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Organic pollutants biodegradation by halophile-isolated bacteria in saline conditions

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ABSTRACT

The present study surveys halophilic bacteria that were isolated from various Tunisian biotopes such as saline, lagoon, fish farm, for their acclimation capacity in fish processing wastewater at 10% NaCl. Hydrolase activities such as protease and lipase were screened. Strains S6 and S10, isolated from fish farm in a lagoon area and showed a protease activity of 503 and 328 U/L in liquid media, were selected. These strains were identified as *Salinivibrio* genus and more precisely to the species *Salinivibrio siamensis* and *Salinivibrio costicola subsp. alcaliphilus* using 16S RNA gene sequencing analysis. Bioremediation of fish processing wastewater was investigated by the use of the selected strains, the species *Salinivibrio siamensis* and *Salinivibrio costicola subsp. alcaliphilus* in comparison with urban activated sludge. The experiments were performed at different salt concentrations (10–20%). As salinity increased, the TOC removal efficiency was decreasing, and then increasing progressively through time. The Total Organic carbon (TOC) removal decreased from 22% to 1% when the salinity increased from 100 $g \cdot L^{-1}$ to 200 $g \cdot L^{-1}$ NaCl after 24 h of incubation and reached 57% and 7%, respectively after 120 h of incubation. Meanwhile, no TOC removal was obtained with the use of urban activated sludge.

Keywords: Bioremediation; Biodegradation; Saline wastewater; Halophile bacteria

1. Introduction

In the context of the preservation of environmental ecosystems, management of saline effluent is an issue with increasing importance. These liquid wastes, which belong to the category of special industrial wastes, up nearly 5% of global effluents [1] are produced by various chemical, pharmaceutical, food and textile industries. High salinity and organic load characterize all these wastewater sources. To avoid oxygen depletion and eutrophication, removing organic pollutants before discharging wastewater into a water is essential.

The presence of salt in wastewater inhibits the proper operation of conventional biological processes [2,3]. Indeed, without acclimatization, conventional bacteria suffer a shock in the contact with the salt, leading to a violent contraction of the cytoplasm, and quickly emptied of their water to compensate for the osmotic shock, which leads most often to plasmolysis [4–6]. For this, the treatment can be done effectively by enrichment with halobacteria and halotolerant bacteria [7–9]. The application of halophilic microbial consortia or even enrichments from non-saline ecosystems, that were adapted to saline conditions, reduces the effect of salt stress on bacterial metabolism [10,11]. Many halophilic bacteria isolated from various saline environments such as *Staphylococcus* sp., *Pseudomonas aeruginosa* and *Halomonas* sp. have been reported to be effective in the carbon removal [12–14].

Moreover, moderately halophilic bacteria, which have adapted to live in a wide range of NaCl (1–25%), constitute an interesting group of microorganisms that could be used as a source of enzymes production [15]. Such enzymes are expected to show optimal activities in extreme conditions and in contrast with their extremely halophilic counterparts can be active in the absence of salts.

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Thus, the possibility to have a wide variety of moderate halophiles producing extremozymes will be of invaluable help for biotechnological applications [16]. Recently, a considerable attention has been given to the enzymes produced by moderately halophilic microorganisms and their biotechnological potentials [17–19].

The main purpose of this research is to isolate moderately halophilic bacteria from various areas in Tunisia such as saline, lagoon and fish farm, which are able to grow in saline wastewater particularly at the disposal of organic matter. Lipase and protease hydrolytic activities are screened. Moreover, the advantages of selected bacteria strains in bioremediation process were studied.

2. Methods

2.1. Sample collection and mixture

The water and sediment samples used in this work were collected from different saline environments in Tunisia cities (Bizerte, Sousse, Monastir, Kerkennah and Sfax) (Table 1). Theses samples were collected at the surface and at a depth of 0.3 m within each site in sterile plastic containers and conserves at 4°C until the use.

A sample was prepared by mixing 150 mL of water and 50 g of sediment from each site under two hours of agitation. Then, the prepared suspension was added with the proportion of 20% (*v*/*v*) to an industrial saline wastewater with a concentration of 100 g·L^{-1} NaCl. In total 7 different samples mixtures were tested (*M*1, *M*2, *M*3, *M*4, *M*5, *M*6, and *M*7). *M*7 sample was taken from urban wastewater plant; in order to compare the different samples to conventional bacteria. The inoculated saline wastewater (ISW1, ISW2, ISW3, ISW4, ISW5, ISW6, and ISW7) were incubated in oxitop bottles at 34°C for 5 d. Non-inoculated saline wastewater sample under the same conditions has served as a control (C) to monitor autochthonous bacteria.

2.2. Saline wastewater

Different samples of saline wastewater were collected from cooking unit of a conservation tuna industry, located in the city of Sfax. Fish processing wastewater samples were stored in cans, transported to the laboratory and stored at 4°C until use.

2.3. Analytical methods

The characterization of sample mixtures from different biotopes was performed. The pH and the salinity were determined using a Swiss multi-parameter analyzer Consort *C*831. Aliquots (100 µl) of 10^{-1} and 10^{-4} dilutions of sample mixture were plated onto Agar medium with 10% NaCl, in order to determine colony-forming unit (CFU).

The TOC in fish processing wastewater was quantified by the use of a TOC Analyzer multi-N/C-1000 (Shimadzu) as described in APHA [20].

2.4. Evolution of BOD inoculated saline wastewater

The respiration of biomass into inoculated saline wastewater medium was evaluated by the respirometric test for 5 d. The oxitop (WTW) method was found to be reliable and accurate to the respirometric test method [21]. The method is based on the measurement of pressure decrease due to the oxygen consumption by microorganisms oxidizing the organic matter [22]. In practice, the oxitop bottles are filled with a measured volume of fish processing wastewater. The microorganisms degrade organic substances using the gaseous oxygen trapped in the closed bottle. The carbon dioxide formed by this process is absorbed in sodium hydroxide pellets.

The pressure changes are measured by a manometer and converted to oxygen consumption by the device to estimate the BOD value which defined as the amount of dissolved oxygen needed by aerobic biological organisms to break down organic material present in inocultad saline wastewater over a specific time period. Respirometric data will be typically used comparatively, that is, in a direct comparison between oxygen uptakes from two different inoculums and from a test sample and a control [23].

2.5. Isolation and growth of halotolerant bacteria

Halotolerant microorganisms were selected from oxitop bottles showing the highest value of oxygen uptakes. Enrichment cultures and isolation procedures to recover

Table 1
Characterization of samples mixtures

Samples mixtures	Isolation site	CFU/L	pH	Salinity (g·L^{-1})
*M*1	Fish farm in Hergla lagoon of Sousse 35° 59 '23" N 10° 30' 10 " E	6 10^7	7.48	105
*M*2	Thyna Saline of Sfax 34° 39' N 010° 43'E	1.7 10^7	7.74	106
*M*3	Saline of Monastir 35° 45'N 010° 46'E	2 10^8	8.06	102
*M*4	Lagoon of Kerkennah Island 34° 47' 25 "N 011° 14' 54" E	3 10^7	7.88	89
*M*5	Bizerte Lagoon 37° 14' N 9° 46' E	4 10^6	7.91	30
*M*6	Wastewater discharge close to sea water of Monastir	2 10^8	8.07	34
*M*7	Activated Sludge of urban wastewater treatment plant of Sfax	6 10^4	7.69	3.75

halotolerant bacteria were performed in medium containing (per liter): NaCl, 90 g; $MgCl_2 \cdot 6H_2O$, 4 g; $MgSO_4 \cdot 7H_2O$, 6 g; KCl, 1 g; $CaCl_2 \cdot 2H_2O$, 0.5 g; NaBr, 0.2 g; $NaHCO_3$, 0.1 g; yeast extract, 5 g; tryptone, 8 g; and glucose, 1 g according to the protocol of Hedi et al. [24]. The pH was adjusted to the value of 7 with the use of 10 M NaOH before autoclaving. Enrichment cultures were subcultured several times under the same conditions. Strains were inoculated in 100 mL of medium in a total volume of 250 mL Erlenmeyer flasks were incubated in a rotatory shaker under agitation at 150 rpm. The adequate temperature chosen for growth was 34°C. Aliquots (100 µL) of 10^{-1} and 10^{-4} dilutions were plated onto Agar medium at salt concentration of 100 $g \cdot L^{-1}$ NaCl. Different colonies were picked and restreaked several times (three times at least) to obtain pure cultures**.** Strains purity was analyzed for 47 isolated halotolerant bacteria using a phase contrast microscope OLYMPUS BX 50.

Growth tests concerned only halotolerant bacteria able to produce different hydrolases. They were performed in flask cultures, under agitation of 150 rpm and a temperature of 34°C. All experiments were performed in duplicate with an inoculum size of 3% (v/v), which had been sub-cultured at least once under the same conditions. Cell growth was monitored by measuring the optical density (OD) at 600 nm (UV 1800, Shimadzu, Japan) [25].

2.6. Screening of isolates for extracellular hydrolase

Considering the importance of organic matter and lipids within the fish processing wastewater, the selection of bacteria strains was focused on the production of extracellular hydrolases such as protease and lipase. In order to detect these enzymes production, different enzymatic agar plates assays were performed with 10% NaCl, as described below.

2.6.1. Screening for lipase activity

For the detection of isolate clones exhibiting lipolytic activity, two types of indicator plates were employed. Lipase activity was detected on Tween Agar medium containing tween 20 (1%, vol/vol) [26] and on the fluorescent dye rhodamine B (0.001%, wt/vol) [27]. Orange fluorescent halos around lipase-positive strains could be seen when these plates were exposed to UV light of 254 nm. Whereas lipolytic activity was detected on tween Agar plates by precipitation around the lipase producing microorganisms. The method is based on the precipitation as the calcium salt of the fatty acids released by hydrolysis of tween. Liberated fatty acids bind with the calcium incorporated into the medium. The calcium complex is visible as insoluble crystals around the inoculation site.

2.6.2. Screening for protease activity

Isolates were spot inoculated on casein/skimmed milk Agar plates and incubated at 30°C. A clear zone of hydrolysis around the inoculation site appeared after 24 h of incubation showing a protease synthesis [28]. In another step, the best isolates strains were inoculated in 50 ml of protease specific medium and were incubated in a rotary shaker (180 rpm) for 3 d. The measurement of enzymatic activity was done at 24, 48, and 78 h of culture according to the method using casein as a substrate [29].

2.7. 16S rRNA genes sequencing and phylogenetic analysis

Genomic DNA of two selected bacteria strains were extracted by the hexadecyl trimethyl ammonium bromide (CTAB) protocol [30]. PCR amplification of 16S rRNA genes was done by using a primer set specific to bacteria: fD1 (5'-AGAGTTTGATCCTGGCTCAG-3') and rD1 (5'-AAGGAGGTGATCCAGCC-3') [31]. Amplification was carried out in a 50 µl reaction mixture containing 1.75 mM MgCl2, 0.2 mM each dNTP, 0.2 µM each primer, 50 ng DNA template and 1.25 U *Taq* DNA polymerase (Fermentas) with reaction buffer supplied by the manufacturer. PCR was started with initial denaturation for 2 min at 94°C. A total of 30 cycles, each including 30 s at 94°C, 45 s at 55°C and 1 min 45 s at 72°C, was followed by a final extension step of 10 min at 72°C. The PCR product was analyzed by gel electrophoresis and purified using an Ez-10 Spin Column DNA Gel Extraction Kit (Bio Basic Inc) according to manufacturer's instructions. Purified PCR products were sequenced using the BigDye® Terminator v3.1 Cycle Sequencing kit on the ABI PRISM 3100-Avant Genetic Analyser (Applied Biosystems). The resulting sequences were compared with those available in GenBank using the BLAST [32] and RDP II [33] online alignment tools. Multiple alignments were generated with the MUSCLE program [34] and phylogenetic trees were constructed with MEGA program version 7 [35] on the basis of evolutionary distances that were calculated by the Neighbor-Joining method [36] with Jukes cantor model. We performed bootstrap resampling analysis [37] for 100 replicates to estimate degrees of confidence in tree topologies. The 16*S* rDNA sequence was deposited in GeneBank under accession numbers: *S*6 (KY421119) and *S*10 (KY421120).

3. Results and discussion

3.1. Identification of the most tolerant inoculum

Table 1 shows the origins and the characterization of sample mixtures from the seven sites. The salinity and the pH of mixture sample varied between 3.75–106 $g \cdot L^{-1}$ and 7.48–8.07, respectively. The highest salinity was observed with *M*1, *M*2, and *M*3. Moreover, the CFU content at salinity 100 $g \cdot L^{-1}$ was varying from $6 \cdot 10^4$/L to $2 \cdot 10^8$/L with the isolation site sample. The highest value of CFU content was found with *M*3 and *M*6 isolated from saline and polluted seawater in Monastir city.

The studied saline wastewater, generated from fish processing industry, was characterized by about 100 $g \cdot L^{-1}$ salinity, 14 $g \cdot L^{-1}$ TOC and pH 6, respectively. The enrichment of fish processing wastewater by different inoculum indicates different evolutions of BOD. The respirometric data obtained through 5 days are summarized in Fig. 1. It was shown that oxygen uptakes from biomass in ISW fluctuated versus of inoculum isolation site. It was observed also that all studied biomass show an oxygen uptake higher than control.

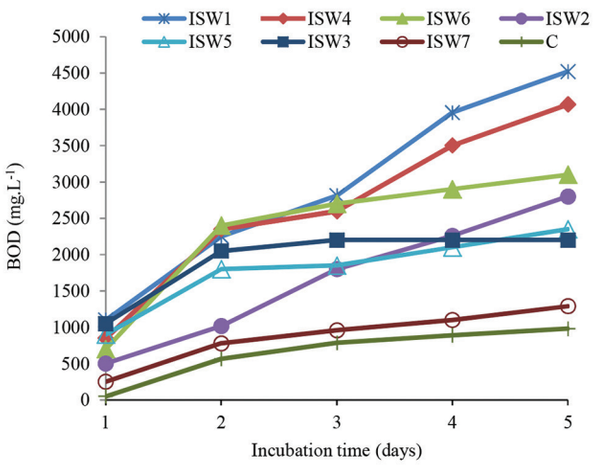


Fig. 1. Evolution of BOD inoculated saline wastewater (ISW1, ISW2, ISW3, ISW4, ISW5, ISW6 and ISW7).

These results may be due to the capacity of some bacteria to the acclimatization to extremophile conditions and to ensure the metabolic activity in the presence of 14 g·L^{-1} TOC and 10% salinity.

There are three major processes involved in the metabolism of a bacterium, growth and division, ingestion and respiration which are very highly coupled or meshed and no one can go faster than the other.

Fig. 1 reveals that the lowest evolution of BOD was observed with both control and inoculated saline wastewater by sample mixture *M*7, which was taken from an urban wastewater treatment plant. The BOD evolution was similar, and the values of this parameter were comparable. The adverse effects of salt on the microbial flora are known. The growth rate is reduced and accompanied by a fall in the rate of respiration. For inoculated saline wastewater (ISW1, ISW4, and ISW6), higher rates of oxygen uptake were observed in the presence of halotolerant bacteria. Samples mixtures *M*1 and *M*4, characterized by high salt and CFU content (Table 1), show a significant higher BOD after 4 and 5 days of incubation ranging from 4000 to 4500 mg O_2 L^{-1}. In the case of samples mixtures *M*2, *M*5, and *M*3, the BOD evolution was comparable and the measured BOD ranged from 2200 to 2800 mg O_2 L^{-1} after 5 days of incubation.

The conditions required for growth depend on the different species of bacteria. It is noticeable that the source of the inoculum is an important factor in the adaptation to salinity.

In this study, it can be confirmed that bacteria from saline natural environments were adapted rapidly to fish processing wastewater. For example, the highest measured BOD during investigation was observed with the sample mixture taken from fish farm at Hergla lagoon at 105 g·L^{-1} of salinity. Instead, sample mixture *M*6 provided from discharge urban wastewater in the sea started to highly grow immediately in fish processing wastewater and then stabilizes with time after two days of incubation. While, the lowest value was detected by the use of the inoculum coming from an urban wastewater plant treatment at 3.75 g·L^{-1} of salinity. The difference in rate growth may be the result of the difference in salinity and biodiversity in isolation sites. These observations suggest that the bacteria growth rate is a result of both genetic and environmental conditions.

The preliminary selection of sample mixture was based on the presence of bacteria able to grow at 100 g·L^{-1} NaCl in fish processing wastewater. Three oxitop bottles showing the highest value of oxygen uptakes were selected.

The fact of different salinity concentrations (1, 100, 150, and 200 g·L^{-1}) on BOD after 5 d of incubation was investigated in the presence of samples mixtures *M*4, *M*6, and *M*1. As can be seen in Fig. 2, at a salinity superior to 100 g·L^{-1}, the growth of bacteria decreased with the increase of salinity. However, between 1 and 100 g·L^{-1} of salinity, BOD did not differ notably especially in samples mixtures *M*6 and *M*1. After an increase in salinity of 200 g·L^{-1}, a great BOD decrease was observed. In fact, the measured value of all tested sample mixture didn't exceed 500 mg·L^{-1}. As previously revealed, the salinity of the isolation site is an important factor for adaptation to salinity variations. Interestingly, the lower salinity of 1 g·L^{-1} allows the highest BOD evolution rate suggesting the presence of moderately halotolerant bacteria.

Comparing the performance of different sample mixture, *M*1 shows the higher BOD after 5 d of incubation value at high salinity concentration. Whereas, at salt concentrations of 1 and 100 g·L$^{-1,}$ the sample mixture *M*4 shows a higher rate activity.

Then the preliminary selection resulted in 47 halotolerant bacteria strains isolated from oxitop bottles in the presence of *M*4, *M*6, and *M*1. These strains were conserved as pure bacteria.

3.2. Research of protease and lipase activities

The objective of the present investigation was to select bacterial strains, from the 47 isolated halotolerant bacteria indicated above, for their abilities to produce protease,

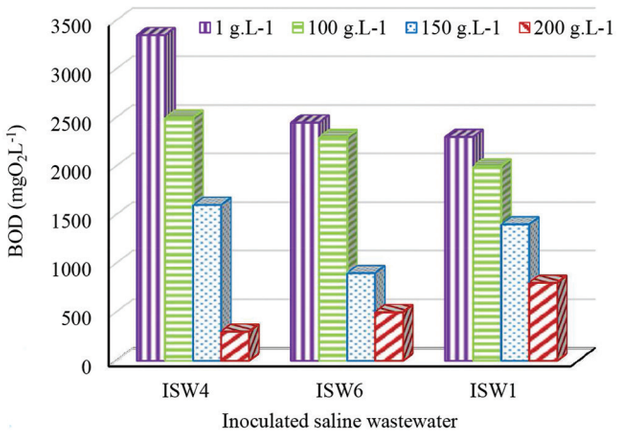


Fig. 2. Influence of different salinity concentrations on BOD evolution after 5 days of incubation.

lipase and tween during their growth on specific medium for enzymes production.

From the 47 isolated halotolerant bacteria on the screening media, 3.77% were lipase, 6.60% Tween and 17% protease producers (including the halo ≥ 2).

The inoculated bacterial strains, which produce high amount of extracellular protease (*S*1, *S*3, *S*5, *S*6, *S*10, *S*11, *S*13, *S*16, and *S*20), were selected and checked for quantitative protease tests (Table 2). It was observed that all the nine isolates secreted protease enzyme at varied levels. The maximum protease activities (503 U/ml, 377 U/ml, and 353 U/ml) were attained after 24; 48 and 72 h respectively by the isolate *S*6. It was found that the maximum production was occurred at the start of exponential phase. The strain *S*20 showed a decreased activity during time. However, the two strains (*S*10, *S*1) show maximum activities after 48 h with a respective picks of 328 and 206 U/ml. Also, the strain *S*5 shows a maximum production at the end of the exponential phase. This strain exhibits no enzyme activity after 24 hours. It starts to produce enzyme activity after 48 and 72 h (76 and 99 U/ml). The lowest extracellular enzyme activity was observed by the isolates *S*3 and *S*1 with enzyme activities (10 and 16 U/ml) respectively.

During this study, 4 strains (*S*1, *S*5, *S*6, and *S*10) have shown to exhibit both lipase and protease. Lipase and protease enzymes are highly commercialized owing to their several industrial applications.

3.3. Characterization of the isolated bacteria strains

3.3.1. Monitoring of growth

The chosen strains (*S*1, *S*5, *S*6, and *S*10) were inoculated into duplicate tubes in both saline wastewater and rich medium at a salinity concentration of 100 g·L^{-1}.

In saline wastewater (Fig. 3a), *S*6 strain showed a faster growth than *S*5, *S*1, and *S*10. In fact, the cell division started without lag time and markedly increased during inoculation. The value of OD reached 2 after 96 h of incubation. The growth of *S*6 and *S*10 strains seems similar. In contrast, the growth of *S*5 strain was the slower one. Effectively, limited growth was observed during its incubation. An increase in *S*10 strain cell number occurred rather similarly on 4 h. *S*1, *S*6, and *S*10 strains showed a similar growth curve between 24 and 72 h. The maximum OD at 600 nm of *S*6 and *S*10, strains corresponding to the beginning of the stationary phase were obtained at incubation time of 96 h, against 72 h for *S*1 and *S*5 strains. The duration of the lag phase and the shape of the curve during the acceleration phase depend on the physiological state of the cells in the inoculum, the fraction of viable cells in the inoculum, and the environmental conditions of the cultures.

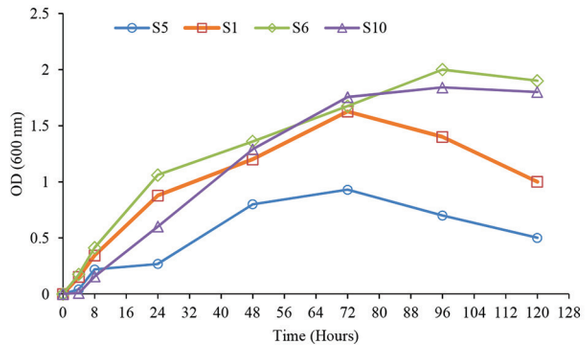
In the case of the use of rich medium for strains cultures (Fig. 3b), the growth curves obtained were comparable with those previously observed in salt effluent but some differences were detected. Effectively, all growth strains were higher and faster in rich medium and an immediate increase in cell number occurred with *S*1 and *S*6. On the other hand, the maximum OD at 600 nm corresponding to the beginning of the stationary phase was later.

Subsequently, the selection of the strains was based on their high capacity to grow and to produce combined enzymes at NaCl concentration of 100 g·L^{-1}. The strains *S*6 and *S*10 were used for further characterization.

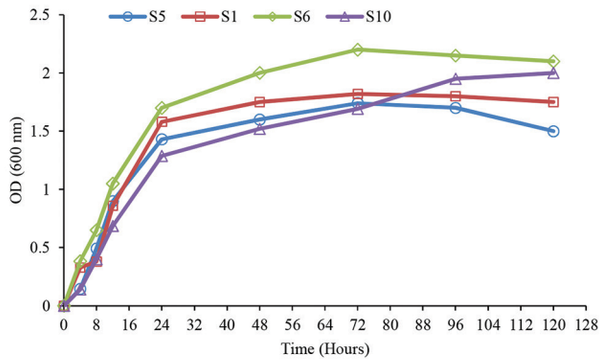
The following test was carried out to determine the tolerance of selected strains to salinity variations (Fig. 4). It was found, that for salinity superior to 150 g·L^{-1}, values of OD at 600 nm greatly decreased following the salinity increase

Table 2
Protease activity of selected moderately halophilic bacterial strains

Strains	24 H	48 H	72 H
*S*1	120	206	187
*S*3	–	7	10
*S*5	42	76	99
*S*6	503	377	353
*S*10	270	328	297
*S*11	–	18	52
*S*13	4	12	16
*S*16	13	34	47
*S*20	70	115	117

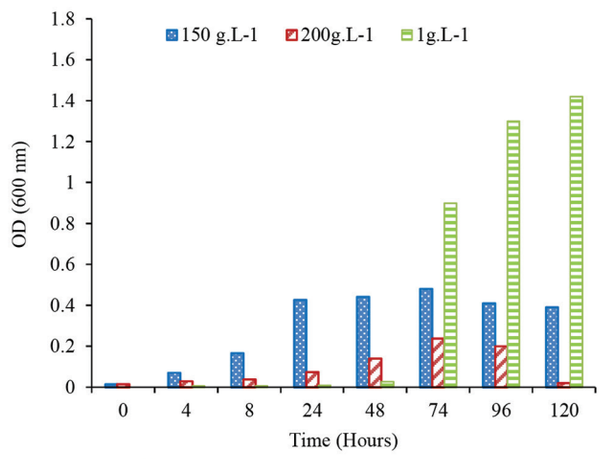


(a) Wastewater

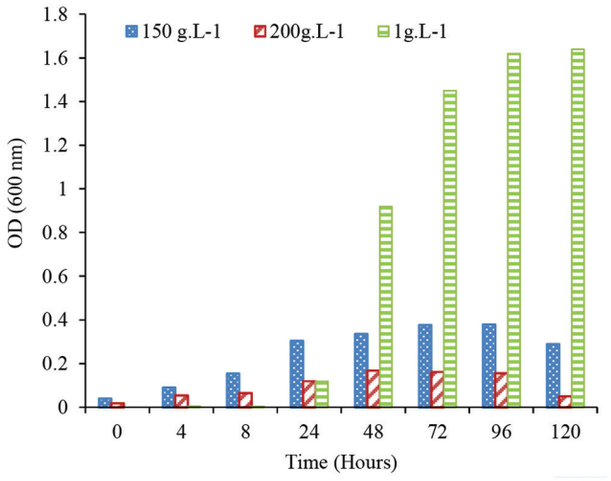


(b) Rich medium

Fig. 3. Evolution growth of selected strains bacteria at a salinity concentration of 100 g·L^{-1} on wastewater (a) and in a rich medium (b).



(a): S 10



(b) : S6

Fig. 4. Effect of different salinity concentrations on bacterial growth strains *S*10 (a) and *S*6 (b).

for both strains. For example the OD of *S*6 strain increases to a maximum of 2 at 100 g·L^{-1} after 78 h of incubation, while at salinity concentrations of 150 and 200 g·L^{-1}, the measured OD decreased markedly and reached a minimum value of 0.38 and 0.16 respectively. As it can be seen, at a salinity concentration of 200 g·L^{-1}, *S*6 and *S*10 cells did not survived for a long period under these conditions as indicated by the decrease of OD during incubation.

It is important to note that at high salinity, *S*6 and *S*10 strains growth was very slow. While, in the case of a low salinity, *S*10 strain growth started after 48 hours of incubation. A similar adaptation time was also observed with *S*6 strain. As it can be seen at 1 g·L^{-1}, the cell growth of *S*6 strain during the lag phase was five to ten times higher than at a salinity concentration of 200 g·L^{-1}.

Among strains, *S*10 growth was better than *S*6 at high concentrations salinity. Whereas at low salinity concentrations, a long period of adaptation and a slow growth of *S*10 strain were observed. Consequently, *S*6 and *S*10 strains can be classified as moderately halophilic bacteria, according to the classification proposed by Kushner [38].

3.4. Identification of the isolated microorganisms and phylogenetic analysis

Bacterial strains *S*6 and *S*10, isolated from the sample mixture *M*1 were identified by PCR. The sequences obtained were compared with the sequences available in the gene bank. Phylogenetic analysis shows that these isolates were belong to the *Gammaproteo bacteria phylum* and were members of the *Vibrionaceae* family (Fig. 5). The isolate *S*6 showed similarity of about 99.9% with the *Salinivibrio costicola* subsp. *alcaliphilus* DSM 16359^{T}, haloalkaliphilic aerobe and protease producer was recovered from a saltish spring with algal mat in the Campania Region (South Italy) [39] and the *Salinivibrio costicola* subsp. *costicola* ATCC 35508^{T} [40].

The isolate *S*10 shows similarity of about 98.8% with the *Salinivibrio siamensis* JCM 14472^{T}, moderately halophilic bacterium was isolated from fermented fish (plara) in Thailand [41]. Phylogenetic analysis based on comparison of 16*S* rRNA gene sequences demonstrated that these isolates were related to species of *Salinivibrio* genus.

Although, these work suggests that *Salinivibrio siamensis* and *Salinivibrio costicola* subsp. *alcaliphilus* can't grow in the absence of NaCl. Our result demonstrates that growth can occur, at a salinity concentration of 1 g·L^{-1}, after a period of adaptation ranging from 24 to 48 hours.

3.5. Biodegradation of saline effluent

In another step, the biodegradation of saline wastewater by two different microbial cultures namely: activated sludge culture from urban treatment plant, and pure *Salinivibrio* strains (100%) was conducted at different salt concentrations: 100, 150, and 200 g·L^{-1}. The salinity was adjusted with NaCl. The specific removal of TOC versus time is presented in Fig. 6.

As it can be seen during the startup period of incubation, the TOC removal efficiency was increased progressively with time at different salt concentrations. While, for 150 and 200 g·L^{-1}, the max removal TOC value was reached after 96 and 48 h of incubation respectively. It is obvious that, the pure *Salinivibrio* culture has the highest TOC removal value (57%) at 100 g·L^{-1} NaCl after 120 h of incubation. Increasing the NaCl concentration to 150 g·L^{-1} clearly reduced the removal rate of TOC to 24%. Further increase of NaCl concentration to 200 g·L^{-1} caused a decrease in the removal TOC which didn't exceed 7% during incubation. These results confirmed that the isolated bacterium was not sufficiently active enough at this salt concentration. On the other hand, the activated sludge resulted in the lowest efficiency for all tested concentrations; this may be attributed to the plasmolysis of the activated sludge organisms at high salt content. Our results were in agreement with the several

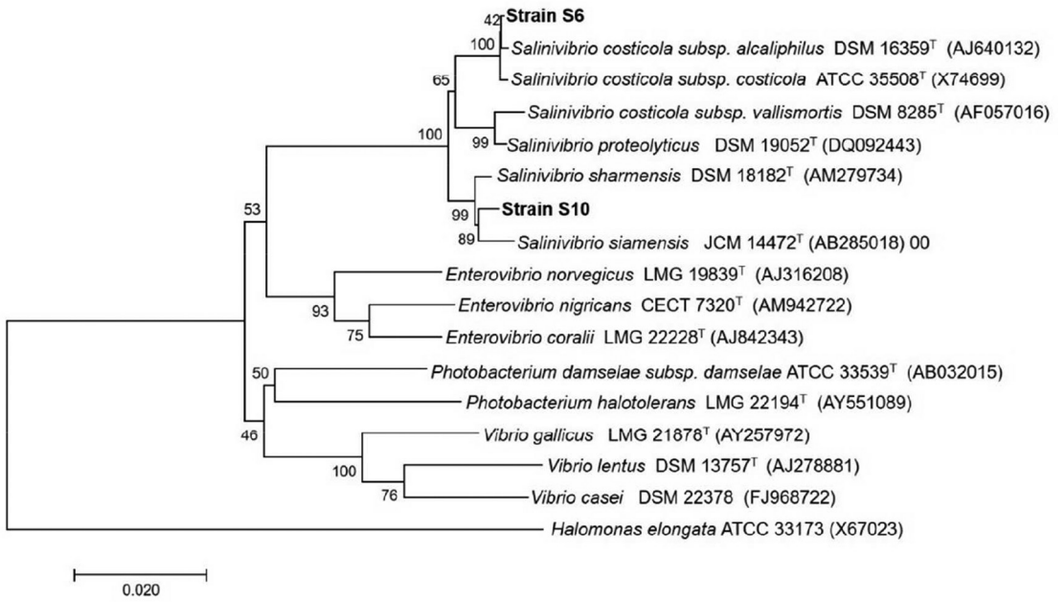


Fig. 5. Phylogenetic tree based on the sequence of 16S rDNA. Bootstrap values are given at the nodes. Scale bar represents the substitution percentage. *Halomonas elongata* was used as outgroup. GenBank accession numbers follow species name in parenthesis.

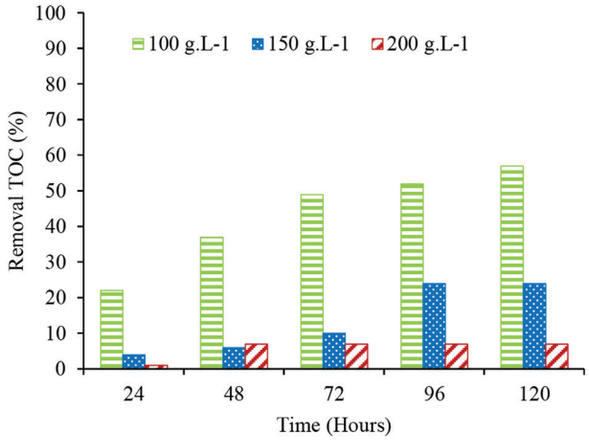


Fig. 6. Biodegradation of saline effluent by *Salinivibrio siamensis* and *Salinivibrio costicola* subsp. *alcaliphilus*.

reports which have pointed out the negative effects of salinity on treatment efficiency. To overcome this problem, salt-tolerant microorganisms should be used in order to alleviate salt inactivation effects. Our result is supported by a previous study of Jemli et al. [42] who reported that the use of halophilic species including *Halomonadaceae* and *Flavobacteriaceae* in MBR biological treatment process resulted in better treatment performances at salt contents above 5%. In this work, the selection of moderate halophylic bacteria (10%) was able to produce extracellular enzymes and to remove organic matter in fish processing wastewater highlights the *Vibrionaceae* family.

4. Conclusions

This study reports the efficiency of moderately isolating halophilic bacteria in wastewater bioremediation at 10% NaCl.

The experiments were performed with the fish processing wastewater containing different concentration of salt, varying between 10–20%. Exploration studies showed that the species *Salinivibrio siamensis* and *Salinivibrio costicola* subsp. *Alcaliphilus,* isolated from fish farm in a lagoon area in Sousse city, are able to remove 22% of TOC after 24 h of incubation at 10% NaCl. As the salt concentration increased from 15 to 20%, the rate of removal dropped from 4% to 1%. However, The TOC removal versus time was gradually increased and their values reached to 57%, 24%, and 7% at 120 h of culture, with salinity concentrations equal to 10, 15, and 20% respectively. This reflects significant effect of salinity larger than 20%. Whereas, the performance of activated sludge was significantly affected at 10% NaCl showing that high salinity destroys conventional bacteria.

In addition, the assessment of selected moderately bacteria shows that the species *Salinivibrio siamensis* and *Salinivibrio costicola* subsp. *alcaliphilus* are able to produce both lipase and protease at 10% NaCl.

Overall, studies on genera *Salinivibrio* must be reinvestigated, as they appear efficient to remove organic pollution in saline wastewater and produce enzymes that should have some applications in various food industries.

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