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Bacteriocin Like Inhibitory Substances (BLIS) Production by a Marine *Lactiplantibacillus plantarum* NEOM-LP1 Isolated from Red Sea (NEOM Region) and Its Antagonistic Activity Against Some Microbial Pathogens.

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Abstract

This study aimed to maximize Bacteriocin Like Inhibitory Substances (BLIS) production by marine isolate *Lactiplantibacillus plantarum* NEOM-LP1 (*L. plantarum* -NEOM LP1) by evaluating inexpensive agricultural by-products as nutrient sources and optimizing the major fermentation parameters. The alternatives to traditional, expensive medium components included corn steep liquor, soybean meal, and sugarcane molasses. In addition, the effects of temperature, pH, and incubation period on BLIS production and antimicrobial activity were analyzed. Optimal BLIS activity, determined by agar well diffusion assay, was observed during growth in de Man, Rogosa, and Sharp (MRS) broth containing 2-3% (w/v) lactose as the sole carbon source, at an initial pH of 6.5 and 35-37 °C after 72 h. Under these conditions, cell-free

culture filtrates generated inhibition zones ranging from 28 to 30 mm against target pathogens, with a minimum inhibitory volume of 0.1 mL. The effect of the carbon source varied with the substrate, and sucrose, and sugarcane molasses had a detrimental effect on growth and BLIS production. Soybean meal promoted cell density but showed little BLIS activity. Conversely, corn steep liquor was a promising low-cost alternative, supporting both growth and BLIS production at levels comparable to those of the standard MRS medium control. These results suggest a potentially economical fermentation process and support the use of corn steep liquor as a low – cost nitrogen source for the large-scale production of *L. plantarum*-NEOM LP1 bacteriocin like inhibitory substances.
Key words: Bacteriocin like Inhibitory Substances (BLIS), NEOM, *L. plantarum*, Corn steep liquor, Cane sugar molasses.

1. Introduction

Probiotics are living microorganisms that, when taken in sufficient quantities, provide health benefits to the host, primarily through their actions in the gastrointestinal tract (Abavisani et al., 2025). This is a positive activity involving regulation of the gut microbiota, improvement of barrier function, and suppression of pathogens, which explains their extensive application in fermented dairy products such as yogurt (Ibrahim et al., 2026; Jena et al., 2025). The most prominent of these useful microorganisms are lactic acid bacteria (LAB) and bifidobacteria. LAB have widespread applications in the food sector as fermentation sources, not only to provide favorable flavor and texture, but also as natural food preservatives due to the generation of antimicrobial compounds of organic acid and bacteriocins (Tyfa and Kunicka-Styczyńska, 2026). On the same note, the use of bifidobacteria, which are selective residents of the human intestine, is also gaining popularity in the context of probiotic effects, such as microbial normalization following the intake of antibiotics and direct antimicrobial activity (Ji et al., 2025). The presence of gut probiotic isolates in marine ecosystems reflects allochthonous input rather than a native, free-living marine community.

One of the major ways through which these probiotics have an antimicrobial effect is by producing ribosomal synthesized antimicrobial peptides termed bacteriocins. They have the potential to inhibit closely related bacterial strains and foodborne pathogens, and they are therefore attractive natural food preservatives and potential therapeutic agents (Diez-Gonzalez, 2007). Recent studies still emphasize the potential of certain probiotic strains, such as *Bifidobacterium* species, to produce new antibacterial compounds with possible use in maintaining human and animal health (Ji et al., 2025; Sachdeva et al., 2025).

Nevertheless, production of microbial metabolites such as bacteriocins on an industrial scale is hindered by a considerable economic obstacle, namely the high cost of traditional synthetic culture media. The standard medium for growing LAB and bifidobacterial is de Man, Rogosa, and Sharp (MRS) broth, which is, however, very expensive and unsuitable for large- scale fermentation (Hummel et al., 1983; Lu et al., 2003). As a result, research has been focused on developing cost-effective and sustainable alternatives. In this regard, recent research highlights the feasibility of using agro-industrial by-products, including fruit juices, molasses, and other vegetal sources,

as inexpensive nutrient sources with the potential to promote or even enhance the growth and metabolic activity of probiotic bacteria (Teng et al., 2026; Tchabou Tientcheu et al., 2025). It is important to optimize both the composition of the medium and the key environmental conditions (e.g., pH, temperature, incubation period) to achieve the highest yield and economic viability of bacteriocins (Zhou et al., 2008; Motta & Brandelli, 2008).

Thus, this study aimed to address this gap by investigating the production of antimicrobial bacteriocin-like substances by *Lactiplantibacillus plantarum*-NEOM LP1 (*L. plantarum*-NEOM LP1*).* The specific objectives were to: (1) test the low-cost, easily obtainable substrates, i.e., corn steep liquor, soybean meal, and sugarcane molasses, as substitutes for components of costly conventional media; and (2) optimize key environmental factors, such as temperature, pH, and incubation period to increase the efficiency of Bacteriocin Like Inhibitory Substances (BLIS) production. The overall objective was to develop a more viable, cost-effective fermentation process to enable the production of this promising antimicrobial agent on a large scale.

2. Materials and Methods

All laboratory-based experimental studies were conducted at the Microbiology laboratory, Biology Department, Faculty of Science, Tabuk University, Saudi Arabia.

2.1 .Site characterization and sampling

On May 29, 2026, surface water was collected at three locations along the NEOM coast, Tabuk region, Saudi Arabia: Gayal beach (28.088853° N, 35.023372° E), Alkhuraybah/Horaibah Marina (28.057635° N, 35.168446° E), and Sharma (28.050681° N, 35.212542° E) (Figure 1). A total of three samples were collected, stored at 4 °C in sterile polypropylene containers, and processed in 4 hours. On-site measurements were made of the temperature, pH, salinity, and dissolved oxygen.

2.2 .Maintenance, enumeration, and isolation

MRS broth, MRS agar, nutritional broth, and nutritional agar were disseminated with serial dilutions in sterile saline (0.1 mL) and incubated for 48 hours at 20, 28, and 37 °C both aerobically and anaerobically (GasPak, Oxoid) (Viju et al., 2017). Colonies were enumerated, purified using repeated streaking on marine nutritional agar, and distinguished by macro- and micromorphology. Stock cultures were kept in 20% (v/v) glycerol at -80 °C, whereas working cultures were kept on marine agar slants at 4 °C with monthly transfers.

2.3 .Isolate screening

For 48 hours, ten pure isolates were cultivated anaerobically at 35 °C in MRS broth and aerobically in Nutrient broth. To produce cell-free supernatant (CFS), cultures were centrifuged (10,000 × g, 10 min, 4 °C) and filtered (0.22 µm). As outlined in section 2.8, activity was measured against *E. coli, S. aureus,* and *Candida albicans*. NEOM-LP1, the isolate that produced the broadest zones against all three indications, was kept.

2.4 .Identification

Gram reaction, cell shape, motility, spore formation, catalase, oxidase, indole, nitrate reduction, and fermentation of glucose, fructose, sucrose, galactose, and maltose were all included in the phenotypic characterization that followed Barrow and Feltham

(1993) (Table 1). The GeneJET Genomic DNA Purification Kit (Fermentas) was used to extract genomic DNA. Primers Eub338 (5′-ACTCCTACGGGAGGCAGCAG-3′) and Eub518 (5′-ATTACCGCGGCTGCTGG-3′) were used to amplify the 16S rRNA gene in a 50 µL reaction that included 25 µL Maxima Hot Start PCR Master Mix (2×), 1 µL of each primer (20 µM), 5 µL template, and 18 µL of water. 35 cycles of 95 °C/30 s, 50 °C/60 s, and 72 °C/90 s were used, followed by 10 minutes at 95 °C and a final 10 minutes at 72 °C. Products were purified using the GeneJET PCR Purification Kit, validated on a 1.0% agarose gel, and sequenced in both directions (ABI 3730xl, GATC). The consensus was deposited as QB034968 after being compared with GenBank using BLAST (Table 2). The recA multiplex PCR of Torriani et al. (2001) or whole-genome sequencing are also necessary for species-level classification because incomplete 16S sequences do not distinguish *L. plantarum* from *L. pentosus* and *L. paraplantarum* (Torriani et al., 2001).

2.5 .Reference antibiotics and indicator strains

The clinical isolates of *Escherichia coli, Staphylococcus aureus,* and *Candida albicans* are isolated, purified and identified by the Microbiology Laboratory of Nozha International Hospital in Cairo, Egypt. At 4 °C, *C. albicans* was kept on potato dextrose agar and bacteria on nutritional agar. In each experiment, sterile uninoculated broth was used as the negative control and streptomycin (15 µg) and fluconazole (150 µg) as the positive controls.

2.6 .Partial filtration and cell-free supernatant

NEOM-LP1 was cultivated in 50 mL MRS broth at 35 °C for 48 hours, centrifuged (10,000 × g, 10 min, 4 °C), and filtered (0.22 µm) for standard tests. The precipitate was collected (10,000 × g, 10 min, 4 °C), dissolved in 10 mM Tris-HCl (pH 7.5), and dialyzed for 24 hours at 4 °C against three changes of the same buffer after solid ammonium sulfate was added to 60% saturation with gentle stirring and left overnight at 4 °C for partial purification (Malheiros et al., 2015). The Bradford test using bovine serum albumin was used to measure protein.

2.7 .Differentiating between proteinaceous, oxidative, and acidic contributions

For mechanistic interpretation, each activity measurement was subjected to three controls. (i) Neutralization: To determine if inhibition continues in the absence of culture acidity, CFS was adjusted to pH 7.0 using sterile 5 M NaOH and re-assayed. (ii) Catalase: To exclude out hydrogen peroxide, CFS was incubated with catalase (1 mg/mL, 25 °C, 30 min) prior to test. (iii) Organic acid standard: To determine the zone that acidity alone can cause, a lactic acid solution at the concentration determined in the CFS was tested against the same three indicators. The protease treatments in Section 2.10 were subsequently applied to a neutralized, catalase-treated CFS that maintained activity; the loss of activity following proteolysis was seen as proof of a proteinaceous principle, and its retention as proof against one.

2.8 .Activity quantification

The agar well diffusion assay was used to measure the antimicrobial activity (Schillinger and López, 1989). A 0.5 McFarland suspension (≈1.5 x 10^2 CFU/mL) in 0.7% soft agar was spread over a base layer to create indicator lawns. A sterile cork borer was used to cut 5 mm wells, which were then filled with 50 µL of the sample

and cultured for 24 hours at the proper temperature. Using a digital caliper, zone diameters were calculated as the mean of two perpendicular measurements to the closest 0.5 mm. According to Barefoot and Klaenhammer (1983), potency was measured in arbitrary units per milliliter (AU/mL), which is the reciprocal of the largest twofold dilution that still produces a discernible zone. Specificity was measured by dividing AU/mL by OD_{600}.

2.9 .Minimum inhibitory volume (MIV) and dose-response

Wells were filled with volumes of crude CFS (0.025–0.25 mL) and left overnight to incubate. The optimal inhibitory volume (OIV) was the smallest volume that produced a zone within 10% of the greatest zone obtained for that indicator; the minimal inhibitory volume (MIV) was the smallest volume that prevented the indicator from growing visibly (Andrews, 2001).

2.10 .Stability of physicochemicals

- Heat stability: After 10 minutes at 60, 80, 90, 100, 110, or 121 °C, CFS was chilled on ice and tested against *S. aureus* and *E. coli* (Tagg and McGiven, 1971; Sidek et al., 2018).
- pH stability: CFS was measured after being adjusted to pH 3.0, 4.5, 7.0, or 9.0 and maintained at 25 °C for two hours.
- Protease sensitivity: proteinase K (50 mM Tris-HCl, pH 7.5), trypsin (50 mM Tris-HCl, pH 8.0, 20 mM $CaCl_2$), and pepsin (0.1 M HCl, pH 2.0) were all combined 1:1 with CFS to a final concentration of 1 mg/mL, incubated for 1–2 hours at 37 °C, neutralized with NaOH as needed, heat-inactivated at 95 °C for 3–5 minutes, and assayed (Ahsan et al., 2021; Twomey et al., 2021). Every enzyme was matched with a water control and an enzyme-free buffer control.

2.11. Optimization Design

Temperature (25, 28–30, 35–37, 40, 45 °C); initial pH (5.5–8.0); carbon source (glucose, lactose, sucrose, and molasses at 1–4% w/v, replacing glucose); and nitrogen source (soybean meal extract or CSL at 1–4% w/v, replacing peptone, beef extract, and yeast extract). The response surface was fitted using second-order multiple regression and verified at the anticipated optimum after the two components with the biggest effects were merged into a central composite design with five center points.

2.12 .Low-cost substrates and cost analysis

Sugarcane molasses was received from the Sugar and Integrated Industries Company, El Hawamdia, Giza, Egypt, and CSL from the Egyptian Starch and Glucose Company, Moustorod, Egypt. Filtration, freeze-drying, and aqueous extraction (1:20 w/v) at 121 °C for 20 minutes were used to create soybean (Sb) meal extract. Each substrate's total nitrogen, total sugar, and solids content (for CSL) were measured. The 2026 supplier costs per liter of prepared medium were used to establish the medium cost, and the process cost was determined as the medium cost for 10° AU of activity recovered.

2.13 .Safety-relevant phenotyping

After 48 hours of anaerobic incubation at 37 °C, hemolysis was measured on blood agar containing 5% (v/v) human blood (Lombardi et al., 2004). Acid tolerance was assessed in MRS broth that had been adjusted to pH 3.0–6.0 and inoculated to approximately 10 CFU/mL. After 24 hours at 37 °C, growth was measured as both log

CFU/mL and OD_{600} (Mourad and Eddine, 2006). The same inoculum was used to test bile tolerance at 0.5–2.0% (w/v) oxgall (HiMedia) (Mishra and Prasad, 2005). Disk diffusion was used to assess antibiotic susceptibility for the EFSA-specified panel, and the results were interpreted using the most recent EUCAST/CLSI breakpoints.

2.14 .Statistics analysis

Values are means ± standard deviation. Each experiment was carried out using three independent biological replicates, each of which was evaluated in duplicate wells. After confirming normality (Shapiro-Wilk) and homogeneity of variance (Levene), zone diameters were compared using two-way ANOVA (treatment × time) and Tukey's HSD test. One-way ANOVA was used to compare a single time point. Second-order regression was used to fit the response surface, and ANOVA was used to assess it. At $p < 0.05$, differences were deemed significant. Design-Expert 13 (Stat-Ease, Minneapolis, MN, USA) and SPSS version 31 (IBM Corp., Armonk, NY, USA) were used for the analyzes.

3. Results

3.1 .Sampling sites characterization

The water's temperature varied between 25.0 and 30.0 °C, its salinity between 38.71 and 42.74 g/L, its pH between 6.0 and 8.0, and its dissolved oxygen between 2.0 and 3.5 mg/L. These values are in line with surface water in the northern Red Sea of Saudi Arabia, where salinity is kept close to 40 g/L due to strong evaporation and minimal freshwater input (Manasrah et al., 2004). In contrast to the oligotrophic, well-oxygenated, and slightly alkaline profile described for this shoreline (Batayneh et al., 2014), the low dissolved oxygen and the locally sub-neutral pH indicate local organic enrichment and increased respiratory activity at the sampled microsites.

3.2 .Isolation and identification

According to the closest database matches, there were ten bacterial isolates isolated. nine belonged to the Enterobacterales (*Enterobacter sp.* (n = 2), *Klebsiella spp.* (n = 3), *Citrobacter sp.* (n = 1), *Serratia spp*. (n = 2), and *Mixta sp.* (n = 1), and one to the *lactobacilli.* The latter was a non-motile rod that was Gram-positive, catalase-negative, and fermented glucose, fructose, sucrose, galactose, and maltose. It also tested negative for oxidase, indole, and nitrate reduction (Table 1). Its partial 16S rRNA sequence, which was deposited as QB034968 (Table 2), was 100% identical to L. plantarum SRCM100442 (NZ_CP028221.1) and *L. argentoratensis* (strain D1). *L. plantarum*-NEOM LP1 was the isolate's designation. Since the fragment is conserved within the species and does not establish the species on its own, a perfect match to a single reference genome does not suggest a particular link to that strain (Section 4.6).

3.3 .Inhibitory activity screening

The three indications were used to test all ten isolates for CFS. The only isolate showing discernible action against *Candida albicans* was NEOM-LP1, which also created the broadest zones against all three. Since Enterobacterales members make their own bacteriocins (Cascales et al., 2007), the isolates' poorer performance can be due to the medium, assay conditions, or indicator selection rather than a lack of antimicrobial ability.

3.4 .The active ingredient is not a traditional bacteriocin

The inhibitory activity was not eliminated by treating CFS with proteinase K, trypsin, or pepsin:

Zones against *S. aureus* and *E. coli* were still discernible and similar to those of the water and buffer controls (Table 3). It would have rendered a proteinaceous bacteriocin inactive. Similarly, activity was maintained between pH 3.0 and 7.0 and after heating at 121 °C for 10 minutes , barely decreasing at pH 9.0. When combined, these findings rule out a heat-labile peptide as the only active principle and are consistent with a small, heat-stable, non-proteinaceous antifungal compound. This class of metabolite is underreported in *L. plantarum* because protease sensitivity is typically assumed rather than tested. The most notable characteristic was the activity against *Candida albicans.* Under most circumstances, zones against the yeast were equivalent to or greater than those against the bacterial indicators (Tables 4–7). In contrast, organic acids are far less effective against yeasts than against bacteria. This level of antifungal activity cannot be solely attributed to acidity.

3.5 .Quantified activity

The baseline culture's CFS activity was 6400 AU/mL against *E. coli*, 3200 AU/mL against *S. aureus*, and 6400 AU/mL against *C. albicans*, which corresponded to specific activities of 2206.9 AU/mL, 1103.4 AU/ml, and 2206.9 AU/mL per OD_{600} unit, respectively (calculated based on baseline biomass OD_{600} = 2.9). The optimization conditions in Sections 3.7–3.9 are compared to these values.. The optimization conditions in Sections 3.7–3.9 are compared to these values.

3.6 .Response to dosage

At 0.05 mL, no inhibition was seen. The minimal inhibitory volume (MIV) was defined by the appearance of zones of 10–14 mm against all three indicators by 72 hours at 0.10 mL. For *E. coli* and *S. aureus*, the optimal inhibitory volume as described in Section 2.9 was 0.15 mL; however, for *Candida albicans*, the zone continued to expand to 0.20 mL (19 mm) before plateauing (Table 3). Zones grew with volume up to 0.25 mL, the maximum volume tested. The assay's diffusion-limited nature is confirmed by its monotonic reliance on volume.

3.7 .The influence of temperature and initial pH

At all temperatures, zones grew wider over the course of 72 hours. At 35–37 °C, the largest zones were found: 26 mm against *E. coli*, 21 mm against *S. aureus*, and 26 mm against *C. albicans* (Table 4). No one temperature is ideal for all three indicators, at 40 °C and 45 °C, when zones did not surpass 16 mm, activity drastically decreased. The same pattern was seen in biomass accumulation, which peaked between 32 and 37 °C at 72 hours. This is consistent with growth-associated production, while acid accumulation may also have played a role.

At 72 hours (26, 21, and 26 mm), the initial pH of 6.5 produced the largest zones against all three markers, which coincided with the highest biomass (OD_{600} 2.9). At pH 5.5 (12–13 mm), pH 7.0 (19–22 mm), and pH 8.0 (11–13 mm), zones were noticeably narrower, suggesting a limited optimum (Table 5).

3.8 .Carbon source

Activity against all three markers increased when lactose was substituted for MRS's glucose (Table 6). 3.0% (w/v) lactose produced 29 mm against *E. coli* and 30 mm

against *Candida albicans* after 72 hours, while 4.0% lactose produced 28 mm against *S. aureus* and 24, 27, and 19 mm against glucose at its optimal concentration (2.0%). The study's biggest effect was on the antifungal response, which increased by roughly 10 mm over glucose. Sucrose and sugarcane molasses provided zones of only 10–12 mm against *Candida albicans,* which were identical to the negative control, but they supported bacterial suppression up to 20 mm against *E. coli* and *S. aureus*. Without a comparable increase in antifungal activity, molasses maintained the highest biomass of any condition examined (OD_{600} 3.8 within 48 h at 4.0%). Given that the isolate ferments sucrose (Table 1), Despite the smaller inhibition zones, it is unlikely that the disaccharide's poor performance indicates a lack of utilization; rather, the sugar was probably consumed and directed toward cell biomass production rather than BLIS synthesis, as shown by the comparable (or higher) cell density and the noticeable drop in medium pH.

3.9 .The nitrogen source and the low cost formulation

Growth was encouraged by Sb meal extract, but it had minimal activity10-12 mm against both bacteria and no discernible suppression of *Candida albicans* at any dosage (Table 7). Both were backed with CSL. The zones at 72 hours were 20 mm against *E. coli,* 21 mm against *S. aureus*, and 20 mm against *C. albicans* at 2.0% (w/v); increasing CSL to 3.0% and 4.0% only increased the bacterial zones to 22 mm while leaving the antifungal zone unchanged; therefore, 2.0% is the lowest concentration achieving the maximal response and is the concentration of practical interest. Zones of 23, 23, and 30 mm against *E. coli, S. aureus,* and *Candida albicans*, respectively, were obtained by combining 2% CSL with 3% lactose in an otherwise MRS-based formulation. These zones were statistically identical to MRS broth at the same time points (23, 23, and 30 mm; Table 8).

Table 8 demonstrates that under the ideal growth conditions (35 °C, pH 6.5) with 3% galactose, BLIS activity was significantly impacted by CSL concentration. 1% CSL had the lowest activity (inhibition zones of 16–20 mm). Raising CSL to 2% significantly increased activity, but increases to 3% and 4% did not result in any further improvement against the tested bacteria (20-23 mm). This suggests that the nitrogen supply had achieved saturation, making 2% CSL the most cost-effective optimum. The greatest and most stable inhibition zones were produced when 2% CSL and 3% galactose were combined. These zones grew or stayed the same for up to 72 hours (21–23 mm against *E. coli* and *S. aureus*), which is consistent with the buildup of a stable extracellular inhibitory substance. In comparison to the zones achieved with higher CSL levels (19-20 mm) and with glucose as the carbon source (18-20 mm), this combination was particularly efficient against *C. albicans* (28-30 mm). Because galactose bypasses glucose-mediated catabolite suppression and reroutes metabolism toward inhibitory metabolite formation, this shows that the type of carbon source, rather than nitrogen content alone, affects the level of anti-Candida effectiveness. The control medium, on the other hand, displayed minimal activity at 24 hours (10-15 mm) that only increased after 48 hours, indicating delayed and decreased production under suboptimal nutritional conditions. In comparison to the control, medium optimization

enhanced bacterial inhibition by up to 6 mm and anti-Candida efficacy by around two times.

As a result, The costly peptone, beef extract, and yeast extract of the typical commercial MRS broth were effectively substituted by the optimized medium that was created with 2% CSL and 3% lactose. The optimized low-cost medium cost only $3.62 per liter, while the normal MRS broth's preparation cost was estimated to be $14.50 per liter based on 2026 supplier pricing. With no appreciable loss of antibacterial or antifungal action, this formulation significantly reduced costs per liter by 75.03% as compared to the conventional MRS medium.(Table 9).

3.10 .Safety-relevant traits

NEOM-LP1 thrived in MRS broth adjusted to pH 3.0–6.0 and in the presence of oxgall up to 2.0% (w/v), however it did not induce any hemolysis on human blood agar (gamma-hemolysis). The strain was resistant to vancomycin, a well-known intrinsic characteristic of the L. plantarum group, but susceptible to ampicillin, gentamicin, and erythromycin among the tested panel defined by the EFSA. These are preliminary results; virulence factors and biogenic amine production were not examined, and the absence of hemolysis does not establish safety.

4 .Discussion

4.1 .An antifungal principle that is heat-stable and resistant to proteases instead of bacteriocins

The study's most significant finding is a negative one: NEOM-LP1's inhibitory action withstood digestion by three proteases with varying specificities. The operational test that separates lactic acid bacteria (LAB) bacteriocins from organic acids and other metabolites is their loss of activity during proteolysis (Arena et al., 2016; Twomey et al., 2021). Therefore, a class I or class II bacteriocin is not an activity that continues after proteolysis and after 10 minutes at 121 °C. There are still two possible explanations: either a peptide that is exceptionally resistant to these enzymes, which is implausible across three cleavage specificities, or a non-proteinaceous metabolite.

The first explanation is supported by the spectrum. Gram-positive membranes are the main target of characterized plantaricins, while organic acids are more frequently responsible for their activity against Gram-negative targets (Arena et al., 2016). Organic acids are far less effective against yeasts, and NEOM-LP1 inhibited *C. albicans* just as effectively as it inhibited *E. coli*, which is predicted of an acid action in a crude acidic supernatant. Thus, in addition to acidity, there seems to be an antifungal component. There are known antifungal metabolites of L. plantarum, such as hydroxy fatty acids, cyclic dipeptides, and phenyllactic acid, some of which are heat stable and protease resistant. Therefore, the critical next step is to identify the component, which can be done using mass spectrometry and standard chromatography.

4.2 .The isolate's ecophysiology

For *L. plantarum*, the physicochemical profile is permissive rather than selective. Strains of the species that grow in medium containing 5–8% NaCl may resist salinities of 39–43 g/L, and marine isolates have been reported on multiple occasions (Frece et al., 2018; Du et al., 2025). Because the species is aerotolerant and facultatively

heterofermentative anaerobe, temperature and pH are also compatible with growth; hence, the low dissolved oxygen would reduce rather than eliminate competition from obligatory aerobes (Kleerebezem et al., 2003). The most economical explanation is that NEOM-LP1 is allochthonous-introduced from a terrestrial or avian source-rather than a resident member of the marine community, given the dominance of Enterobacterales among the ten isolates, which is a sign of fecal or sewage influence. The lack of nutrient, organic-load, and faecal-indicator measurements in this study makes it impossible to identify the source, and the isolate should not be described as indigenous to the Red Sea.

4.3 .Uncoupling of growth and metabolite production

The substrates separated into three behavioural classes. Molasses gave the highest biomass of any condition tested and yet failed to inhibit the yeast, reproducing the uncoupling of growth from secondary-metabolite yield that is characteristic of LAB (De Vuyst et al., 1996; Leroy and De Vuyst, 1999). Soybean meal extract behaved similarly, supporting growth with almost no activity, as expected of a nitrogen source rich in peptides but poor in the precursors or cofactors the pathway requires.

4.3 .The generation of metabolites and growth are not related

Three behavioral classes emerged from the substrates. The uncoupling of growth from secondary-metabolite yield that is typical of LAB was replicated by molasses, which produced the maximum biomass of any environment examined but failed to suppress the yeast (De Vuyst et al., 1996; Leroy and De Vuyst, 1999). Similar to a nitrogen source rich in peptides but deficient in the precursors or cofactors the pathway needs, Sb meal extract supported growth with virtually little activity. Both were modestly and evenly supported by CSL. The most cost-effective explanation, which is qualitative rather than quantitative, is that CSL provides directly usable forms of soluble organic nitrogen, free amino acids, minerals, and B vitamins.

4.4 .Catabolites regulation of lactose and other carbon sources

The antifungal zone increased from 19–20 mm to 30 mm when lactose was substituted for glucose; this was the biggest single boost in activity, not temperature or pH. Since lactose is a less effective carbon source for this strain than glucose, the slower glycolytic flow it imposes may alleviate the biosynthetic pathway's carbon catabolite repression, a mechanism that has been well documented for the formation of bacteriocin in LAB (De Vuyst et al., 1996; Gänzle et al., 1999). Instead of the descriptive treatment that is feasible here, a dedicated research with transcriptional or metabolic readout is warranted because the effect is significant and consistent across concentrations.

4.5. Practical implications:

Two findings have practical implications:

The first is that 2% CSL, when combined with lactose, replaced the peptone, beef extract, and yeast extract of MRS with no discernible loss of activity, addressing the main cost barrier to LAB fermentation at scale (Trinetta et al., 2008; Wiese et al., 2010; Tchabou Tientcheu et al., 2025). The second is the antifungal activity, which is rare in *L. plantarum* and could be useful for food preservation if the compound turns out to be a defined metabolite. The economic claim is now restricted to the medium's price;

downstream recovery, purification losses, and capital costs are not included in the analysis, and a thorough techno-economic evaluation would be necessary before the method is characterized as less expensive rather than less expensive to prepare.

4.6 .Identification limits

L. plantarum, L. pentosus, and *L. paraplantarum* share nearly identical 16S sequences and are typically only distinguished by recA or gyrB sequencing, multiplex PCR, or whole-genome comparison. A 100% match over a partial 16S rRNA sequence establishes membership of the L. plantarum group but not of the species (Torriani et al., 2001). Here, NEOM-LP1 is utilized as an isolate identification; it does not imply any particular lineage. The question would be answered by species-level assignment using the recA multiplex assay or by average nucleotide identity from whole-genome data, which would also enable the strain's biosynthetic gene clusters to be examined for potential antifungal pathways. This analysis would replace speculation about the active compound with a testable prediction.

4.7. Effect of CSL Concentrations and 3% Galactose on BLIS Production by L. plantarum NEOM-LP1

Table 8 demonstrates that under the ideal growth conditions (35 °C, pH 6.5) with 3% galactose, BLIS activity was significantly impacted by CSL concentration. For Lactobacillus cultivation, CSL, a cheap by-product high in vitamins, minerals, and amino acids, has been employed as a low-cost source of nitrogen instead of peptone and yeast extract (Manzoor et al., 2017). Additionally, it has helped lactic acid bacteria produce BLIS and bacteriocin (Coetzee et al., 2007; Shahrir et al., 2024). The lowest activity was found with 1% CSL (16–20 mm), indicating that BLIS biosynthesis and growth were both limited by nitrogen. Increasing CSL to 2% significantly increased activity, but 3% and 4% produced little additional benefit against the studied bacteria (20–23 mm), suggesting nitrogen saturation. It has also been demonstrated that balanced nitrogen and sugar supply, rather than just CSL content, determines bacteriocin output in CSL-based media (Coetzee et al., 2007). The greatest and most stable inhibitory zones were produced by combining 2% CSL with 3% galactose. These zones grew or stayed the same for up to 72 hours (21–23 mm against E. coli and S. aureus), and they were particularly efficient against Candida albicans (28–30 mm vs. 18–20 mm with glucose). Although galactose can also cause catabolite repression in some lactic acid bacteria (Filimon et al., 2024), this explanation is still speculative. The superior activity with galactose, which is metabolized through the Leloir pathway (Fiocco et al., 2019), may therefore reflect weaker glucose-type repression. In L. plantarum, the effects of carbon and nitrogen combinations on growth and bacteriocin activity are likewise strain-dependent (Zheng et al., 2024). The control medium, on the other hand, displayed minimal activity at 24 hours (10–15 mm) that only increased after 48 hours, indicating delayed and decreased production under suboptimal nutritional conditions.

5 .Conclusion

A cell-free supernatant produced by a *L. plantarum* isolate from NEOM coastal water inhibited S. aureus*, E. coli,* and, surprisingly, *Candida albicans*. The activity was heat

stable to 121 °C, retained between pH 3.0 and 7.0 and not abolished by proteinase K, trypsin or pepsin, which precludes a classical bacteriocin and suggests a heat-stable, protease-resistant antifungal principle as the topic of further study.

Declarations

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Author contributions:

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

Ethics approval

The study did not involve human participants or animal experimentation. Human blood-bank material was used solely as a supplement of the hemolysis assay medium, under [UT-SCI-2026-0514]. All microbiological work was performed under biosafety level 2 conditions.

Data availability

The 16S rRNA sequence is deposited in GenBank under accession QB034968.

All the data is available with the authors and shall be provided upon request

Conflicts of interest :

The authors report no financial or any other conflicts of interest in this work.

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727

Tables

Table 1. Phenotypic characteristics of isolate NEOM-LP1.

Test	Result
Gram reaction	Positive
Cell morphology	Rod (bacillus)
Motility /	Negative
Spore formation	Negative
Oxygen requirement	Facultative anaerobe, aerotolerant
Catalase	Negative
Oxidase	Negative
Indole	Negative
Nitrate reduction	Negative
Fermentation of glucose	Positive
Fermentation of fructose	Positive
Fermentation of sucrose	Positive
Fermentation of galactose	Positive
Fermentation of maltose	Positive

Table 2. Molecular identification of isolate NEOM-LP1 by partial 16S rRNA sequencing.

Parameter	Result
Closest database match	*Lactiplantibacillus plantarum* SRCM100442 (NZ_CP028221.1)
Query coverage	100%
Sequence identity	100%
GenBank accession	QB034968
Deposited sequence length	698 bp
Interpretation	Member of the L. plantarum group; species-level confirmation requires recA or whole-genome data

Table (3): The effect of MIV values on BLIS production as inhibition zone (mm)

Culture filtrate conc.(ml)	*Escherichia coli*			*Staphylococcus aureus*			*Candida albicans*		
	24h	48h	72h	24h	48h	72h	24h	48h	72h
0.05	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
0.10	10±0.1	11±0.1	11±0.05	10±0.1	12±0.07	12±0.1	12±0.1	14±0.07	14±0.07
0.15	13±0.1	15±0.1	15±0.1	14±0.13	16±0.12	16±0.12	15±0.15	16±0.1	16±0.15
0.20	16±0.1	16±0.2	18±0.05	14±0.1	15±0.2	20±0.15	14±0.12	15±0.15	19±0.14
0.25	16±0.1	16±0.1	17±0.1	14±0.1	16±0.1	20±0.1	15±0.15	15±0.13	19±0.10

Table (4): The effect of different incubation temperatures values on BLIS activity as inhibition zone (mm)

Temp.	*E. coli*			*S. aureus*			*Candida albicans*		
	24h	48h	72h	24h	48h	72h	24h	48h	72h
25°C	9±0.15	12±0.2	13±0.05	-ve	-ve	13±0.10	11±0.15	12±0.11	12±0.10
28 -30°C	14±0.12	17±0.15	23±0.14	14±0.15	18±0.2	15±0.15	11±0.15	14±0.2	23±0.15
35 -37°C	16±0.15	16±0.2	26±0.11	16±0.11	19±0.12	21±0.13	15±0.12	15±0.12	26±0.1
40°C	13±0.13	13±0.12	13±0.14	11±0.11	12±0.12	16±0.11	10±0.11	11±0.11	11±0.12
45°C	12±0.14	12±0.15	12±0.05	10±0.15	11±0.11	12±0.12	10±0.14	11±0.11	10±0.12
control	15±0.13	20±0.11	20±0.11	15±0.13	20±0.14	20±0.11	10±0.15	13±0.15	14±0.10

Positive control; streptomycin 15 µg for bacteria and fluconazole 150 µg for yeast fungus.
Negative control; uninoculated broth culture medium

Table (5): The effect of different pH values on BLIS activity as inhibition zone (mm).

pH	*E. coli*			*S. aureus*			*Candida albicans*		
	24h	48h	72h	24h	48h	72h	24h	48h	72h
5.5	9±0.11	10±0.5	13±0.12	9±0.12	10±0.11	13±0.15	11±0.11	11±0.12	12±0.13
6.0	13±0.11	14±0.4	15±0.12	11±0.12	13±0.15	15±0.15	12±0.12	13±0.11	15±0.12
6.5	17±0.11	19±0.2	26±0.16	16±0.12	19±0.15	21±0.15	15±0.12	15±0.11	26±0.5
7.0	16±0.12	18±0.15	22±0.15	16±0.13	19±0.13	20±0.15	14±0.11	15±0.11	19±0.4
7.5	15±0.11	15±0.14	16±0.14	17±0.12	17±0.14	19±0.15	13±0.11	13±0.12	14±0.13
8.0	12±0.12	13±0.15	13±0.15	11±0.11	12±0.12	12±0.12	10±0.12	11±0.11	11±0.11

Positive control; streptomycin 15 µg for bacteria and fluconazole 150 µg for yeast fungus.
Negative control; uninoculated broth culture medium

Table (6): Effect of carbon source and concentration on the inhibitory activity of cell-free supernatant from NEOM-LP1 (zone of inhibition, mm).

Carbon source	*E. coli*			*S. aureus*			*Candida albicans*		
	24h	48h	72h	24h	48h	72h	24h	48h	72h
glucose									
1%	13±0.15	14±0.15	16±0.10	13±0.12	14±0.12	20±0.1	13±0.1	14±0.1	16±0.1
2%	15±0.13	15±0.15	24±0.12	16±0.12	19±0.15	27±0.1	15±0.1	15±0.1	19±0.1
3%	15±0.12	17±0.14	19±0.12	17±0.12	19±0.15	27±0.1	15±0.1	15±0.1	19±0.1
4%	15±0.13	16±0.12	18±0.13	17±0.11	18±0.12	25±0.1	15±0.1	15±0.1	19±0.1
lactose									
1%	13±0.15	19±0.12	14±0.13	14±0.13	20±0.15	15±0.1	15±0.1	23±0.1	25±0.1
2%	13±0.2	20±0.13	27±0.14	21±0.11	20±0.12	20±0.1	17±0.1	22±0.1	28±0.1
3%	14±0.13	24±0.11	29±0.14	25±0.12	20±0.13	20±0.1	17±0.1	22±0.1	30±0.1
4%	15±0.12	22±0.13	26±0.14	16±0.12	21±0.12	28±0.1	17±0.1	22±0.1	30±0.1
sucrose									
1%	15±0.11	17±0.14	18±0.15	17±0.12	19±0.15	20±0.1	10±0.1	11±0.1	11±0.1
2%	18±0.12	20±0.12	20±0.12	18±0.12	18±0.13	20±0.1	10±0.1	12±0.1	11±0.1
3%	18±0.11	20±0.15	20±0.16	18±0.11	19±0.12	20±0.1	12±0.1	11±0.1	11±0.1
4%	18±0.11	20±0.15	20±0.15	19±0.11	19±0.12	20±0.1	11±0.1	11±0.1	11±0.1
sugar cane molasses									
1%	13±0.1	20±0.1	20±0.1	15±0.1	13±0.1	15±0.1	10±0.1	11±0.1	10±0.1
2%	13±0.1	17±0.1	21±0.1	15±0.1	14±0.1	16±0.1	10±0.1	12±0.1	12±0.1
3%	13±0.1	17±0.1	17±0.1	15±0.1	16±0.1	17±0.1	11±0.1	12±0.1	12±0.1
4%	13±0.1	17±0.1	18±0.1	14±0.1	16±0.1	17±0.1	11±0.1	12±0.1	12±0.1

Positive control; streptomycin 15 µg for bacteria and fluconazole 150 µg for yeast fungus.
Negative control; uninoculated broth culture medium

Table (7): Effect of nitrogen source and concentration on the inhibitory activity of cell-free supernatant from NEOM-LP1 (zone of inhibition, mm).

Protein source	*E. coli*			*S. aureus*			*Candida albicans*		
	24h	48h	72h	24h	48h	72h	24h	48h	72h
Soy bean (%)									
1	10±0.05	10±0.12	11±0.12	10±0.11	11±0.12	12±0.13	-ve	-ve	-ve
2	10±0.10	11±0.11	12±0.12	10±0.12	11±0.11	12±0.12	-ve	-ve	-ve
3	10±0.10	11±0.12	12±0.11	10±0.12	11±0.11	12±0.13	-ve	-ve	-ve
4	10±0.15	11±0.12	12±0.12	10±0.11	12±0.12	12±0.13	-ve	-ve	-ve
Corn steep liquor (%)									
1	20±0.11	18±0.12	17±0.11	20±0.11	19±0.11	19±0.11	16±0.11	17±0.12	17±0.11
2	20±0.13	22±0.11	20±0.11	22±0.12	21±0.11	21±0.11	18±0.12	20±0.12	20±0.12
3	20±0.12	22±0.11	22±0.11	22±0.11	21±0.11	22±0.12	18±0.11	20±0.12	20±0.11
4	20±0.13	22±0.12	22±0.11	22±0.11	21±0.13	22±0.12	18±0.11	20±0.12	20±0.11

Positive control; streptomycin 15 µg for bacteria and fluconazole 150 µg for yeast fungus.
Negative control; uninoculated broth culture medium

Table 8: The effect of using 1-4% CSL and 3% lactose under optimized growth conditions (Temp.35°C & pH 6.5) on BLIS activity by *L. plantarum* -NEOM LP1as inhibition zone diameter(mm).

Protein source	*E.coli*			*Staph. Aureus*			*Candida albicans*		
	24h	48h	72h	24h	48h	72h	24h	48h	72h
Galactose 3 %									
1% CSL	20±0.11	18±0.12	18±0.11	20±0.11	20±0.11	20±0.11	16±0.11	18±0.12	18±0.11
Optimized MRS broth*	21±0.11	22±0.12	23±0.11	21±0.11	21±0.11	23±0.11	28±0.11	30±0.12	30±0.11
3% CSL	21±0.12	22±0.10	23±0.12	22±0.10	21±0.11	22±0.12	19±0.10	20±0.10	20±0.11
4% CSL	20±0.13	22±0.10	22±0.10	22±0.10	21±0.10	22±0.12	19±0.10	20±0.12	20±0.10
2% CSL+ standard 2% glucose	20±0.13	22±0.11	20±0.11	22±0.12	21±0.11	21±0.11	18±0.12	20±0.12	20±0.12
Control (un optimized)	15±0.13	20±0.11	20±0.11	15±0.13	20±0.14	20±0.11	10±0.15	13±0.15	14±0.10

Positive control; streptomycin 15 ug for bacteria and fluconazole 150 ug for yeast fungus.
Negative control; uninoculated broth culture medium
Optimized MRS broth*; it is MRS broth medium using 2%CSL & 3%lactose instead of both N & C sources.

Table 9: Comparative overview of BLIS production by *L. plantarum* -NEOM LP1 and other reported strains.

Strain	**Isolation Source**	**Cultured Medium**	**Optimized Conditions**	**Potency**	**Target microorganisms**	**Thermal stability**	**Novelty/ Key Contributions**
NEOM-LP1 (this study)	Red Sea coastal water, NEOM	MRS with 2% CSL; 3% lactose	35–37 °C, pH 6.5, 72 h	Not determined	*E. coli, S. aureus, C. albicans*	Stable at 121 °C, 10 min	High antifungal efficiency utilizing hyper-low-cost marine /agro-industrial inputs
ST16Pa	Waste milk	MRS	30 °C, pH 6.0	3200 AU/mL	*L. monocytogenes, Enterococcus spp.*	Stable at 100 °C, 30 min	Standard commercial medium
ZDY2013	Fermented acid milk	MRS	37 °C, pH 6.5	1600 AU/mL	*S. aureus*	Stable up to 80 °C	Probiotics characterization with moderate thermal tolerance
F-10	Fermented olives	Whey permeate	30 °C, pH 5.5	2500 AU/mL	*B. cereus*	Stable at 121 °C, 15 min	Cost reduction using whey, but lacked potent antifungal spectrum

Figure 1: Geographic map showing the sampling sites of NEOM region



Figure 2: Phylogenetic Tree of Query Sequence and Closely Related Strains

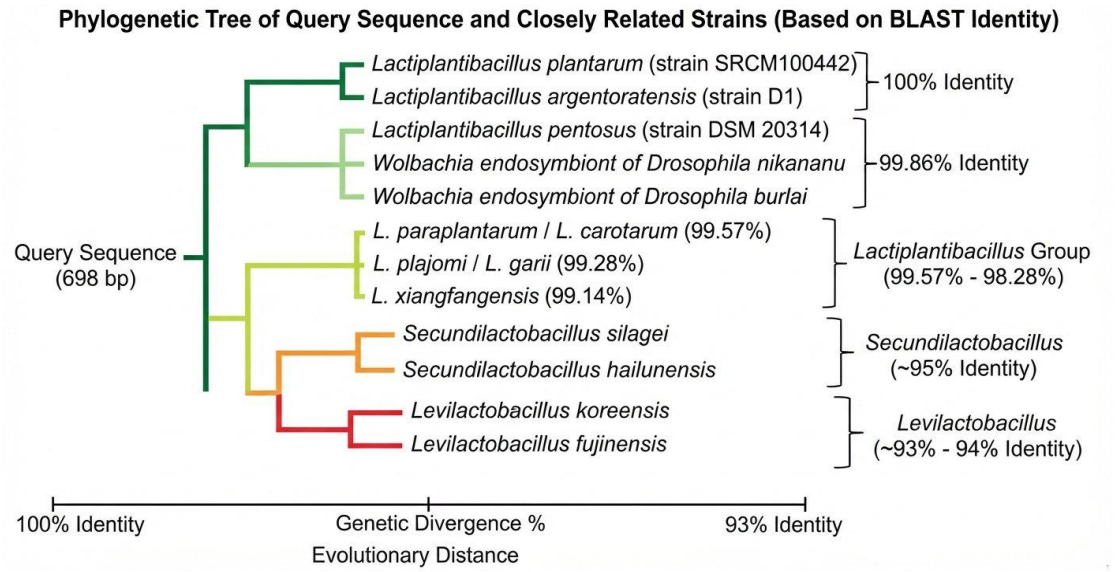


Figure 3: The effect of different incubation temperatures on *L. plantarum-NEOM-LP1* cell growth

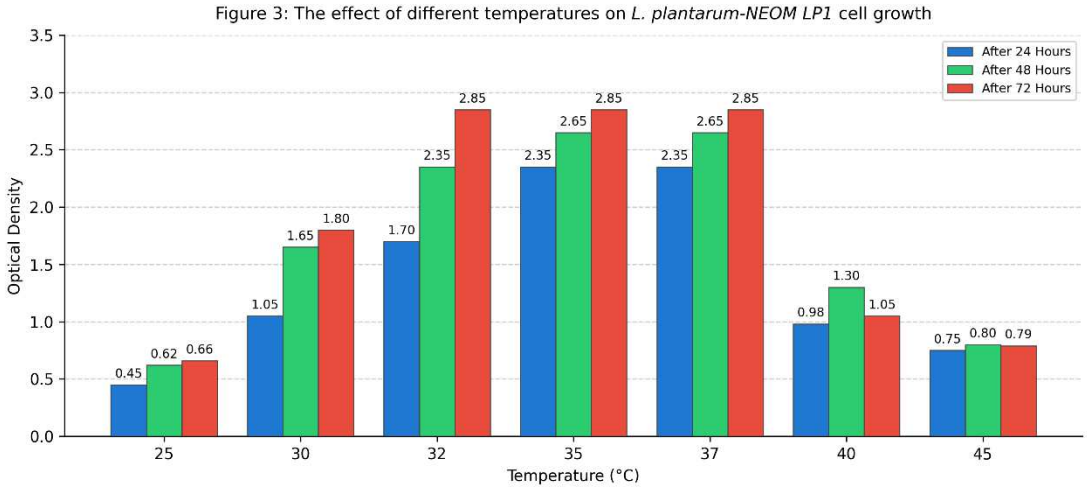


Figure 4: The effect of different initial pH values on *L. plantarum-NEOM LP1* cell growth

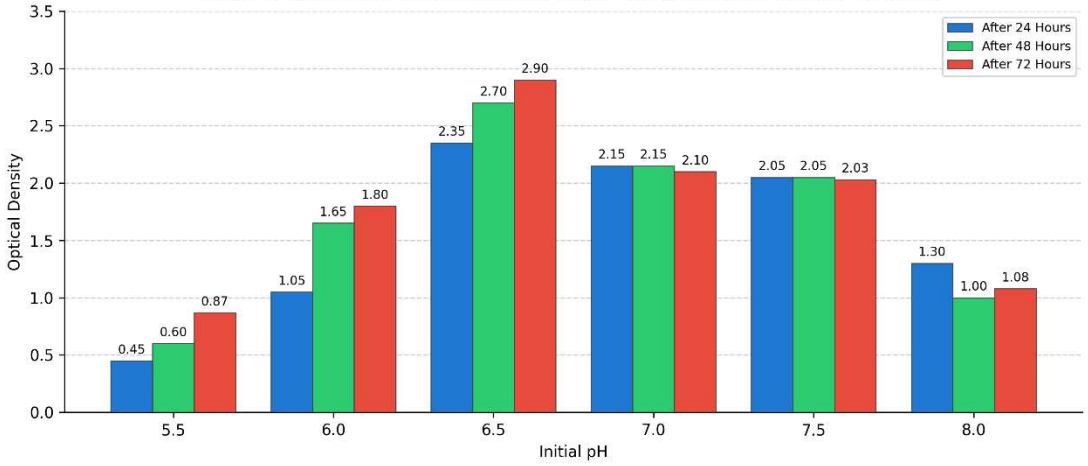


Figure 5: The effect of different glucose concentrations on *L. plantarum-NEOM LP1* cell growth

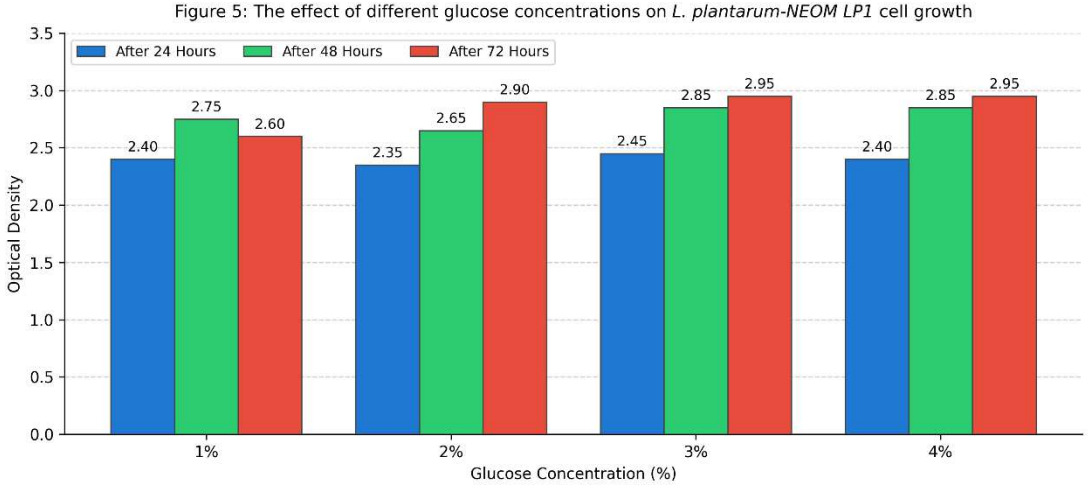


Figure 6: The effect of different lactose concentrations on *L. plantarum*-NEOM LP1 cell growth

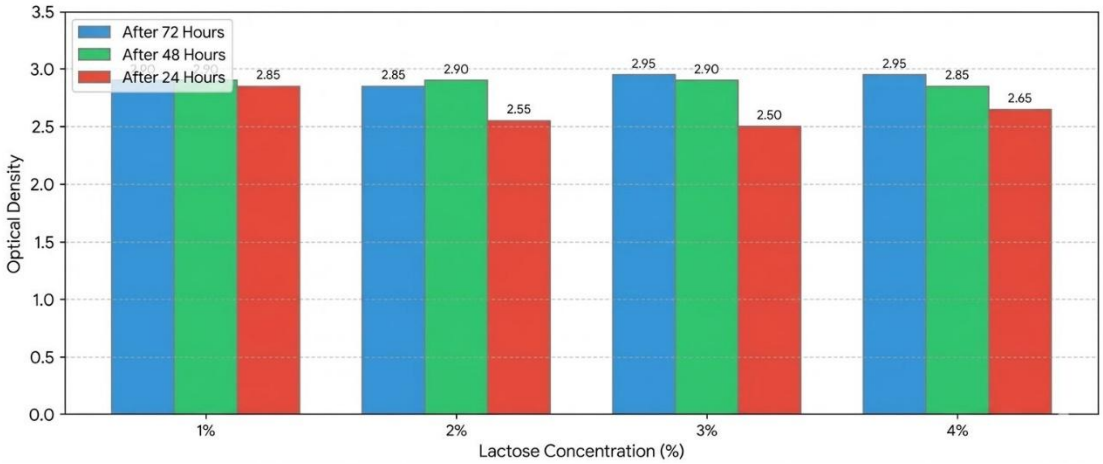


Figure 7: The effect of different sucrose concentrations on *L. plantarum*-NEOM LP1 cell growth

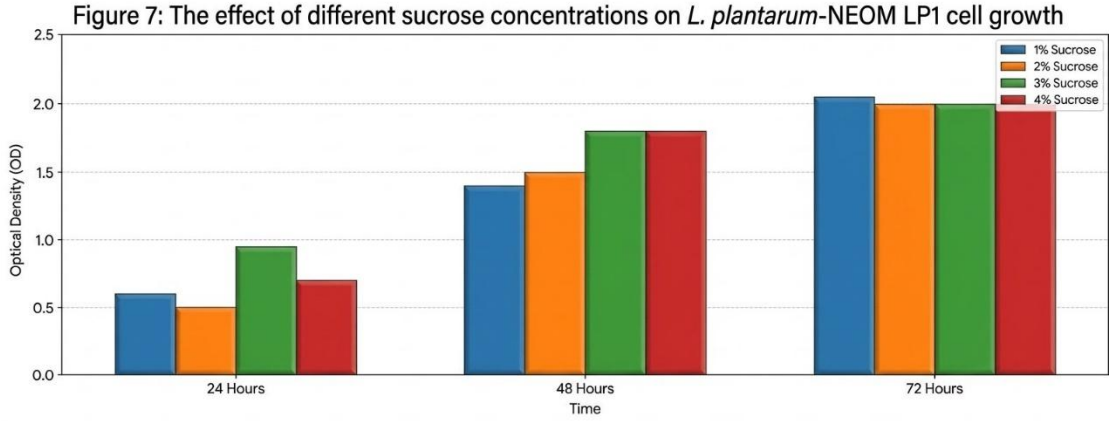


Figure 8: The effect of different cane sugar molasses concentrations on *L. plantarum*-NEOM LP1 cell growth

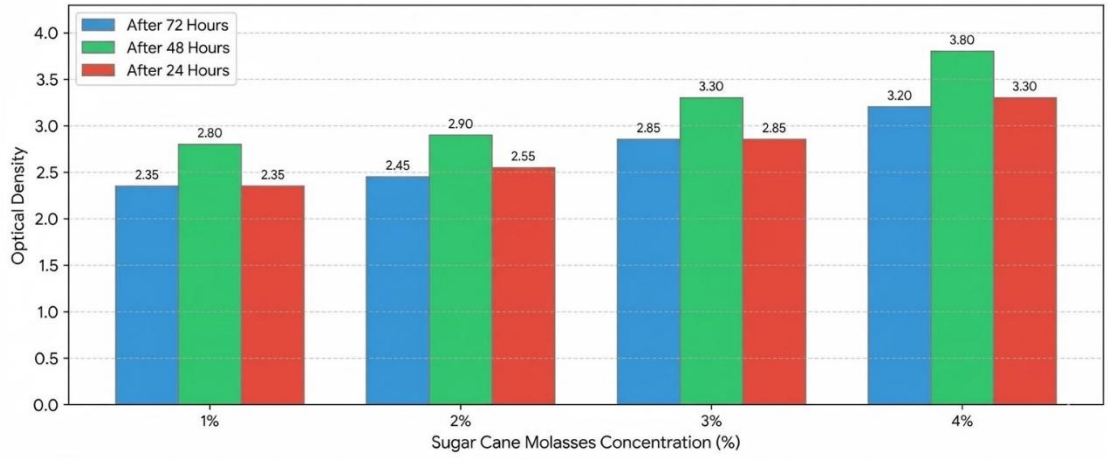
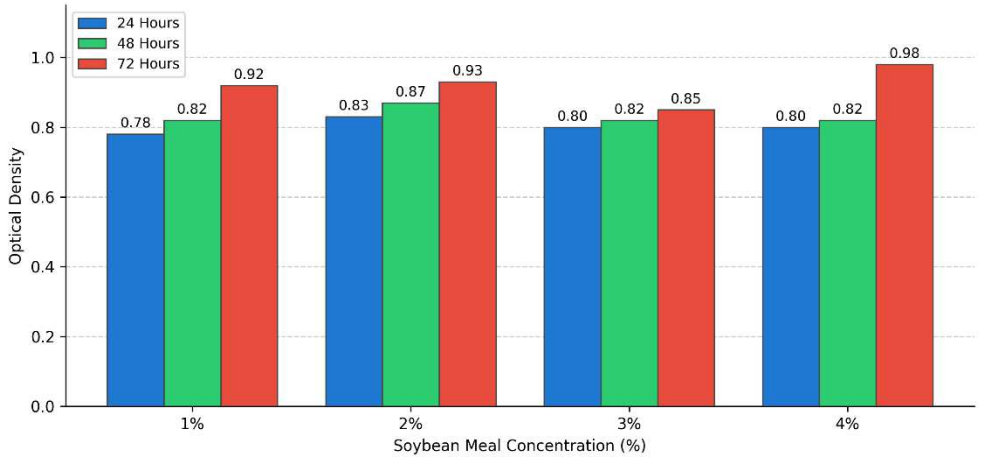
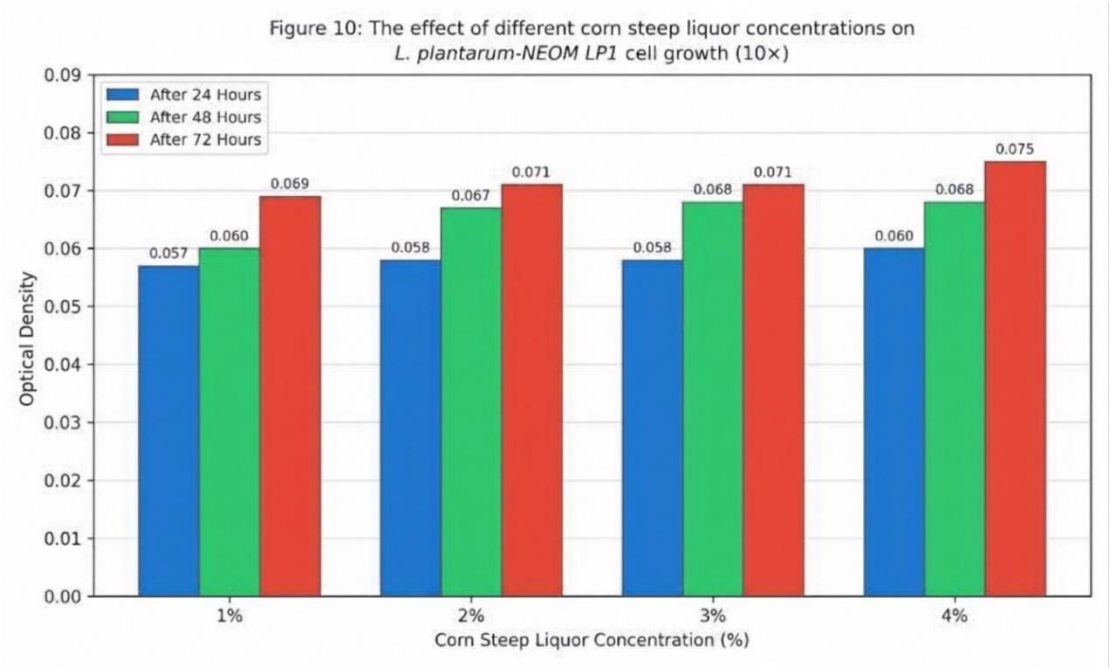


Figure 9: The effect of different soybean meal concentrations on the cell growth of *L. plantarum-NEOM LP1* (diluted 10×)




Figure 10: The effect of different corn steep liquor concentrations on
L. plantarum-NEOM LP1 cell growth (10×)
After 24 Hours
After 48 Hours
After 72 Hours
Optical Density
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
0.057
0.060
0.069
0.058
0.067
0.071
0.058
0.068
0.071
0.060
0.068
0.075
1%
2%
3%
4%
Corn Steep Liquor Concentration (%)

805