Antibacterial activity of Lactic acid bacteria strains Isolated from Marine Algae against Pathogenic Bacteria

Nouhaila Belasla[1], *Imane* Brahimi[1], *Meryem* Benyamane[1], *Faouzi* Errachidi[2], *Chakib* El Adlouni[1], *and Abdellah* Zinedine[1,*]

[1]Laboratory of Marine Biotechnologies and Environment (BIOMARE), Faculty of Sciences, Chouaib Doukkali University, El Jadida 24000, Morocco.
[2]Functional Ecology and Environmental Engineering Laboratory, Faculty of Science and Technology, Sidi Mohamed Ben Abdellah University, P.O. Box 2202, Fez 30050, Morocco.

Abstract. This work aims at evaluating the antibacterial activity of 14 lactic acid bacteria (LAB) strains isolated from two species of marine algae (*Sargassum muticum* and *Ulva lactuca)* against six pathogenic bacteria, including *Escherichia coli* ATCC 10536, *Staphylococcus aureus* ATCC 9144, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 33019, *Bacillus* sp. CIP 104717, and *Salmonella* sp. LAB inhibitory capacity was assessed by using agar well diffusion test. Results showed that significant inhibitory zones of 30.33, 23.33, 13, 12, 9, and 8 mm were obtained against *E. coli*, *B. subtilis*, *B. cereus*, *Bacillus* sp., *Salmonella* sp. and *S. aureus*, respectively. These findings underscore the potential use of marine LAB for producing antibacterial substances, suggesting their applications in biotechnological processes targeting food spoilage and human infections.

1 Introduction

The emergence of antibiotic resistance and nosocomial diseases has compelled the scientific community to conduct extensive investigations aimed at addressing this predicament. Contemporary scientists are increasingly focused on discovering novel alternative biological molecules produced by microorganisms thriving in hostile ecological niches marked by extreme physicochemical parameters, including temperature, salinity, and/or acidity.

The escalating prevalence of antibiotic-resistant microorganisms and the anticipated ineffectiveness of current therapeutic approaches are causing growing concerns [1]. These challenges underscore the imperative to explore alternative strategies to avoid chemicals and reduce antibiotic resistance. Simultaneously, heightened interest in the biopreservation of food has arisen due to the detrimental effects of chemical preservatives on human health [2].

The presence of lactic acid bacteria (LAB) has thus prompted research into their biochemical and biological properties, holding significant promise for biotechnological applications in the industrial and medical fields. Lactic acid bacteria, a group of fastidious, non-spore-forming, Gram-positive, catalase-negative microorganisms, have garnered considerable attention for their pivotal role in various biological processes [3].

Found in diverse natural biotopes, LAB strains are renowned for their capacity to produce lactic acid and other secondary metabolites, including bacteriocins, diacetyl, acetic acid, reuterin, hydrogen peroxide, and ethanol [4]. These bacteria constitute one of the most crucial bacterial groups in the food industry, having long been consumed in dairy products worldwide, with most being classified as 'generally recognized as safe' (GRAS) microorganisms [5].

Their metabolic activities and properties are subjects of ongoing studies for industrial applications, such as acidification affecting the production of fermented foods and preservation [6,7]. Furthermore, LAB strains exhibit various health-related effects, including antimicrobial, antioxidant, immunomodulatory, antitumor, antihistamine, and anti-cholesterol effects. Notably, LAB produce inhibitory substances called bacteriocins with antimicrobial activity, employed in both medicine and the food industry [8].

Thus, the purpose of this research study was to investigate the antibacterial effect of LAB isolated from marine algae (*Sargassum muticum* and *Ulva lactuca)* against the growth of pathogenic bacteria implicated in nosocomial diseases and/or food poisoning.

* Corresponding author: zinedine.a@ucd.ac.ma

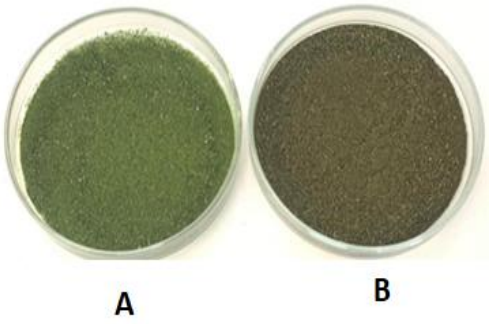
2 Materials and methods

2.1 Algae sampling

Marine algae species (Figure 1) were collected from Sidi Bouzid coast at El Jadida city (Morocco). To eliminate salts excess, collected samples were washed with tap and distilled water. Marine algae were dried at ambient temperature, then underwent grinding by a high-speed blender to obtain a fine powder (Figure 2).

A B

Fig 1. Algae species collected in the coast of Sidi Bouzid (El Jadida-Morocco). **A**: *Ulva lactuca*, **B**: *Sargassum muticum*



A B

Fig 2. Algae powder obtained after grinding: **A**: *Ulva lactuca*, **B**: *Sargassum muticum*

2.2 LAB strains isolation

Under sterile conditions, one (1) g of each algae powder was added to a sterile tube containing 9 mL of MRS broth (De Man, Rogosa and Sharpe, Biokar, France), previously sterilized, the tubes were incubated at 37 °C for 24 h. Decimal dilutions ranging from 10^{-1} to 10^{-7} were prepared in the MRS broth. Several successive subcultures were carried out on solid MRS agar medium until very distinct and homogeneous colonies were obtained. Purification of isolates was performed on MRS agar medium by striation followed by microscopic observation. Presumed LAB strains were examined macroscopically based on their colony morphology and catalase reaction, and microscopically by Gram staining [9].

2.3 Strain revivification

LAB strains stored on MRS slant at +4°C were reactivated by inoculating one by one into 5 mL of MRS broth, followed by its incubation at 30°C for 24 h.

2.4 Antibacterial activity

The inhibitory effect of the marine LAB isolates was evaluated against six pathogenic bacteria (table 2). The antibacterial effect was studied by using the agar well diffusion test, as previously reported [10]. This method involves the inoculation of the Mueller Hinton medium surface with a bacterial suspension of the target bacteria. Subsequently, a well with a diameter of 6 mm was created using a Pasteur pipette. Following this, 100 µL of the bacterial supernatant were introduced into the well of petri dishes and incubated for 16-18 h at 37°C. The diameter (mm) of the inhibition zone surrounding the wells of petri dishes was measured to determine the antibacterial activity of each tested LAB strains.

Table 1. Pathogenic bacteria strains used in this study (Source: BIOMARE Laboratory Collection).

N°	Code	Bacteria	Gram
1	ATCC 10536	*Escherichia coli*	Negative
2	ATCC 9144	*Staphylococcus aureus*	Positive
3	ATCC 6633	*Bacillus subtilis*	Positive
4	ATCC 33019	*Bacillus cereus*	Positive
5	CIP 104717	*Bacillus* sp.	Positive
6	-	*Salmonella* sp.	Negative

3 Results and Discussion

The purpose of this study was to assess the antibacterial activity of 14 marine LAB strains against specific pathogens (BIOMARE Laboratory collection), including *E. coli* (ATCC 10536), *Staphylococcus aureus* (ATCC 9144), *Bacillus subtilis* (ATCC 6633), *Bacillus cereus* (ATCC 33019), *Salmonella* sp., and *Bacillus* sp. (CIP 104717). The antibacterial activity of LAB isolates was evaluated through a well diffusion agar test, and the diameter of the inhibition zone (mm) was measured following an incubation period of 18 h at 37°C. Results showed that all the tested LAB strains (n=14) exhibited an antibacterial activity against the tested pathogenic strains, as evidenced by distinct inhibition diameters. Notably, strains S2, S4, and U2 demonstrated substantial inhibitory effects, surpassing a diameter of 25 mm against *E. coli.* Similarly, strains S1, S6, U1, and U4 exhibited noteworthy inhibition, with diameters exceeding 20 mm against *B. subtilis*. Moreover, strains S3, U2, U3, U4, and U5 displayed inhibitory zones greater than 10 mm against *B. cereus*. Other strains also manifested significant antibacterial activity with inhibition diameters of 8-9 mm against *S. aureus*, *Salmonella sp.,* and *Bacillus sp.* (Table 2).

Table 2. Diameter of the inhibition zones (mm) obtained by LAB strains isolated from *Sargassum muticum* against the tested pathogenic bacteria strains

Pathogenic strains	1	2	3	4	5	6
LAB strains						
S1	23.3±2.9	6± 0	20.3±0.5	6±0	6±0	6±0
S2	25.3±5.03	6 ± 0	6 ±0	6±0	6±0	6±0
S3	21.6±10.4	6 ± 0	6±0	13.3±1.2	6±0	6±0
S4	30.3±4.5	6± 0	6±0	8.6±2.4	6±0	6±0
S5	23 ±7	6± 0	16.3 ±1.5	6±0	6±0	6±0
S6	14.3 ±2.8	6± 0	20.3 ± 3	11±2.5	6±0	9.1± 2
S7	11.3±6.3	6±1.4	6± 0	10±3.5	6±0	6±0
S8	6.4 ± 2.8	6± 0	6±0	6±0	6±0	6±0
S9	15.3±9.5	6 ± 0	19.1± 2.8	6±0	6±0	6±0
STR	23.3 ± 0	12.8 ± 0	20 ± 0	26.5 ± 0	20 ± 0	15.5±0
TET	25.3 ± 0	6 ± 0	20 ± 0	38.5 ± 0	44 ± 0	24±0

TET: Tetracycline ; STR: Streptomycin ; 1: E. coli ; 2: S. aureus ; 3 : B. subtilis ; 4 :B. cereus ; 5 : Bacillus sp. ; 6 : Salmonella sp.

Table 3. Diameter of the inhibition zones (mm) obtained by LAB strains isolated from *Ulva lactuca* against the tested pathogenic bacteria strains.

LAB strains	U1	U2	U3	U4	U5	STR	TET
Pathogenic strains							
1	13.6 ±10	25 ±10	8.6 ±4.6	8.6±2.3	9 ±5.1	23.3 ±0	25.3 ± 0
2	6 ± 0	6 ± 0	6.8 ± 0	7.2 ± 2	6 ±0	12.8 ±0	6 ± 0
3	23.3 ±2.8	6±0	6±0	20.6±0.6	6±0	20 ± 0	20 ± 0
4	6±0	10.2±7.2	6±0	8±3.5	6±0	26.5 ±0	38.5 ± 0
5	9.2±3.2	12.2±1.2	10.3±1.9	10.3±1.9	9.8±3.3	20 ± 0	44 ± 0
6	6±0	6±0	6±0	6±0	8.2±3.7	15.5±0	24±0

TET: Tetracycline ; STR: Streptomycin ; 1: E. coli ; 2: S. aureus ; 3 : B. subtilis ; 4 :B. cereus ; 5 : Bacillus sp. ; 6 : Salmonella sp.

The highest activity (Figure 3) was detected by the LAB strain S4 against *E. coli* (ATCC 10536) with an inhibition diameter of 30.33 mm ± 0.00). It should be noted that LAB strains (S4, S6, U1, U2, U3, U5) exhibiting inhibitory effects against pathogenic strains have also demonstrated a high acidifying potential. This correlation suggests that the antibacterial effect of these strains could be attributed to their capacity for acidification.

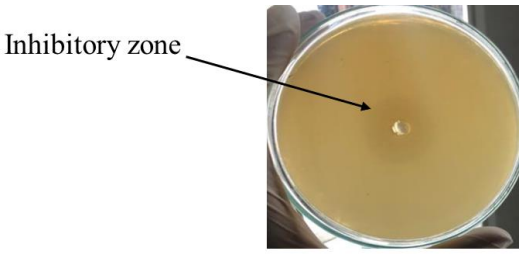


Fig 3. Inhibitory effect of the LAB strain (S4) isolated from *Sargassum muticum against E. coli* (ATCC 10536).

On the other hand, LAB strains (S1 and S2) exhibited a low acidification potential, indicating that the antibacterial effect of these strains might be attributed to other substances secreted by bacteria, such as bacteriocins.

Arrioja-Bretón *et al.* [11] tested the antibacterial activity of six LAB strains. Among these candidates, *L. plantarum* and *Pediococcus Pentosaceus* demonstrated the highest effectiveness against *E. coli* and other pathogens.

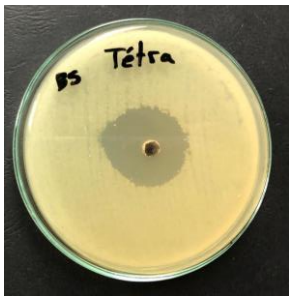


Fig 4. Inhibitory effect of tetracycline against *Bacillus subtilis* (ATCC 6633).

Väkeväinen *et al.* [12] examined the antibacterial effect of 7 LAB strains, and revealed that 3 LAB strains exhibited activity against *E. coli*, presenting a zone of inhibition ranging between 16-19 mm. Additionally, 5 strains demonstrated inhibition against *S. aureus*, with an inhibition diameter falling within the range of 10 to 15 mm [13,14].

Previous studies, encompassing LAB strains belonging to *Lactobacillus delbrueckii sub. sp. Bulgaricus, L. acidophilus, Limosilactobacillus fermentum, Lacticaseibacillus casei subsp. casei, Pediococcus pentosaceus, Leuconostoc mesenteroides subsp. mesenteroides, Leuconostoc mesenteroides sub sp. dextranicum, Enterococcus faecalis*, and *Streptococcus thermophilus* showed the inhibition of *E. coli* with inhibition diameters varying from 8 to 20 mm. Interestingly, *Lactococcus lactis* was found not to affect *E. coli* but exhibited an effect against *S. aureus*.

The antibacterial activity of LAB holds significant importance for the food industry as it plays a crucial role in preserving food products and extending their shelf life [15,16]. This phenomenon is highly desirable as it contributes to the reduction of chemical preservatives in foods [17,18]. Indeed, LAB are known as producers of a spectrum of metabolites that exert inhibitory effects on the growth of pathogenic bacteria [19,20]. Their antimicrobial efficacy is primarily rooted in the creation of unfavorable conditions for bacterial development, notably the reduction in pH due to the production of lactic acid in substantial quantities [21]. Furthermore, LAB stimulates the generation of other inhibitory substances, including antibacterial agents such as bacteriocins, contributing to the inhibition of pathogens [22].

4 Conclusion

In summary, LAB strains isolated from marine algae showed potential antibacterial activity, particularly evident in the complete inhibition of *E. coli* and high efficacy against *Bacillus subtilis*. This holds promising implications for the food industry, offering natural alternatives to chemical preservatives in response to the increasing consumer demand for healthy, minimally processed foods. The selection of LAB strains from marine sources demonstrates the potential for innovative starter cultures, meeting market preferences for high-quality, safe products. Moving forward, molecular identification and exploration of

antibacterial molecules present exciting avenues for future research in this field.

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References

1. Z. Erginkaya, G. Konuray-Altun, *Potential biotherapeutic properties of lactic acid bacteria in foods*. Food Bioscience, **46**, 9 (2022)
2. J. Raman, J.S. Kim, K. R. Choi, H. Eun, D., Ko, Y. J Yang, D., S.J. Kim, *Application of Lactic Acid Bacteria (LAB) in Sustainable Agriculture: Advantages and Limitations*. International Journal of Molecular Sciences, **23**, 14 (2022)
3. C. Shi, M. Maktabdar, *Lactic Acid Bacteria as Biopreservation Against Spoilage Molds in Dairy Products – A Review*. Frontiers in Microbiology, **12**, 18 (2022)
4. S. Mohammed, N. Özdemir, A.H. Çon, *Isolation and characterization of potential probiotic lactic acid bacteria from traditional cheese*. LWT - Food Science and Technology, **152,** 9 (2021)
5. L. Matevosyan, I. Bazukyan, A. *Trchounian, Antifungal and antibacterial effects of newly created lactic acid bacteria associations depending on cultivation media and duration of cultivation*. BMC Microbiology, **19**, 7, (2019)
6. S.P. Bangar, S. Suri, M. Trif, F. Ozogul, *Organic acids production from lactic acid bacteria: A preservation approach*. Food Bioscience, **46**, 16 (2022)
7. J.A. Mora-Villalobos, J. Montero-Zamora, N. Barboza, C. Rojas-Garbanzo, J. Usaga, M. Redondo-Solano, L. Schroedter, A. Olszewska-Widdrat, J.P. Lopez-Gomez. *Multi-Product Lactic Acid Bacteria Fermentations*: Fermentation, **6**, 21 (2020)
8. L. Matevosyan, I. Bazukyan, A. *Trchounian, Antifungal and antibacterial effects of newly created lactic acid bacteria associations depending on cultivation media and duration of cultivation*. BMC Microbiology, **19**, 7, (2019)
9. I. Brahimi, M. EL Boujamaai, F. Errachidi, A. Zinedine, *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*. Euro-Mediterranean Journal for Environmental Integration, **9**, 8, (2023)
10. D. Arrioja-Bretón, E. Mani-López, E. Palou, A. López-Malo, *Antimicrobial activity and storage stability of cell-free supernatants from lactic acid bacteria and their applications with fresh beef*. Food Control, **115**, 11 (2020)
11. K. Väkeväinen, A. Valderrama, J. Espinosa, D. Centurión, J. Rizo, D. Reyes-Duarte, G.Díaz- Ruiz, A. von Wright, P.Elizaquível, K. Esquivel, A. I. Simontaival, R. Aznar, C. Wacher, C. *Plumed-Ferrer, Characterization of lactic acid bacteria recovered from atole agrio, a traditional Mexican fermented beverage*. LWT - Food Science and Technology, **88**, 9, (2018)
12. S. Sakoui, Derdak, R., Addoum, D. Pop, C.Vodnar, R. Suharoschi, A.Soukri, B. El Khalfi, *The first study of probiotic properties and biological activities of lactic acid bacteria isolated from Bat guano from Er-rachidia, Morocco*. LWT - Food Science and Technology, **159**, 9 (2022)
13. Z. Islam, E. Uddin, T. Rahman, M. Ashiqul, *Isolation and characterization ofdominant lactic acid bacteria from raw goat milk: Assessment of probiotic potential andtechnological properties*. Small Ruminant Research, **205**, 11 (2021)
14. H. Fidan, T. Esatbeyoglu, V. Simat, M. Trif, G. Tabanelli, T. Kostka, C. Montanari, S. A. Ibrahim, F. Özogul, *Recent developments of lactic acid bacteria and their metabolites on foodborne pathogens and spoilage bacteria: Facts and gaps*. Food Bioscience, **47**, 18 (2022)
15. P. Petrova, *Lactic Acid Fermentation of Cereals and Pseudocereals: Ancient Nutritional Biotechnologies*. Nutrients, **12**, 26 (2020)
16. W. H. Holzapfel, P. Haberer, R. Geisen, J. Björkroth, U. Schillinger, *Taxonomy and important features of probiotic microorganisms in food and nutrition.* American Journal of Clinical Nutrition, **73**, 9 (2001)
17. A. Zapaśnik, B. Sokołowska, M. Bryła, *Role of Lactic Acid Bacteria in Food Preservation and Safety*. Foods, **11**, 17 (2022)
18. T. Badji, N. Durand, F. Bendali, I. Piro-Metayer, A. Zinedine, J. Ben Salah-Abbès, S. Abbès, D. Montet, A. Riba, C. Brabet. *In vitro detoxification of aflatoxin B1 and ochratoxin A by lactic acid bacteria isolated from Algerian fermented foods*. Biological Control, **179**, 11 (2023)
19. K. Khedid, M. Faid, A. Mokhtari, A. Soulaymani, A. Zinedine. *Characterization of lactic acid bacteria isolated from the one humped camel milk produced in Morocco*. Microbiological Research, **164**, 11 (2009)
20. A. Ndaw, A. Zinedine, M. Faid, A. Bouseta, *Effect of controlled lactic acid bacterial fermentation on the microbiological and chemical qualities of Moroccan sardines (Sardina pilchardus)*. Acta Microbiologica et Immunologica Hungarica, **55**, 15 (2008)
21. M. El Boujamaai , N. Mannani, A. Aloui, F. Errachidi, J. Ben Salah-Abbès, A. Riba, S. Abbès, J.M. Rocha, E. Bartkiene, C. Brabet, A. Zinedine, *Biodiversity and biotechnological properties of lactic acid bacteria isolated from traditional Moroccan sourdoughs*. World Journal of Microbiology and Biotechnology **39**,12 (2023)

22. J. Ben Salah-Abbès, M. Mannai, H. Belgacem, A. Zinedine, S. Abbès. *Efficacy of lactic acid bacteria supplementation against Fusarium graminearum growth in vitro and inhibition of Zearalenone causing inflammation and oxidative stress in vivo*. Toxicon, **202**, 8 (2021)