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Short Communication

# The Combined effect of *Rosmarinus officinalis* L essential oil and Bacteriocin BacLP01 from *Lactobacillus plantarum* against *Bacillus subtilis* ATCC11778

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ABSTRACT

Combining bacterial metabolites and natural products of plants is one proposed alternative to antibiotic usage. Reports on such combinations are starting to emerge. This study aimed to determine the antibacterial potential of bacteriocin (BacLP01) produced by Lactobacillus plantarum and the essential oil of *Rosmarinus officinalis* L (ROEO) alone and their combination. Each antibacterial agent was tested against food-borne pathogens: *Bacillus subtilis*, *Bacillus cereus*, *Escherichia coli*, and *Staphylococcus aureus* using the agar well diffusion method. The antibacterial agents were evaluated from their minimum inhibitory concentration (MIC) against the test organisms. The checkerboard technique was performed to assess the resulting combination to determine the nature of the sum effects between antibacterial agents against *B. subtilis* ATCC11778. BacLP01 achieved the highest activity against *B. subtilis*, equivalent to 6400 AU/mL. ROEO showed an inhibition zone ranging from 10.23 ± 1.92 to 14.52 ± 0.83mm. *S. aureus* expressed the highest sensitivity to ROEO (14.52 ± 0.83 mm), and B. subilis presented low susceptibility with a 10.23± 1.92 mm diameter inhibition zone. Likewise, the synergistic effect of their combination on *B. subilis* ATCC11778 follows the same trend. The MICs for bacteriocin (BacLP01) and ROEO were 5 and 6.25 µL/mL, respectively. The result from FICI for the combination of BacLP01 and ROEO was 0.49, suggesting a synergistic interaction effect against *B. subtilis*. The study concluded that a combination of BacLP01 and ROEO could be an efficient means to control the presence of pathogenic bacteria in food.

***Keywords*:** Bacteriocin, *Lactobacillus Plantarum*, Checkerboard, *Rosmarinus officinalis*, Essential oil.

## Introduction

In 1906, an outbreak of severe diarrhoea, stomach cramps, and vomiting occurred in a sanatorium, affecting around 300 inmates and staff. This event led to the first documented reports linking Bacillus spp. with food poisoning.[1] Bacillus species have been acknowledged as a notable impediment in the food industry owing to the resistance exhibited by their endospores to various stress factors, including but not limited to heat, cold, pressure, radiation, drying, and chemicals. Additionally, several studies have postulated that the surface hydrophobicity of specific spore-forming bacilli may augment their propensity for adhering to food-processing surfaces, such as stainless steel.[2] The adhesion of bacteria to surfaces can lead to the formation of biofilms that causes a significant challenge to the food industry due to their profound resilience to cleaning protocols.

A diverse range of food products, including eggs, rice, spices, milk powders, and cereal products, have been found to harbour Bacillus spores, primarily *B. cereus*, *B. subtilis*, *B. licheniformis*, and *B. megaterium.*

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While *Bacillus subtilis* has GRAS status meaning "Generally Recognised as Safe" bacteria by the FDA for specific applications such as enzyme production, concerns exist regarding the potential pathogenicity of other Bacillus species.[3] Numerous instances of food poisoning have been attributed to the *Bacillus subtilis* group, specifically due to the production of heat-stable lipopeptides, namely lichenysin A and surfactin, which are toxic to mammalian cells.[4] In recent times, investigations have facilitated the delineation of an innovative toxin-antitoxin (TA) mechanism in *B. subtilis* isolates originating from comestibles. Consequently, it is pertinent to assess the categorisation of this species as a plausible food-borne pathogen.[5]

Growing interest revolves around Lactic acid bacteria as a suitable substitute for synthetic preservatives due to their inherent capacity to generate diverse antimicrobial substances, including but not limited to the production of organic acids, returicyclin and reuterin, hydrogen peroxide, diacetyl, and new bacteriocins, among other natural agents with antimicrobial properties.[6,7] Although multiple hypotheses have been proposed to elucidate the antimicrobial potentials of lactic acid bacteria, the most widely accepted explanation attributes this effect to bacteriocins, a class of specific antimicrobial substances. These proteinaceous molecules, which can be peptides or proteins, exhibit bactericidal or bacteriostatic activity that is selectively effective against particular microorganisms. Bacteriocins are a diverse group of ribosomally synthesised small molecules which exhibit potent antimicrobial activity at specific concentrations.[8,9] Various bacteriocins derived from LAB exhibit significant antagonistic effects against a broad spectrum of microorganisms, Listeria, Clostridium, Staphylococcus, Bacillus spp., Brochothrix, Aeromonas, and Vibrio spp.[10,11]

Essential oils (EOs) are increasingly employed with other food preservatives as part of the hurdle technology approach. These oils are aromatic plant extracts possessing a broad spectrum of antimicrobial activity, including but not restricted to antiviral, antifungal, antimycotic, antiparasitic, and insecticidal properties.[12–14] Numerous studies have documented the *in vitro* effectiveness of EOs against a diverse range of food-borne pathogenic microorganisms. Notably, natural EOs such as Rosemary (*Rosmarinus officinalis* L) have been traditionally employed as antimicrobial agents and natural antioxidants in the food industry.[13,15,16] Rosemary (*Rosmarinus officinalis*), family Lamiaceae, originating from the Mediterranean region, has been the subject of numerous antibacterial and antioxidant studies. Owing to this plant's impressive characteristics, rosemary extract has become a popular natural food preservative in the meat and fish industry.[17]. Its antibacterial activity has been linked to 1,8-cineole,[18] verbenone,[19] α-pinene,[20] camphor,[21] myrcene,[22] and β-caryophyllene.[23] Despite the abundance of information regarding the biopreservative properties of bacteriocins and essential oils, limited attention has been given to the potential synergistic effects of these two antimicrobial agents.

The primary objectives of this investigation were twofold: firstly, to verify the bacteriocinogenic activity of six strains of Lactic bacteria screened from Klila, a traditional cheese handmade in the southwestern territories of Algeria, and to assess the antibacterial potential of essential oil extracted from *Rosmarinus officinalis* and secondly, to evaluate the efficacy of BacLP01, a bacteriocin produced by Lactobacillus plantarum and Rosmarinus officinalis essential oil against *Bacillus subtilis* ATCC11778, with a view to applying this combination as a natural food preservative.

## Materials and Methods

*Bacterial strains origins*

For the current investigation, six strains from the screened lactic bacteria (LABs) were evaluated from a larger pool of 41 LABs isolated from Klila cheese (Algeria). These strains had previously been identified using RNA 16S sequencing by LGC Genomics (Berlin, Germany), and the resulting sequences were analysed using the NCBI database. This identification was conducted as part of a study by Benamara *et al*.[24]

Bacterial Strains and Culture Conditions

The LAB strains are *Lactobacillus plantarum* and *Enterococcus durans* (a) (a: isolated from sheep cheese). *Leuconostoc pseudomesenteroides*, and Enterococcus durans (b) (b: isolated from cow milk cheese). *Pediococcus pentosaceus* and *Enterococcus durans* (c) (c: isolated from goat cheese). LABs were grown at 30°C for 24 H in MRS Broth, and 4 spoilage germs were used. B. subtilis ATCC11778 and B. cereus ATCC14579 were obtained from the Laboratory of Biodiversity and Microbial Ecology (LUBEM)-France. *E. coli* ATCC 25922 and *S. aureus* ATCC 43300 were obtained from the Central Laboratory of the Public Hospital Rouiba-Algeria. The bacteria strains were kept at –20°C. Cultures were preserved at -20 °C (with 30% glycerol as a cryoprotectant).

*Antibacterial effects of LAB strains*

The antibacterial activity of LAB strains was initially evaluated using the agar spot test methodology described by Fleming *et al.*[25] The LAB strains was individually cultivated on Man, Rogosa, Sharpe (MRS) media by the spot method followed by anaerobic incubation at 30°C for 24 hours. Following this, 7 mL of Muller-Hinton semi-solid media containing 100 µL of the test bacteria (adjusted to $10^6$ CFU/mL) was poured on top of the first layer and then aerobically incubated at 37°C up to 48 hours. Pathogenic bacteria, including *B. subtilis*, *Bacillus cereus*, *E. coli*, and *Staphylococcus aureus*, were aerobically cultured for 24 h at 30°C. A positive inhibition was considered when a visible clearance zone surrounding the colony of the producer strain could be observed.

*Bacteriocin bioassay*

In this study, the agar well diffusion method was utilised to screen for Bacteriocin production as described by Castro et al.[26] The LAB strains were grown in MRS broth (37°C for 18-24 hours) followed by centrifugation, 5 minutes at 10,000 rpm. The cell-free supernatant was calibrated to pH 6.5 to prevent inhibition by organic acids, and catalase at a final concentration of 1mg/mL was added to eliminate the inhibitory activity of hydrogen peroxide. The remaining activity was determined using *Bacillus subtilis* ATCC11778 as the indicator microorganism.[27 28] The untreated samples were used as a control. The agar plates were incubated for 24 hours at 37°C under microaerophilic conditions. The diameter of the inhibition zone was determined by subtracting the well diameter from the total inhibition zone diameter. A positive outcome was denoted by an inhibition zone diameter exceeding 2mm.

Cell-free supernatants (CFS) exhibiting activity after the treatments mentioned above were subjected to further treatment with 0.2 mg/mL of proteinase K (pH 7.0). Subsequently, the solutions were subjected to filter sterilisation and were mixed with supernatants in a 1:1 (v/v). Untreated bacteriocin and enzyme solutions were used as control measures. All samples and controls were incubated at 37°C for 2 h.[29] The agar well diffusion technique was employed to assess any remaining antibacterial activity of the treated extracts.

*Partial purification of bacteriocin*

To partially purify bacteriocin from the CFS, the Lactic Bacteria strains were grown in 2 L MRS broth at 30°C for 16 h, followed by the removal of cells via centrifugation. The final pH of the supernatant fluid was 6.5, and it was precipitated with 80% ammonium sulfate [$(NH_4)_2SO_4$] (Summarized in Table 2). The resulting pellets were resuspended in sodium phosphate buffer (50 mmol L-1, pH 7) and stirred at 4°C for 24 hours. The mixture was subsequently tested for antibacterial activity, and salt elimination was achieved by dialysis through a 3.5 kg $mol^{-1}$ cut-off membrane (Snake Skin, Pierce, Rockford, USA) against sterile distilled water. The resulting suspension was referred to as a proteinaceous fraction or crude bacteriocin fraction. The antimicrobial activity of the bacteriocin was quantified as the reciprocal of the most diluted sample that hindered the growth of the indicator bacteria. The resulting value was reported in units of activity per millilitre (AU $mL^{-1}$).[30]

*R. officinalis Essential oil*

Rosemary leaves used in this study were harvested in Mascara (North West of Algeria) in March 2021. The essential oils were extracted by the hydrodistillation method in a Clevenger-type apparatus. The process involved boiling 100 g of Rosemary leaves in 1000 ml of distilled water for 4 hours. The process was repeated three times. A refrigerant was used for the condensation of the oils. The essential oil yield was calculated based on the dry weight of the sample, and the resulting rosemary oil extract (ROEO) was collected and preserved in sealed vials, shielded from light, at a temperature of 4°C.[31]

*Antibacterial activity of R. officinalis essential oil (ROEO)*

The antibacterial activity was evaluated according to the Clinical and Laboratory Standards Institute[32] using the agar well diffusion method. In this study, the bacteria strains were cultured overnight at 37°C, and the turbidity of each strain was adjusted to $10^8$ CFU/mL. To assess the susceptibility of the bacterial strains to ROEO, standardized inoculum suspensions were added to Mueller Hinton agar plates, and 6 mm diameter wells were bored in the agar. Subsequently, 20 µL of ROEO was placed into each well, and the plates were incubated for 24 hours at 37°C. The inhibition zone diameter surrounding each well was measured in millimetres. The bacterial strains were classified based on the size of the zone as insensitive (diameter less than 8 mm), sensitive (diameter between 9-14 mm), highly sensitive (diameter between 15-19 mm), or extremely sensitive (diameter greater than 20 mm).[33]

*Determination of MICs*

The minimum inhibitory concentration (MIC) was determined in sterile 96-well microplates, following the guidelines of the EUCAST (European Committee on Antimicrobial Susceptibility Testing).[34] BacLP01 and ROEO were evaluated for their antibacterial activity using a microdilution technique. Initially, 100 µL of each antibacterial agent was Supplemented to the first well of the plate and then serially

diluted by transferring 50 µL aliquots to subsequent wells. Subsequently, *B. subtilis* inoculum with a $10^5$-$10^6$ CFU/mL concentration was added to each well, resulting in a final volume of 200 µL. The microtiter plate was then incubated at 37°C for 18-24 hours. ROEO was dissolved in 10% DMSO and diluted in the concentration range of 100 to 0.097 µL/mL, while BacLP01 was diluted from 160 to 1.25 µL/mL in L-broth. The MIC was determined as the lowest concentration of the antibacterial agent that inhibited bacterial growth. Negative controls using untreated cells and DMSO were included, and the experiments were conducted in triplicate.

*Determination of the combined effect of bacteriocin and ROEO against Bacillus subtilis ATCC11778*

The checkerboard method or fractional inhibitory concentration (FIC) technique is employed in evaluating the combination of two antimicrobial substances. The synergistic or antagonistic interaction between the antibacterial agents was assessed by determining the fractional inhibitory concentration index (FIC Index) using the minimum inhibitory concentration (MIC) values, following the equations proposed by Sanz *et al.*[35] The fractional inhibitory concentration index (FICI) was calculated by adding the FIC of drug A and the FIC of drug B, which can be represented as

$\sum$FICI = FIC (A) + FIC (B).

$$(1)\ \text{FIC (A)} = \frac{\text{MIC (A)in combination}}{\text{MIC (A) alone}})$$

$$(2)\ \text{FIC (B)} = \frac{\text{MIC (B) in combination}}{\text{MIC (B) alone}}$$

The FICI values were interpreted as follows: FICI values of ≤0.5 indicated a synergistic interaction, FICI values ranging from <0.5 to 0.75 showed partial synergy, FICI values ranging from ≤0.76 to 1.0 indicated an additive effect, FICI values ranging from >1.0 to ≤4.0 indicated no interaction (i.e., no significant difference), and FICI values greater than 4 indicated an antagonistic interaction.

*Statistical analysis*

The data were statistically analysed by one-way ANOVA with the Statistical Program (SPSS, USA, ver. 16.0). Pearson's correlation analysis was done to correlate the bacteriocin BacLP01 and the ROEO potential in the samples.

## Results and Discussion

Consuming food contaminated with pathogenic bacteria remains a global public health issue. Therefore, the development of effective sanitising procedures has received much attention in recent years. One potential solution is using essential oils from plants and bacteriocins as safe additives for controlling food-borne pathogens and spoilage bacteria while also extending the shelf life of food. This study investigated two natural compounds, bacteriocins and R. Officinalis essential oil (ROEO). The LAB strains tested in this study displayed varying antibacterial (summarized in Table 1) activity against at least two of the tested antibacterial strains, with *Lactobacillus plantarum* and *Enterococcus durans* exhibiting remarkable activity against all pathogenic bacteria screened. All LAB strains inhibited S. aureus and *E. coli* growth, possibly due to nutritional competition or the secretion of antimicrobial metabolites such as ethanol, hydrogen peroxide, and bacteriocins.[36,37,38]

Among the six Lactic Acid Bacteria (LAB) strains, only two maintained their antibacterial activity after neutralising and treating supernatants with catalase: *Lactobacillus plantarum* and *Enterococcus durans* (a) isolated from sheep cheese. However, there was a loss of antibacterial activity after adding proteinase K. This confirms its proteinaceous nature and can be considered a Bacteriocin-like inhibitory substance (BLIS). Numerous literature reviews have been conducted to explore the capacity of Enterococcus spp. to generate various bacteriocins, known as enterocins. These enterocins display antimicrobial properties, impeding the proliferation of a wide range of food-borne and spoilage-associated pathogens, including *Staphylococcus aureus, Escherichia coli, Bacillus spp., Pseudomonas spp., Listeria monocytogenes*.[39 40]

The present study observed that bacteriocin (BLIS) produced by E. durans inhibited *B. subtilis* ATCC11778, *Bacillus cereus* ATCC14579, *Escherichia coli* ATCC 25922, and S. aureus ATCC 43300. Also, the bacteriocin (BLIS) produced by *L. plantarum* inhibited the four pathogenic bacteria used in this study. As stated by other authors, various strains of L. plantarum isolated from fermented foods have been found to produce bacteriocins with antibacterial activity against pathogenic microorganisms. One such strain, *L. plantarum* BR12, isolated from traditional Bulgarian dairy products, produces a bacteriocin that inhibits the growth of *B. subtilis, S. aureus,* and *Listeria monocytogenes*. Additionally, other studies have reported that *Lactobacillus plantarum* AMA-K isolated from naturally fermented milk can produce peptides that effectively inhibit the growth of *Listeria innocua* and *Enterococcus faecalis.*[41,42]

**Table 1:** Antibacterial effects of LAB strains screened from Klila cheese prepared with: Sheep's milk, cow's milk, and goat's milk.

| | **Origine** | ***S. aureus* ATCC 43300** | ***E. coli* ATCC 25922** | ***B. cereus* ATCC 14579** | ***B. subtilis* ATCC 11778** |
|---|---|---|---|---|---|
| *L. plantarum* | Sheep cheese | ++ | ++ | +++ | +++ |
| *E. durans (a)* | Sheep cheese | ++ | ++ | ++ | ++ |
| *Leu. pseudomesenteroides* | Cow milk cheese | ++ | + | - | - |
| *E. durans (b)* | Cow milk cheese | + | + | - | + |
| *P. pentosaceus* | Goat cheese | ++ | + | - | - |
| *E. durans (c)* | Goat cheese | + | + | + | - |

– : absence of antimicrobial activity; + : an inhibition zone less than 10 mm; ++ : an inhibition zone greater than 11 mm; +++: an inhibition zone greater than 20 mm

**Table 2:** Performance of the bacteriocins (crude extract) of *Lactobacillus plantarum* and *Enterococcus durans* isolated from sheep: preceding and after precipitation in ammonium sulphate

| | **BacLP01(AU/ml)** | | **BacEd01(AU/ml))** | |
|---|---|---|---|---|
| | **before precipitation** | **after precipitation** | **before precipitation** | **after precipitation** |
| *S. aureus* | 1600 | 3200 | 1600 | 3200 |
| *B. subtilis* | 1600 | 6400 | 800 | 3200 |
| *B. cereus* | 1600 | 3200 | 800 | 3200 |
| *E. coli* | 800 | 3200 | 800 | 1600 |

The bacteriocins BacLP01 from L. plantarum and BacEd-a from E. durans were obtained by precipitating the culture broths with 80% ammonium sulphate saturation. Upon precipitation, changes in the activity of each bacteriocin were observed. BacLP01, produced by L. plantarum, exhibited the highest activity against *B. subtilis* ATCC11778 with a titre of 6400 AU/mL. In comparison, BacEd01 produced by E. durans (a) showed the lowest activity with a titre of 1600 AU/mL against E. coli (Table 3). Thus, the crude extract of BacLP01 was selected for further study. Notably, the exposure of the bacteriocin crude extract to ammonium sulphate resulted in an increase in the bacteriocin titre by at least one to two-fold dilutions. Numerous studies have documented that treating bacteriocin crude extract with ammonium sulphate enhances its antibacterial activity.[43,44,45]

The antibacterial activity of the Rosemary essential oil was observed against the bacteria used in this study. A previous study by Jiang *et al*. reported ROEO was significantly effective against Staphylococcus aureus, *Bacillus subtilis, Proteus vulgaris, Pseudomonas aeruginosa*, and *Escherichia coli*.[46]

Similar findings have been documented by Bozin *et al*.[47] and Santoyo *et al.*[48] However, comparing the current results with the literature showed that our ROEO showed a moderate activity with a diameter of inhibition zone ranging from 14.52± 0.83 to 10.23± 1.92 mm. This finding agrees with Hendel et al.[49], Mehalaine et al.[50], and Zaouali *et al.*[51] Besides, another study revealed that the essential oil of Rosemary officinalis presented a weak antimicrobial activity than Thymus vulgaris L.[52], cinnamon (*Cinnamon zeylanicum*), and clove (*Syzygium aromaticum*).[53] However, Raeisi *et al*.[54] and Bozin *et al.* [55] reported a higher inhibition zone ranging from 21.4±0.2 to 31.3±0.3 and from 16.2 ± 0.45 to 43.2 ±1.92 mm, respectively. The observed variation in antimicrobial activity can be attributed to the origin of Rosemary and the extraction method. For instance, Zaouali *et al.* [53] reported that the essential oil extracted from two endemic varieties of *Rosmarinus officinalis L*. in Tunisia, var. *typicus* and var. *troglodytorum*, exhibited different diameter of inhibition zones depending on the sampling area, either sub-humid or upper arid zone. As presented in Table 4, the essential oil of Rosemary displayed the highest susceptibility towards *S. aureus*, with a diameter of inhibition zone of 14.52±0.83mm, whereas B. subtilis showed low susceptibility with a diameter of inhibition zone of 10.23±1.92mm. These findings are consistent with those of previous studies from Santoyo *et al*.[48] and Pintore *et al.* [56], which demonstrated the effectiveness of rosemary essential oil against *Staphylococcus Spp* compared to *E. coli.*

The inhibitory activity of the essential oil of Rosemary could be associated with the presence of some compounds such as borneol, camphor, verbenone, and 1,8-cineol. Their antibacterial effect has been previously highlighted and reported by Santoyo *et al*.[48], Fu *et al*.[57], and Roomiani *et al*.[58] In a study conducted by Fu *et al*.[59], the bacteria Propionibacterium acnes was inhibited by the Rosemary essential oil in a concentration-dependent manner. The authors disclosed that elevated EO concentrations impelled the treated bacteria with critical damage expressed in native shape losses, cell wall desquamation, Cytoplasm emanation, and apoptosis.

Several limitations hinder the industrial utilisation of bacteriocins. These include a limited range of inhibition, low solubility, susceptibility to degradation by native proteolytic enzymes, and binding to food components. As a result, the effectiveness of bacteriocins like nisin as antimicrobial agents is reduced.[60] Combining bacteriocins with other treatments may lead to synergistic effects. It could be an effective and practical approach to control microorganisms in food while preserving their sensory qualities.[36] In this context, the current work represents an evaluation of the efficiency of Bacteriocin LP01 produced by *Lactobacillus plantarum* and *Rosmarinus officinalis* L essential oil against *Bacillus subtilis* ATCC11778 as synergic antibacterial components. The results presented in Table 4 showed that MICs for bacteriocin BacLP01 and ROEO were 5 and 6.25 µL/mL, respectively. The study also explored the combination of these antibacterial agents. The FICI for the combined effect of BacLP01 and ROEO against *Bacillus subtilis* ATCC11778 was 0.49, which suggested a synergistic interaction.

The observed outcomes are congruent with those presented by Raeisi et al., who reported that the combination of rosemary essential oil and nisin exhibited the highest antibacterial activity against Salmonella typhimurium, Listeria monocytogenes, *Escherichia coli*, and *Staphylococcus aureus*.[61] Gao *et al*.[62] study agrees with Raeisi et al. that Nisin/Rosemary extract combination improved the sensory quality of pompano fillet without changing its physicochemical originality and inhibited microbial growth in contrast to treatment with either compound alone, owing to the significant extension shelf life of the fillet.[62] Also, Turgis et al. reported that an association between Pediocin and Satureja montagna oil synergistically affected E. coli O157:H7.[63] In another study, Dimitrijević *et al*.[64] noted that the antilisterial activity was achieved by an increased sublethal dose of lactic acid.[64] A previous study also demonstrated a combination of Thymus vulgaris essential oil and the bacteriocin BacLP17 produced from Enterococcus mundtii exhibited an excellent synergistic effect[65]. According to Olasupo et al., the amalgamation of nisin with carvacrol, eugenol, or thymol manifested cooperative action against B. subtilis and L. innocua.[66] Additionally, the inclusion of 0.6% oregano EO, 500 or 1000 IU/g nisin in minced sheep meat demonstrated a cumulative antimicrobial influence against S.enteritidis during refrigerated storage at 4°C or 10°C. Based on the works of Cobo *et al*, the efficacy of enterocin AS-48 in suppressing Listeria was notably augmented in a salad when administered in combination with essential oils.[67] Specifically, essential oils such as thyme verbena, thyme red, Spanish oregano, ajowan, tea tree, clove, and sage oils (evaluated at a 1% concentration), as well as 2% rosemary oil, were found to enhance antilisterial activity. Lastly, Singh et al., in addressing the challenge of nisin resistance in Gram-positive bacteria, suggest the application of nisin (0-200 IU/ml) and garlic extract (0-6 mg/mL) as a viable intervention.[68] Furthermore, the concurrent utilisation of bacteriocins and essential oils may prove beneficial in managing pathogenic microorganisms present in food, thereby safeguarding their sensory characteristics and preventing the proliferation of bacteriocin-tolerant strains.

**Table 3:** Antibacterial activity of R. officinalis essential oil

| | ROEO (mm) |
|---|---|
| S. aureus | 14.52 ± 0.83 |
| B. subtilis | 10.23 ± 1.92 |
| B. cereus | 12.05 ± 2.07 |
| E. coli | 13.26 ± 1.09 |

**Table 4:** The minimum inhibitory concentration of the products tested

| | BacLP01 | | ROEO | | FICI -Index of BacLP01 and ROEO |
|---|---|---|---|---|---|
| | MIC | FIC A | MIC | FIC B | |
| B. subtilis ATCC11778 | 0.5 | 0.25 | 06.25 | 0.24 | 0.49 (S) |

FIC-Index: [Synergy (S); ≤0.5], [indifference (I); between 0.5 to 4.0], [antagonism (A): above 4.0]

## Conclusion

The food industry faces a considerable challenge in inactivating Bacillus sp. However, this study has shown that a combination of Rosmarinus officinalis L essential oil and Bacteriocin BacLP01, produced by Lactobacillus plantarum, can effectively control the growth of Bacillus subtilis ATCC11778 synergistically. This finding opens up new opportunities for developing novel preservatives that

can prevent food-borne pathogens without affecting the organoleptic properties of the food. Further research is necessary to evaluate the feasibility of this combination in food models and to fully characterise its potential benefits.

## Conflict of Interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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