1 and *Halomonas sp*. were 65 and 66 kDa, respectively [47]. The lipase from *Pseudomonas sp.* 7323 has an apparent molecular weight of 64kDa [48]. Previous works described lipases of molecular weight in the range of 60 to 65kDa produced by other microorganisms, for example [49].

The current work mentioned that the strain Mn8 of *H. zhanjiangensis* exhibits biosurfactant activity revealed by the emulsification essay and the test drop collapse method. In literature, *Halomonas meridiana* was able to convert olive oil into biosurfactants [50]. Gutiérrez et al., described two marine Halomonas producers of glycoproteins as higher stable emulsifiers at extreme conditions [51]. On the other hand, many reports mentioned the production of lipase/biosurfactants by the same bacterial strain, which present benefits in lipid industrial applications. In this context, several works described glucolipid biosurfactants produced by Halomonas sp. stable at extreme conditions [52].

Additionally, biosurfactants from *Halomonas zhanjiangensis* was identified as glycolipids by the FTIR spectrum, in literature, the glycolipids were the most BSs studied. The GC-MS analysis shows that the major lipid of the Glycolipids of *H. zhanjiangensis* strain Mn8, is 2-Hendecanone C1, these lipids are well-known as a pesticide. This finding was confirmed by the strong antifungal activity against Fusarium. Furthermore, our data were in accordance with those obtained by Anila et al. [52], who also used GC-MS and FTIR to the characterization of the biosurfactants produced by halophilic bacteria, indicating the presence of fatty acid and glucolipid derivate with strong antimicrobial activity. These data revealed the potential use of halophilic BSs as effective antimicrobial agents in the formulation of antimicrobial drugs [52].

In addition, Dhasyan et al. [53], describe a glucolipid produced by *Halomonas sp.* exhibiting strong antimicrobial action against bacterial and fungal pathogens. Moreover, the present work, show the highest emulsifying activities of biosurfactants produced by strain Mn8 as compared to the positive control Triton X. These finding exhibits the promising behavior of these biomolecules in the development of the industrial lipid process. Moreover, Mnif et al. showed that glucolipid biosurfactants have highly diverse structures and are able to reduce the surface and interfacial tension and the surface and interface [54]. Donio et al. [55], reported BSs from *Halomonas sp.* with strong antimicrobial action.

Conclusions

In conclusion, this work highlighted, that a novel strain Mn8 of *Halomonas zhanjiangensis* appears to be a promising candidate for the development of natural emulsifiers as compared to commercial ones. The thermos-resistant lipase described in this work and their activity at extreme pH of 5 and 8 enhance their use in industrial processes applying extreme conditions and their application in detergent formulations.

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