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Biotechnological properties and antifungal activity of lactic acid bacteria isolated from two marine algae Ulva lactuca and Sargassum muticum collected from the Moroccan coast of Sidi Bouzid-El Jadida

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Abstract:	Isolation of new strains of lactic acid bacteria (LAB) from extreme environments is currently in great demand given their biotechnological properties and their potential integration into food biotransformation and valorization processes. Thus, the purpose of this work was to isolate LAB strains from two marine algae (Ulva lactuca and Sargassum muticum) collected from the coast of Sidi Bouzid-El Jadida (Morocco). A total of 15 potential LAB strains were isolated on MRS agar on the two marine algae species. Microscopic and phenotypic identification of LAB isolates showed that all strains were Gram-positive, cocci-shaped and showed negative catalase reaction. Biochemical tests showed that all LAB isolates were homofermentative and 10 LAB strains (66.6%) showed lipolytic and/or proteolytic enzymatic activities. Out of the 15 LAB isolates, 8 strains exhibited a considerable antifungal activity against fungal strains of Aspergillus flavus and A. niger ranging from 60 to 90%. The ability of LAB isolates to grow on culture media formulated by the two algae powders showed a significant acidifying capacity and a decrease in pH from an initial value of 7.04 to 2.63 after growth for 10 days. These results suggest the potential use of LAB isolates in fermentation and biotechnological processes for the valorization of marine algae into value-added products for various applications.
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1
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5

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13 ## Abstract

14 Isolation of new strains of lactic acid bacteria (LAB) from extreme environments is currently
15 in great demand given their biotechnological properties and their potential integration into food
16 biotransformation and valorization processes. Thus, the purpose of this work was to isolate
17 LAB strains from two marine algae (*Ulva lactuca* and *Sargassum muticum*) collected from the
18 coast of *Sidi Bouzid*-El Jadida (Morocco). A total of 15 potential LAB strains were isolated on
19 MRS agar on the two marine algae species. Microscopic and phenotypic identification of LAB
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21 reaction. Biochemical tests showed that all LAB isolates were homofermentative and 10 LAB
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26 and a decrease in pH from an initial value of 7.04 to 2.63 after growth for 10 days. These results
27 suggest the potential use of LAB isolates in fermentation and biotechnological processes for
28 the valorization of marine algae into value-added products for various applications.

29 **Keywords:** Marine algae, *Ulva lactuca, Sargassum muticum,* Lactic acid bacteria, Antifungal,
30 Fermentation, Valorisation, Biotechnological process.

31
32 ## Introduction
33 Global revolution in food production, social and environmental sustainability, health, nutrition
34 and economic development has increased attention to marine resources, including the
35 production and use of algae. Compared to terrestrial plants, marine algae can grow faster and
36 year-round, resulting in multiple harvests per year and higher yields per unit area. Marine algae
37 are considered a rich resource of various bioactive compounds, such as antimicrobial,
38 antioxidant, antiproliferative and anticancer substances (Hmani et al. 2021). In addition, marine
39 algae production has many environmental advantages over land-based agriculture, such as not

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40 needing fertilizer, pesticides or fresh water. However, traditional techniques used to extract
41 bioactive compounds from marine algae such as acid hydrolysis using high temperature or using
42 chemical solvent extraction, are often costly and produce large amounts of wastes, some of
43 which could be complex and may be toxic or have negative impact on the environment. On the
44 other hand, heat-sensitive compounds, as bioactive compounds (phenolic compounds,
45 carotenoids, etc.) may be degraded during algae extraction process. As a result, green
46 technologies such as fermentation and enzyme treatment have emerged as alternatives to
47 traditional extraction techniques because they can obtain the desired compounds without
48 producing toxic residues or using solvents that may alter the bioactive compounds activity
49 (Pérez-Alva et al. 2022).
50 Fermentation of marine algae with LAB strains has recently been reported in the literature, and
51 the use of LAB strains for the fermentation of algal by-products to produce lactic acid is
52 considered a promising approach. Furthermore, marine algae are considered as important
53 natural resources of bioactive substances such as phenolic compounds, carotenoids, vitamins,
54 proteins, lipids and polysaccharides (Sumardianto Riyadi et al. 2021). Moreover, marine algae
55 are renewable substrates rich in fermentable sugars, with green and red algae (macro-algae)
56 generally containing 30-50% complex sugars that are fermentable after pretreatment.
57 Despite the abundance of scientific information in the literature on LAB isolated from terrestrial
58 matrices especially from cereal products, vegetables, plants, and fruits, etc., few studies are
59 available on LAB strains isolated from marine origin and their use for marine algae
60 fermentation and transformation. Indeed, lactic acid production from algae was described for
61 the first time in Japan but is still poorly studied. Currently, marine algae are mainly used in food
62 and feed, for the production of algae-based fertilizers, biofuels, and bioplastics. However, the
63 use of algae as culture medium for lactic acid production by homofermentative LAB is less
64 studied compared to conventional substrates, since homofermentative LAB produce 2 times
65 more lactic acid that the heterofermentative LAB (Hwang et al. 2012, Sudhakar and Dharani
66 2022). According to recent studies, many algal species could be used as good substrates for
67 LAB fermentation, and may open the possibility of obtaining many add value products to be
68 incorporated as new ingredients in human diets, animal feed or to be used as new organic soil
69 fertilizers, etc. (Uchida and Miyoshi 2013).
70 Morocco, a North African and Mediterranean country, with a total of 3500 km of coastline, is
71 considered as a country with the longest coastline on the African continent. Moroccan coasts
72 are particularly rich in marine algae and constitute a reserve of species with economic, social
73 and ecological potential. Previous studies have reported the isolation of several LAB species
74 from different matrices as example of raw camel milk (Khedid et al. 2009), poultry waste
75 manure (El-Jalil et al. 2008) and sardines (Ndaw et al. 2008). Thus, this study aimed to isolate,
76 for the first time, new strains of LAB from two abundant marine algae *Ulva lactuca* and
77 *Sargassum muticum* collected from the Moroccan coast of *Sidi Bouzid* at El Jadida city, as well
78 as, to evaluate their multifunctional properties (biotechnological and antifungal) for their
79 potential application in various biotechnological processes.
80

81 **Material and Methods**

82

83 **Algae sampling and preparation**

84 Two species of abundant marine algae (*Ulva lactuca* and *Sargassum muticum*) were collected
85 on the coast of *Sidi Bouzid* in El Jadida city (Morocco) during the period April-June 2022. After

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86 washing with tap water and distilled water for the removal of salts, algae samples were dried at
87 room temperature (25°C), then ground with a Blender laboratory, and finally the ground
88 material was then sieved to obtain a fine algae powder and stored at 4°C until analysis.
89
90 **Isolation and characterization of LAB**
91 One (1 g) of each algae powder sample was mixed with 10 mL of MRS broth (De Man, Rogosa
92 and Sharpe, Biokar, France) and incubated at 30°C for 24h under aerobic conditions. LAB
93 strains isolation was carried-out on MRS agar supplemented with bromocresol purple (0.002
94 %) as a color indicator and yellow colonies were selected and purified (Shobharani et al. 2012).
95 Potential LAB isolates were examined macroscopically based on the morphology of colonies,
96 microscope observation and catalase reaction (Prastujati et al. 2022). Gram-positive LAB
97 isolates tested as catalase negative were stored on MRS slant at 4°C for further biochemical
98 characterization.
99
100 **Growth at different temperature values**
101 LAB isolates were inoculated in sterile MRS broth tubes and incubated at different temperature
102 values (30°C, 37°C and 45°C) for 48 h to distinguish mesophilic LAB strains from thermophilic
103 LAB strains (Belkacem et al. 2009).
104
105 **CO_2 and exopolysaccharides (EPS) production**
106 The homo- or heterofermentative properties of LAB isolates were studied based on their ability,
107 or not, to produce CO_2 on MRS semi-agar enriched with sugar (5% glucose) after incubation at
108 30°C for 48 h under aerobiosis. For EPS production, LAB isolates were streaked onto MRS
109 agar medium supplemented with 5% of glucose and incubated aerobically at 30°C for 48 h.
110 EPS production was considered positive if colonies showed mucoid or viscous shapes on the
111 agar medium (Khalil et al. 2018).
112
113 **Lipolytic and proteolytic activity**
114 Proteolytic activity of the LAB isolates was assessed by depositing 20 µL of LAB cultures on
115 sterile Whatman paper disks, previously placed on the surface of MRS agar supplemented with
116 10% skimmed milk. Proteolytic activity was evaluated as a clear zone around the discs after
117 incubation at 30° C for 24 h under aerobiosis, while lipolytic activity was tested by inoculating
118 LAB isolates on agar spot (MRS supplemented with 1% of tributyrin). After incubation at 30°
119 C for 72 h under aerobic conditions, positive lipolytic activity was elucidated as an opaque zone
120 around the disc due to the formation of fatty acids and esters released by calcium.
121
122 **Antifungal activity**
123 The antifungal activity of LAB isolates was tested against the growth of fungal strains of *A.*
124 *flavus* and *A. niger* (fungal collection of BIOMARE Laboratory, UCD University, El Jadida).
125 Mycelium growth inhibition by the cell-free supernatants (CFS) of LAB strains was determined
126 on Dichloran Rose-Bengal Chloramphenicol (DRBC) Agar (Biokar, France). Plates containing
127 DRBC medium, added at 10% (v/v) with sterile CFS of each LAB strain, were inoculated in
128 the center with disc (5 mm) of *A. flavus* and *A. niger*. After aerobic incubation at 25°C for 5
129 days, the diameters of fungal colonies were measured. A negative control was used by adding
130 MRS broth to DRBC medium (Wang et al. 2002).
131

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132 **LAB application for algae fermentation**
133
134 **Screening of LAB growth on algae media**
135 In order to test the growth of LAB isolates of algae-based media, two culture media were
136 separately prepared using all MRS components, except glucose that was replaced with algae
137 powder (*S. muticum* or *U. lactuca*) as carbon sources. Since LAB strains are demanding in terms
138 of nutritional substances, the first objective of this study was to check if LAB isolates are
139 capable of using algae-carbohydrate. For this, LAB strains were inoculated on the algae media
140 (*S. muticum* culture medium or *U. lactuca* culture medium) and incubated at 30°C for 48 h
141 under aerobic conditions, as previously reported by Shobharani et al. (2012).
142
143 **Algae pre-treatment and fermentation assay**
144 Algae fermentation assays with LAB strains were carried out by preparing a hydrolysate from
145 the two studied algae species. Thus, 2 g of each algae powder were mixed with 100 mL of
146 distilled water and added to 1% of H_2SO_4 (Sudhakar and Dharani 2022). The mixture was
147 sterilized at 121°C for 20 min to obtain a juice extract from each algae species. The pre-treated
148 algal extracts potentially rich in polysaccharides were then filtered and supplemented with the
149 following nutrients (g/L): yeast extract (5.5), peptone (12.5), K_2HPO_4 (0.25), KH_2PO_4 (0.25),
150 CH_3COONa (10), $MgSO_4$ (0.1), $MnSO_4$ (0.05 mg), $FeSO_4$ (0.05 mg) and the pH was adjusted
151 to 7. Two algae extract media (AEM) were prepared and sterilized at 121 °C for 15 min. Later,
152 LAB strains isolated from each algal species were grown on the AEM medium prepared by the
153 respective algae powder. Indeed, 50 mL each prepared AEM were inoculated with a preculture
154 of each LAB strain at 10% (v/v), and incubated at 30°C for 10 days under aerobiosis. The pH
155 evolution, as an indicator of LAB growth was followed during the fermentation assay of each
156 AEM. This step is required for the selection of the most growing and acidifying isolates on
157 AEM. Performing strains will be selected to be used as starters for bulk algae fermentation.
158
159 **Statistical analysis**
160 All experiments in this study were performed in triplicate (n = 3). The Student t test was used
161 to confirm the significant difference between the two means of inhibition activity of LAB
162 isolated from the algae studied against the two fungal strains tested. The difference was
163 significant at $p<0.05$. The digital tool to perform this statistical analysis is the Excel 2013
164 software. Concerning the analysis by the principal component analysis (PCA) method and LAB
165 strains clustering, we used the free software Past 4.
166
167 **Results and discussion**
168
169 **LAB isolation**
170 This first step consisted of a phenotypic identification of cultivable LAB strains isolated from
171 the two algae powders. Purification of isolates on the MRS culture medium resulted in fifteen
172 (15) pure strains. It should be highlighted that all studied strains have cocci forms and there
173 were no lactobacilli-form among the isolates. Furthermore, all strains were Gram positive and
174 catalase negative as main characteristics. These traits suggest their probable membership in
175 lactic acid bacteria group.
176
177 **Biochemical properties of LAB isolates**

178 Results of the growth at different temperature values showed that out of the 15 strains tested, 8
179 strains (S1, S6, S3, S5, S7, U2, U3, U5) were able to grow at 45°C. LAB growth at this
180 temperature is manifested by the presence of a bacterial cloud (ring) at the bottom of the tubes
181 when compared to the control. According to Boudjema et al. (2009), LAB are known for their
182 good lactic acid production (9.06 g/L) at temperature values of 42-45°C, compared to other
183 temperature growth values. On the other hand, all tested LAB isolates belong to the
184 homofermenters group. Given that isolated strains are mesophilic and others are thermophilic,
185 we can suggest that mesophilic bacteria could belong to the *Streptobacterium* group, while
186 thermophilic bacteria belong to *Thermobacterium* group (Ruiz Rodríguez et al. 2019).
187
188 **Biotechnological properties of LAB**
189 Studying the biotechnological properties of LAB is essential to characterize the isolates for their
190 potential use in future laboratory and large-scale pilot applications. Properties of LAB isolated
191 from the two studied marine algae species are summarized in table 1.
192 Regarding EPS production, all LAB strains (except S5 and S10) were positive. These results
193 showed that algae powders are rich in LAB, from which EPS-producing strains can be isolated.
194 EPS production by LAB strains varies from strain to strain and is considered as one of the most
195 sought after and desired biotechnological properties since this property plays a critical role on
196 the texture of fermented products. Some LAB strains, belonging to genera *Leuconostoc,*
197 *Weissella* and *Pediococcus,* are able to synthesize and excrete EPS from sucrose by
198 extracellular glucansucrases or fructansucrases (İspirli et al. 2020). It was reported that EPS
199 production by LAB is considered as one of the most desired biotechnological properties since
200 EPS play a crucial role on the texture of fermented matrices (EL Boujamaai et al. 2023). Our
201 results are similar to those that already reported the production of EPS by LAB strains such as
202 *Enterococcus faecium, Limosilactobacillus fermentum, Lacticaseibacillus casei, Leuconostoc*
203 *mesenteroides and Streptococcus thermophilus*, etc. (Zannini et al. 2016; Lynch et al. 2018).
204 Differences between LAB strains in EPS production suggest that EPS amounts is a strain-
205 dependent characteristic, influenced by the bacterial strain and growth phase, including the
206 environmental conditions of the culture medium. These conditions include carbon source,
207 nitrogen and physicochemical conditions for bacterial growth, especially temperature, pH,
208 oxygen levels, etc. (Khalil et al. 2018).
209 Regarding enzyme production by LAB isolates, the lipolytic activity showed that out of 15 LAB
210 strains, 11 isolates (73.3%) have significant lipolytic activity in the presence of 1% tributyrin.
211 It is well known that LAB are generally considered to be weakly lipolytic, as compared to other
212 groups of microorganisms. It should be highlighted that algae contain varied forms of lipids
213 owing to their extreme habitat diversity, especially n-6 and n-3 polyunsaturated fatty acids
214 (PUFAs), glycolipids, and phospholipids and sterols (Kumari et al. 2013). These lipids are
215 known with high nutritional value and with important bioactive properties (Domingues and
216 Calado 2022). Thus, the presence of a significant lipolytic activity in LAB isolated from marine
217 algae could be explained by their adaptation to their natural substrates (marine algae)
218 characterized by a richness in lipids, where the lipolytic activity of such LAB could play an
219 important role during algae fermentation by LAB isolates. In comparison, previous studies
220 isolated 29 lipase-producing strains from over 100 different LAB representing the genera of
221 *Lactobacillus, Lactococcus, Leuconostoc, Pediococcus* and *Streptococcus* (Meyers et al.,
222 1996). Recently, Aziz *et al.* (2021, 2022) reported the ability of *L. plantarum* 12-3 to convert

223 linoleic acid at different concentrations to different health-beneficial fatty acid and other
224 metabolites.
225
226
227 **Table 1** : Metabolic properties and phenotypic characteristics of the isolated LAB strains

Studied algae	LAB Strains code	Growth temperature (°C)			Metabolic properties			
		30	37	44	EPS	Lip	Prot	Hom
S. muticum	S1	(+)	(+)	(+)	(+)	(+)	-	(+)
	S2	(+)	(+)	-	(+)	-	-	(+)
	S3	(+)	(+)	(+)	(+)	(+)	(+)	(+)
	S4	(+)	(+)	-	(+)	-	-	(+)
	S5	(+)	(+)	(+)	-	-	-	(+)
	S6	(+)	(+)	-	(+)	-	-	(+)
	S7	(+)	(+)	(+)	(+)	(+)	(+)	(+)
	S8	(+)	(+)	-	(+)	(+)	(+)	(+)
	S9	(+)	(+)	(+)	(+)	(+)	(+)	(+)
	S10	(+)	(+)	-	-	(+)	(+)	(+)
U. lactuca	U1	(+)	(+)	-	(+)	(+)	-	(+)
	U2	(+)	(+)	(+)	(+)	(+)	(+)	(+)
	U3	(+)	(+)	(+)	(+)	(+)	(+)	(+)
	U4	(+)	(+)	-	(+)	(+)	(+)	(+)
	U5	(+)	(+)	(+)	(+)	(+)	-	(+)

228 *EPS: Exopolysaccharides; Lip : lipolytic activity; Prot : Proteolytic activity ; Hom :*
229 *Homolactic fermentation, (+): positive reaction; -: negative reaction*

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Results of the proteolytic activity showed that out of 15 isolates, 8 LAB (5.33%) exhibited a positive activity. A bacterial strain is called proteolytic if it has a lysis zone of diameter between 5 and 15 mm. It therefore appears that the strains S3, S7, S8, S9, S10, U2, U3, and U4 are strongly proteolytic. LAB strains U1 and U5 revealed a moderate proteolytic activity, since the diameter of the proteolysis zones were between 9 mm and 12 mm, and the others (S1, S2, S4, S5, S6, U1 and U5) are not proteolytic since the clear zone does not exceed 3 mm. Marine algae are considered a viable source of protein and some species are known to contain protein levels similar to those of traditional protein sources (meat, egg, soybean, and milk), which would constitute alternative protein sources required to fulfil the consumers demand and to meet predicted global protein requirements (Bleakley and Hayes 2017). Proteolytic activity of LAB is a very important property because it is necessary for LAB growth in fermented matrices and improving the organoleptic properties (texture and flavor) of these products (Zareie et al. 2023). The analysis of distances between the characteristics illustrated in table 1 relating to LAB strains isolated from the two algae species (*S. muticum* and *U. lactuca*) allowed the neighborhood grouping represented in figure 1.

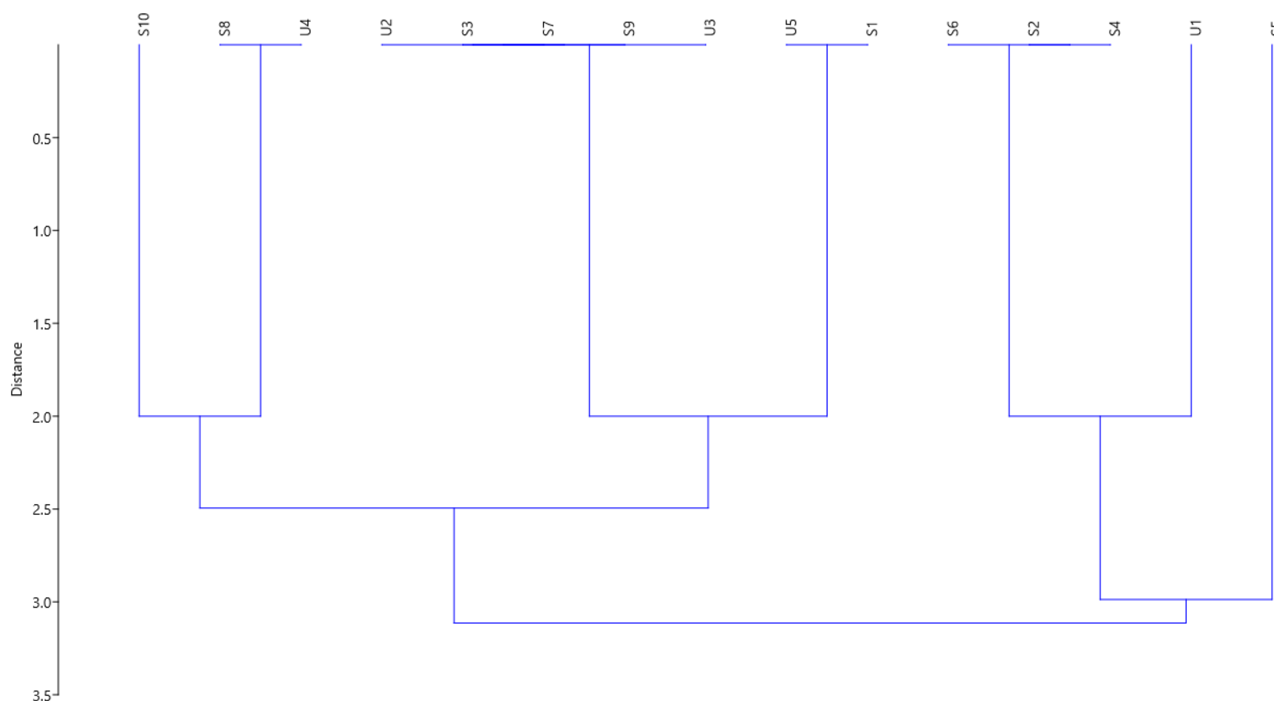

Figure 1 : Neighborhood grouping of LAB strains isolated from the studied algal species.

From this illustration, it can be seen that the bacteria are classified into two groups, namely a small group combining strains U1, S2, S4, S5 and S6 and a large group formed of strains U2, U3, U4, U5, S1, S3, S7, S8, S9 and S10. In the first group, the strains S2, S4 and S6 are brought closer to the strain U1 and the strain S5 forms a singleton which remains linked to the others by the base of the classification branch. The large group according to data shown in figure 1 is

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256 formed by the strains U4, S8 and S10 which form a small group and the strains S1, U2, U3, U5,
257 S7 and S9. This classification reflects a metabolic and taxonomic link between the isolated LAB
258 strains. The results illustrated in Figure 1 show that the isolated strains of the two algae share
259 certain genetic characteristics that allow them to live and survive in the small niches formed by
260 the two algae separately.
261
262 **Antifungal activity of LAB**
263 Currently, food preservation is based on the use of chemicals that can have adverse
264 consequences on consumer health (Leyva Salas et al. 2017). To this end, scientists are always
265 looking for natural compounds and beneficial microorganisms, such as new LAB strains or
266 their metabolites, as alternatives to avoid chemical additives use during food processing.
267 Results on the antifungal activity of LAB against the two fungal species of *A. niger* and *A.*
268 *flavus* showed that out of 15 total LAB strains, 8 strains (S1, S2, S6, S8, U2, U3, U4 and U5)
269 showed considerable antifungal activity (> 60%) against *A. flavus* and/or *A. niger* (Figure 2).
270 while the other LAB strains showed lower to moderate antifungal activity. Moreover, all LAB
271 isolates are acidifying, suggesting that their antifungal activity is due to the acidification in
272 addition to the potential production of other molecules active against the tested fungal species.
273 Similar results were reported by Aderiye (2010), who reported that 68% of LAB isolated from
274 Nigerian fermented foods had antifungal activity against *A. niger* and *A. flavus*. These results
275 are in agreement with those already reported by Muhialdin et al. (2018), Abouloifa et al. (2020),
276 and Ben Salah-Abbès et al., 2021) on the ability of LAB to inhibit the growth of various fungal
277 species, and to reduce mycotoxin (Belgacem et al. 2019; Badji et al., 2023). Indeed, LAB strains
278 are able to produce multiple antimicrobial compounds, including organic acids (lactic, acetic,
279 citric, formic and succinic acids, among others), bacteriocins, phenyllactic acid, hydroxy-fatty
280 acids, proteinaceous compounds, reuterin, and many other compounds with antimicrobial
281 effects (Bartkiene et al. 2019; Matevosyan et al. 2020).
282

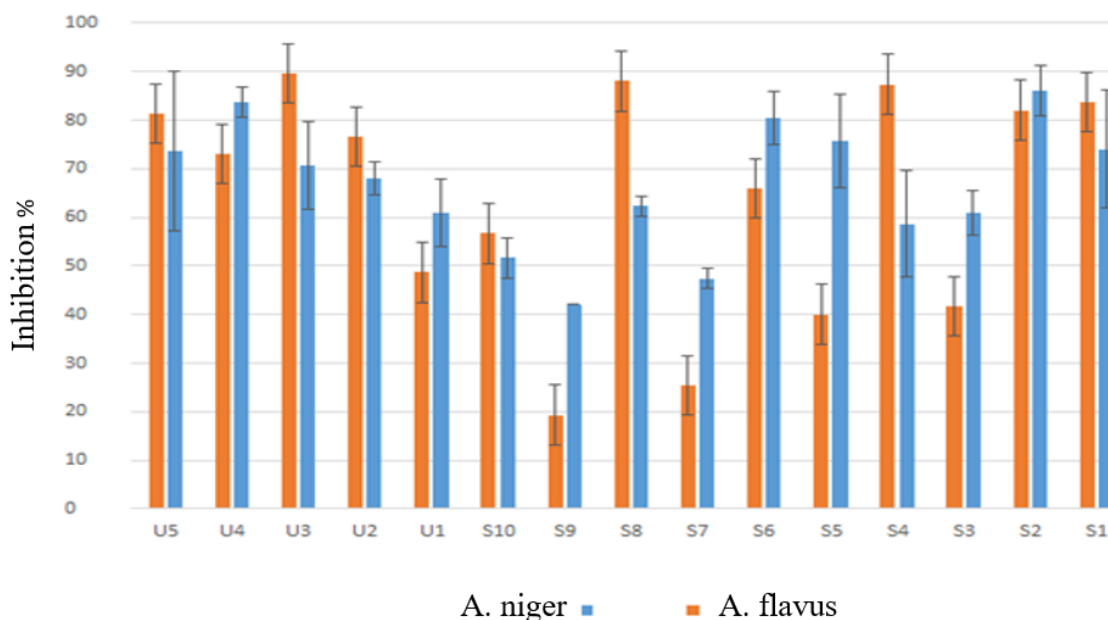

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Figure 2 : Antifungal activity expressed as % inhibition of *A. niger* and *A flavus* fungal growth.

Dimensional analysis (Figure 3) performed by the principal component analysis (PCA) technique illustrates an antifungal activities distribution done by LAB strains (S1 to S10 and U1 to S5) isolated from *S. muticum* and *Ulva lactuca* algae as well than those of fungal strains tested (*A. niger* and *A. flavus*). According to figure 4, the LAB strains contribute in negative and positive parts of component 1 while the fungal strains contribute in the positive part (A. *niger*) and the negative part (*A. flavus*) of component 2. pH variation (Δ pH) contributes to both components (1 and 2) at level 0. Figure 4 also shows that the strain *A. niger* is correlated (sensitive) to strains U4, S2 and S6 and the strain *A. flavus* is correlated (sensitive to strains U2, U3, U5, S1, S4 and S8). The other strains form a third group not correlated with the fungal strains tested.

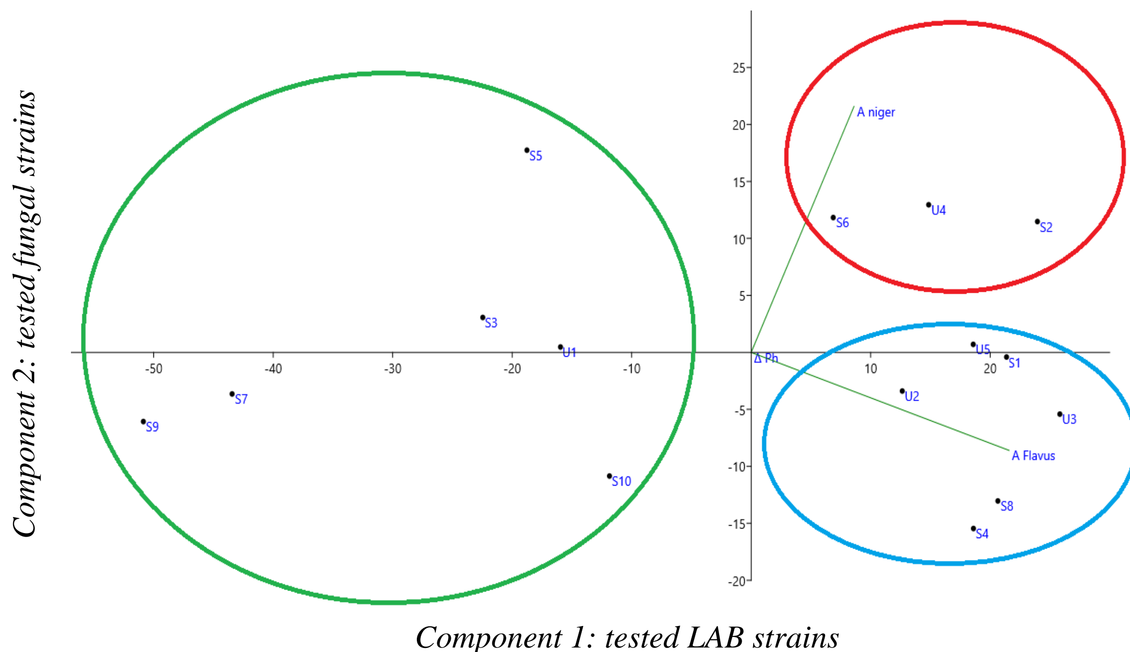

Figure 3: Dimensional analysis of antifungal activities of LAB strains (S1 to S10 and U1 to U5) isolated from *S. muticum* and *U. lactuca*.

Application of LAB for algae fermentation

LAB growth on algae media

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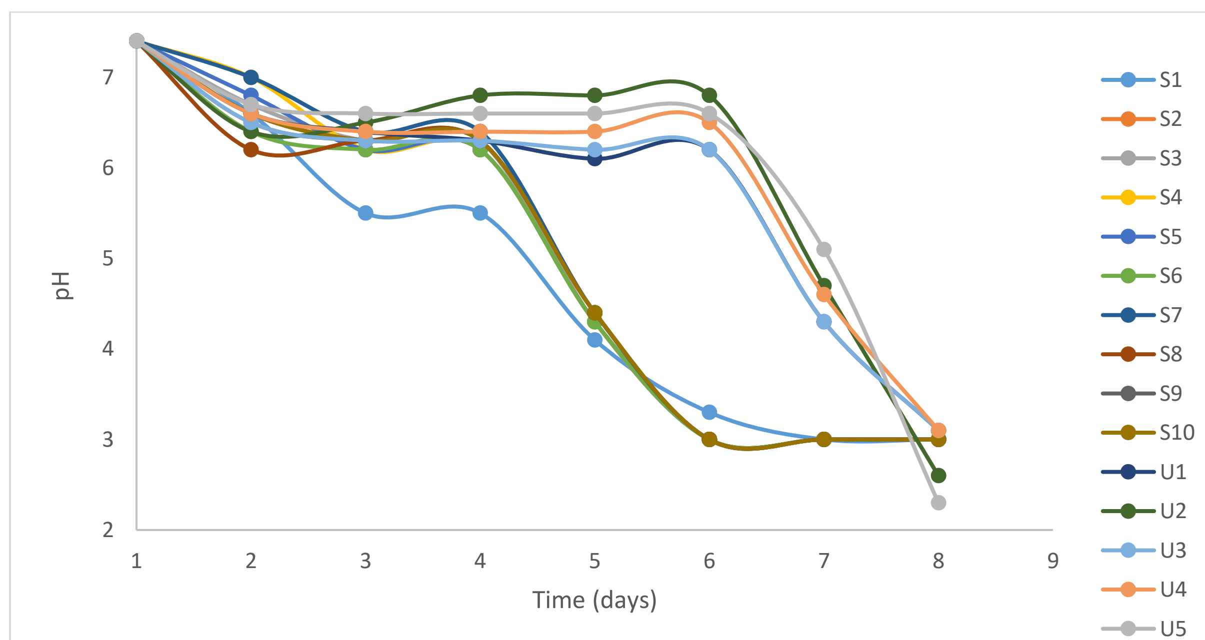
313 The growth of LAB isolates on modified MRS agar media was assessed by replacing the
314 amount of glucose in the conventional MRS medium with an equivalent amount of marine algae
315 powder of *S. muticum* or *U. lactuca*. Results showed a good growth of all LAB isolates on the
316 AEM (both agar and broth).
317
318 **pH monitoring during fermentation**
319 LAB isolates were firstly tested on algae autolysate and strong bacterial growth was observed
320 at the bottom of the tubes, which allowed to conclude about the ability of LAB strains to growth
321 on the algae based medium and to ferment algal components, especially algae polysaccharides.
322 Fermentation assays were performed by pH measurement. Results of this experiment are
323 reported in figure 4. As shown, all LAB strains were able to acidify AEM (prepared using *U.*
324 *lactuca* or *S. muticum* powder) by lowering pH from an initial value of 7.4 to a final pH value
325 of 3 for LAB strains S1, S6 and S10 isolated from *S. muticum* strains, and to a final pH value
326 2.3 and 2.6, respectively, for LAB strains U5 and U2 isolated from *U. lactuca*. It should be
327 highlighted that all reached pH values are close or below to a pH value of 3, which be explained
328 by the very good growth of isolated strains on the AEM and the high production of organic
329 acids, mainly lactic acid, by LAB isolates during the fermentation assay.
330
331 As shown in figure 4, it can be noted that the pH drop follows a bimodal phenomenon (a slow
332 phase and a fast phase). The LAB strains (U1 to U5) isolated from *U. lactuca* present a first
333 slow phase for 6 days then a linear fast phase. The linear model identified in the strains isolated
334 from the *U. lactuca* presents a long organic acid production metabolism. The drop in pH values
335 only occurred after the 6th day of growth. This rapid drop resulted in low record pH values,
336 especially for the LAB strains U2 and U5 (*U. lactuca*) that decreased the pH at the end of
337 bacterial growth until very low values of 2.6 and 2.3, respectively.
338
339



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342 **Figure 4**. pH evolution of AEM by LAB strains isolated from the studied algal species.

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345 Whereas the LAB strains isolated from *S. muticum* present a first slow phase which lasts only
346 4 days followed by a rapid logarithmic phase. Theses strains exhibits a remarkable adaptation
347 to the massive production of organic acids by fermentable sugars bioconversion (Straathof
348 2023). Indeed, the strain S1 (*S. muticum*) resulted in a drastic extreme decrease from an initial
349 pH value of 7.4 to final pH values of 4.1 and 3.1 after 5 and 6 days of growth, respectively.
350 This pattern has been observed with other LAB strains isolated from *S. muticum*, especially the
351 strains S6 and S10. Few studies have been undertaken on LAB of algal origin and their power
352 of fermentation of algal extracts. Compared to similar studies, fermentation of lyophilized
353 commercial *Chlorella vulgaris* microalgae with *Lactiplantibacillus plantarum* and *Lp. pentosus*
354 resulted in a high decrease of pH until 4.7 after 12h incubation (Carmine 2022). In another
355 study, *Sargassum* spp. was used for lactic acid production using different LAB strains, resulting
356 in 1.4 g/L concentration (Sudhakar and Dharani 2022). Acidifying capacity of LAB strains is
357 essentially based on the conversion of carbohydrates into lactic acid. This conversion is one of
358 the most important steps for the food industry. To date, lactic acid has been reported to be
359 produced from starchy and lignocellulosic biomass sources, such as corn and sugar cane waste,
360 but there are very few reports of lactic acid production from algae (Lin et al. 2020).
361
362 **Conclusion**
363 Results of this study showed that marine algae can be considered as an excellent source for the
364 isolation of new LAB strains potentially endowed with interesting metabolic capacities. The
365 obtained results showed good performance of LAB isolates to ferment bulk marine algae. The
366 ability of the isolated strains to ferment algae extracts opens up future perspectives and
367 biotechnological applications, in particular, the isolation of new antimicrobial substances
368 (bacteriocins, antifungal compounds, etc.) that could be used as natural biopreservatives in food
369 industry, extraction of biomaterials (EPS, polylactic acid, etc.) and production of extracellular
370 enzymes from marine LAB. Finally, isolation of LAB strains from other marine biotopes (other
371 algae species, sediments, seawater, etc.) and characterization of their properties is needed. The
372 molecular identification of marine LAB strains also appears very relevant.
373
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379
380 **Declaration:**
381
382 **Conflict of interest.** The authors declare that they have no conflict of interest

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