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OPTIMIZATION STUDIES OF BACTERIA ISOLATED FROM MARINE WATER FOR BIOACTIVE COMPOUNDS

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ABSTRACT

The objective of this research was to identify and select the bacteria from samples of marine water that produce antimicrobial agents and to optimize different growth conditions for the production of a significant quantity of antibacterial metabolites that are effective against the test pathogens. The present study focus on the isolation of bacteria from marine water of Bheemli beach Bay of Bengal, Visakhapatnam, Andhra Pradesh, India. The isolated bacteria were labelled initially as C1, C6 and C7 identified by 16S rRNA sequencing as *Bacillus paramycoides* (*Bp*), *Lysinibacillus fusiformis* (*Lf*) and *Bacillus oshimensis* (*Bo*). These bacteria had shown to be the most potent strains in exhibiting the considerable growth inhibition of the test organisms. By adjusting one parameter at a time, the effects of factors such as temperature, pH, incubation time, sodium chloride concentration and the effect of carbon and nitrogen sources on the production of antibacterial metabolites were examined. It was observed that environmental factors had a significant impact on the isolate's ability to secrete metabolites. The suitable conditions for optimum synthesis of bioactive compounds were identified as incubation period for *Bacillus paramycoides* (72 hrs), *Lysinibacillus fusiformis* (96 hrs) and *Bacillus oshimensis* (72 hrs) and other conditions of pH 7.0, temperature 30° C, NaCl 2% concentration with the nutritional components of ribose as

carbon source and sodium nitrate as nitrogen source were same for all these three isolates. The results of the study showed that the growth of test microorganisms was inhibited by these isolated bacterial strains namely *Bacillus paramycoides, Lysinibacillus fusiformis* and *Bacillus oshimensis* in the primary screening and the inhibition was further increased by optimizing the conditions required for the cultivation.

Keywords: Antibacterial activity, Bioactive compounds, Marine bacteria, Optimization

1. INTRODUCTION

Since the dawn of the 21st century, microbial secondary metabolites have played a crucial role in advancing modern medicine, enabling the treatment of a wide range of diseases. These compounds, while not essential for basic survival under laboratory conditions, provide significant ecological advantages in natural environments **[1]**. Secondary metabolites serve multiple functions in microbial ecology include signalling molecules that facilitate communication between organisms within ecosystems **[2]**; protective agents that help microbes defend themselves against competitors or predators **[3]**; mediators that influence relationships between different species in the ecosystem **[4]**; components in programmed cell death **[5]**; chemical defense enhancers **[6]**.

The multifaceted roles of these compounds in nature have made them valuable resources for pharmaceutical research and development that inspire new approaches in drug discovery and design **[7]**. Extensive research has been conducted to identify, isolate and optimize the production of secondary metabolites from various natural sources, with a particular emphasis on microorganisms due to the immense biodiversity of microbes and their ability to produce a wide range of bioactive compounds **[8]**. The primary goal of these studies has been to harness the compounds especially in three key areas such as Antitumor agents (Doxorubicin) **[9]**; Anti-inflammatory substances (Cyclosporin A) **[10]**; Antibiotics (Penicillin, Streptomycin) **[11]**. For instance, salinosporamide A compound, isolated from the marine actinomycete *Salinispora tropica*, is a potent proteasome inhibitor **[12, 13]**. Dolastatin 10, originally isolated from sea hares but later found to be produced by marine cyanobacteria, led to the development of the anticancer drug brentuximab vedotin **[14]**. Kahalalide F, cyclic depsipeptide produced by a green alga-associated bacterium has shown potential as an anticancer and anti-infective agent **[15]**. Largazole isolated from a marine cyanobacterium, which is a histone deacetylase inhibitor has shown promise in cancer treatment **[16]**.

Key factors that influence the synthesis of bioactive compounds include: nutrient availability such as carbon, nitrogen, and phosphorus sources **[17]**; pH, temperature, oxygen levels and salinity **[18]**. To optimize culture conditionor maximum productivity, effective techniques are designed including one-factor-at-a-time (OFAT), involves varying one factor while keeping others constant **[19]**.

Bacillus species demonstrate exceptional resilience to a wide range of environmental stressors, include extreme temperatures of both high and low, elevated salinity levels, varied pH values, immense pressures and scarcity of essential nutrients **[20]**. A study by Ramachandran *et al*., 2022 **[21]** found that *Bacillus* species isolated from marine sediments in the Bay of Bengal were screened for antioxidant and cytotoxic activities against human breast cancer cell lines.

Few examples of antibacterial compounds from marine *Bacillus* species include: Bacillamide C isolated from a marine *Bacillus* sp. **[22]**. It was found that antibacterial metabolites, such as Lichenin, Bacillocin 490, and P40 are three bacteriocin-like peptides from *Bacillus licheniformis* **[23]**. Macrolactin F, 7-O-succinylmacrolactin F, and 7-O-succinylmacrolactin A compounds were identified from *Bacillus* sp. Sc026 **[24]** and novel thiopeptide compounds were found from *Bacillus cereus* QN03323 **[25]**. The goal of this study is to isolate secondary metabolite producing bacteria from the marine environment and evaluate their potential by studying the impact of various physical parameters on the highest possible production of antibacterial metabolites.

2. MATERIALS AND METHODS

2.1 Secondary metabolite producing bacterial isolates

Bacillus paramycoides, Lysinibacillus fusiformis and *Bacillus oshimensis* were isolated from the marine water samples collected from the coast of Bheemli Beach, Visakhapatnam, Bay of Bengal, Andhra Pradesh. The antibacterial activity of these marine isolates was tested using agar well method. These isolates were characterized up to the genus level by comparing it with Bergey's manual of systematic bacteriology **[26]**. 16S rRNA sequencing was used to identify the phylogenetic relationship of the bacterial isolates.

2.2 Effect of different parameters on metabolite production

The isolate's ability to produce antibacterial metabolites under environmental culture conditions was assessed. The study examined a few characteristics, including temperature, pH, incubation period, sodium chloride and nutritional components of carbon and nitrogen. Each result shown is the mean ± standard deviation of three separate analyses.

2.2.1 Effect of temperature

To determine the optimal temperature for antibacterial metabolite production, the isolates were cultured in production medium at various temperatures: 30°C, 35°C, 40°C and 45°C while other parameters were kept at their optimal conditions. The temperature that resulted in the largest inhibition zone against the test organism was considered optimal for antibacterial metabolite yield.

2.2.2 Effect of pH

By varying the pH of the production medium between 2 to 9, the effect of pH on the strain's production of antibacterial metabolites was ascertained. The pH value that resulted in the highest yield of antibacterial metabolites was identified as the optimal pH for the strain.

2.2.3 Impact of incubation time

Conical flasks (250ml) containing 100 ml of Zobell broth inoculated with active isolates of *Bacillus paramycoides*, *Lysinibacillus fusiformis* and *Bacillus oshimensis* and kept in shake flask fermentation. For maximum yields, the flasks were incubated at 30°C for several durations of time (24, 48, 72, 96 and 120 hrs) on a rotary shaker revolving at 200 rpm. The flasks were collected; the crude product was removed at each interval and the production of antibacterial metabolite was assessed against the test organisms.

2.2.4 Effect of salt concentration

Sodium chloride has a significant impact on the synthesis of antimicrobial compounds by microorganisms as it affects the osmotic pressure of the medium, The production medium was incubated with active isolates of *Bacillus paramycoides*, *Lysinibacillus fusiformis* and *Bacillus oshimensis* at various concentrations ranging from 1.0% to 5.0% in order to find the optimal concentration of NaCl for antibacterial metabolite yield keeping the optimized parameters of temperature, pH and incubation time.

2.2.5 Effect of carbon sources

The effect of different carbon sources with 1% concentration on the production of antibacterial metabolites was examined by employing monosaccharide pentose sugar (ribose), hexose sugars (glucose & fructose), disaccharide sugar (lactose) and polysaccharide sugar (starch). The production medium was inoculated with the active isolates of *Bacillus paramycoides*, *Lysinibacillus fusiformis* and *Bacillus oshimensis* aseptically and incubated at 30°C for 72 hrs (*B. paramycoides* and *B.oshimensis*) and 96 hrs (*L. fusiformis*) with 2% NaCl at pH 7. After incubation the growth was measured and the extract was centrifuged to obtain cell free extract which was used to test antibacterial activity against the test organisms.

2.2.6 Effect of nitrogen sources

Different nitrogen sources including both inorganic ($NaNO_3$, NH_4OH, NH_4Cl) and organic (urea and yeast extract) at 1% were used to assess the effect of nitrogen sources on antibacterial metabolite production by *Bacillus paramycoides*, *Lysinibacillus fusiformis* and *Bacillus oshimensis.* The medium mixed with these nitrogen sources was inoculated with the isolates, incubated at previously optimized conditions. After incubation, the growth was measured and the broth was centrifuged to obtain cell free extract which was used to test antibacterial activity against the test organisms.

3. RESULTS AND DISCUSSION

The synthesis and production of antimicrobial bioactive metabolites by bacteria is influenced by a multitude of factors and by both nutritional and environmental factors, such as temperature, pH, incubation time, inorganic salt content and so forth. The mix of medium components affects the growth and metabolite synthesis in addition to these parameters **[27]**.

3.1 Screening of active isolates

The 16S rRNA sequencing revealed the identification of the isolates that are initially labelled as C1, C6 and C7 as *Bacillus paramycoides* (*Bp*) *Lysinibacillus fusiformis* (*Lf*), *Bacillus oshimensis* (*Bo*) respectively. Using the agar diffusion method, antibacterial metabolite producers were screened wherein the isolates *Bacillus paramycoides*, *Lysinibacillus fusiformis* and *Bacillus oshimensis* exhibiting the largest zone of growth inhibition was chosen **[28]** and employed for further study of optimization.

3.2 Optimization of culture conditions

3.2.1 Effect of temperature on metabolite production

In the present study, different temperatures - 30°C, 35°C, 40°C, and 45°C were used. The isolates of *Bacillus paramycoides*, *Lysinibacillus fusiformis* and *Bacillus oshimensis* produced antibacterial metabolites at optimum temperature of 30°C. Islam *et al*., revealed that 30° C was the ideal temperature for the synthesis of antibiotic compounds from the strain *Bacillus subtilis.* Also, Adinarayana *et al*., 2002 **[29]** found that *Streptomyces marinensi*s produced maximum quantities of neomycin at 30°C.

Figure 1 presents data on the zone of inhibition in mm for different test organisms by *Bacillus paramycoides, Lysinibacillus fusiformis* and *Bacillus oshimensis* at different temperatures (30°C, 35°C, 40°C and 45°C).

At 30°C, *Bacillus paramycoides* showed the highest zone of inhibition against *M.luteus* (23.8±0.4mm) and *E. coli* (20.4±0.5 mm) indicating good antimicrobial activity. *Lysinibacillus fusiformis* exhibited the highest zone of inhibition against *M.luteus* (21.4±0.4 mm)

and *Alkaligens faecalis* (20.8 ±0.4 mm). *Bacillus oshimensis* showed the highest zone of inhibition against *Alkaligens faecalis* (23.2±0.6 mm) and *M.luteus* (20.4±0.4 mm)

At 35°C, *Bacillus paramycoides* demonstrated the highest zone of inhibition against *Salmonella enterica* (22.4 ±0.4 mm). *Lysinibacillus fusiformis* exhibited the highest zone of inhibition against *M.luteus* (20.6 ±0.4 mm) followed by *Salmonella enterica* and *S.aureus* (20.3 ±0.4 mm). *Bacillus oshimensis* showed the highest zone of inhibition against *Alkaligens faecalis* (20.2 ±0.4 mm) and *M.luteus* (18.9 ±0.1 mm).

At 40°C *Bacillus paramycoides* demonstrated the highest zone of inhibition against *M.luteus* (20.4±0.5 mm). *Lysinibacillus fusiformis* exhibited the highest zone of inhibition against *M.luteus* (19.3±0.4 mm) and *L. acidophilus* (17.2±0.2 mm). *Bacillus oshimensis* showed the highest zone of inhibition against *M.luteus* (18.3 ±0.4 mm) and *R. rhodochrous* (18.2±0.3mm).

At 45°C, *Bacillus paramycoides* demonstrated the highest zone of inhibition against *S.aureus* (22.1±0.4 mm) and *Salmonella enterica* (21.2±0.8mm). *Lysinibacillus fusiformis* exhibited the highest zone of inhibition against *S.aureus* (20.8 ±0.4 mm) *and Salmonella enterica* (20.7 ±0.3mm). *Bacillus oshimensis* showed the highest zone of inhibition against and *Salmonella enterica* (18.4±0.3mm).

Bacillus paramycoides, *Lysinibacillus fusiformis* and *Bacillus oshimensis* exhibited varying degrees of antimicrobial activity against different test organisms at different temperatures. The highest zones of inhibition were generally observed against *M.luteus*, *Salmonella enterica*, *Alkaligens faecalis*, *L. acidophilus* and *E. coli*.

3.2.2 Effect of pH on metabolite production

Various levels of hydrogen ion concentrations were used to grow the active isolates in order to find the ideal pH for optimal metabolite synthesis. *Bacillus paramycoides*, *Lysinibacillus fusiformis* and *Bacillus oshimensis* produces antibacterial metabolites at an ideal pH of 7.0, however this range is somewhat variable. pH significantly influences both the composition of marine bacterial communities and their metabolic output as per the findings by Jin, X., and Kirk, M.F. 2018 **[30]**.

Figure 2 presents the zones of inhibition in mm for *Bacillus paramycoides*, *Lysinibacillus fusiformis* and *Bacillus oshimensis* against various test organisms at different pH values (2, 4, 6, 7, 8, and 9).

At pH 2, none of the isolates showed any inhibition against the test organisms,

suggesting that this pH level was not favorable for the isolates to exhibit antimicrobial activity. At pH 4 and 6 the inhibitory activity was less when compared to pH 7, where all the three isolates showed inhibition against all the test organisms, with *Lysinibacillus fusiformis* and *Bacillus oshimensis* generally exhibiting higher zones of inhibition compared to *Bacillus paramycoides*. At pH 4 the highest activity was observed against *M.luteus* (13.2±0.4 mm) by *Bacillus paramycoides;* at pH 6 the highest inhibition was found against *S.aureus* (14.7±0.7 mm) by *Lysinibacillus fusiformis.* At pH 7, *Bacillus paramycoides* had shown highest inhibition of 16±0.7 mm against *R. rhodochrous*, *Lysinibacillus fusiformis* was found to be highly effective with the zone of 15.6±0.2 mm while *Bacillus oshimensis* exhibited highest zone of 15.9±0.1mm against *S.enterica.*

At pH 8 and 9, *Lysinibacillus fusiformis* and *Bacillus oshimensis* exhibited the zones with moderate inhibition. The inhibition zones are low for both *Bacillus paramycoides* and *Lysinibacillus fusiformis* at pH 8 and 9 against all the test organisms in contrast to pH 7 ; but *Bacillus oshimensis* exhibited high inhibition zones against two organisms such as *A.protophormiae* (15.9±0.9mm), *R.rhodochrous* (16.3±0.5mm) at pH 8 and *A .faecalis* (13.7±0.7mm), *S.aureus* (14.4±0.5mm) at pH 9. Among the test organisms, *E. coli* and *P.vulgaris* were found to be significantly susceptible particularly at pH 7,8 and 9. *Bacillus oshimensis* exhibited promising antimicrobial activity against few bacterial species, especially under slightly alkaline conditions (pH 8 and 9). The ideal pH was found to be 7 for all these three isolates for the optimum production of bioactive metabolites.

3.2.3 Influence of incubation period on the production of bioactive metabolites

The production of antibacterial compounds by microbial isolates was evaluated up to 120 hrs fermentation period, starting from 24 hrs after inoculation. Peak metabolite production was observed on 72 hrs of incubation for both *Bacillus paramycoides* and *Bacillus oshimensis* whereas 96 hrs for *Lysinibacillus fusiformis* followed by a gradual decline in subsequent days on concurrence with the study by Smith *et al*., 2020 **[31]**. To maximize the yield of antibacterial substances, the fermentation process was extended to 120 hrs, as recommended by Johnson and Lee (2019) **[32]**. All experiments were conducted in triplicate to ensure statistical reliability **[33]**.

The **Figure 3** displays the zones of inhibition (in mm) for three isolates of *Bacillus paramycoides*, *Lysinibacillus fusiformis* and *Bacillus oshimensis* against various test organisms at different time

intervals (24, 48, 72, 96 and 120 hrs). The antimicrobial effectiveness **increased with incubation time** with most test organisms showing minimal to no inhibition at 24 hrs, followed by progressive enhancement through 120 hrs.

Bacillus paramycoides showed inconsistent and generally lower zones of inhibition at 24 hrs and 48 hrs against most of the test organisms. At 72 hrs , it exhibited the inhibition against *Arthrobacter protophormiae* of (28.1±0.2mm), *R.rhodochrous* (23.4±0.4mm), *Streptococcus mutans* (19.6±0.5mm), *Enterococcus faecali*s (18.3±0.3mm), *S.enterica* (18±0.1mm), *E.coli* (16.3±0.2mm), *Alkaligens faecalis* and *M.luteus* (16.2±0.2mm), *P.vulgaris* (14.2±0.3mm), *S.aureus* (12.6±0.1mm), *Lactobacillus acidophilus* (12.4±0.4mm) and with the highest zones of 28.1±0.2mm (*A. protophormiae*).

Lysinibacillus fusiformis demonstrated effective and more consistent inhibition against most of the test organisms against both Gram-positive and Gram-negative bacteria compared to *Bacillus paramycoides* and *Bacillus oshimensis*. It showed considerable inhibition zones at 96 hrs against *E. faecalis* (22.3±0.3mm), *E.coli* (21.3±0.2mm), *S.mutans* (19.2±0.3mm), *P.vulgaris* (18.4±0.4mm), *L.acidophilus* (18.3±0.4mm), *R.rhodochrous* (18±0.1mm), *Salmonella enterica* (16.2±0.3mm), *A.protophormiae* (15.5±0.5mm), *M.luteus* (13.5±0.5mm), and *S.aureus* (12.6±0.1mm) with the highest inhibition zone of 22.3±0.3 mm was observed against *E. faecalis* .

Bacillus oshimensis displayed moderate inhibition against most test organisms, generally lower than *Lysinibacillus fusiformis* but higher than *Bacillus paramycoides*. It showed consistent inhibition at 72 hrs against *A. protophormiae* (17.4±0.4mm), *R. rhodochrous* (18±0.1mm), *Alkaligens faecalis* (16.2±0.2mm), *E. faecalis* (17.4±0.4mm), *S.mutans* (18.3±0.3mm), *Salmonella enterica* (23.4±0.4mm), *E. coli* (19.4±0.4mm), *P.vulgaris* (19.4±0.4mm), *M.luteus* (12.3±0.3mm), *L. acidophilus* (14.4±0.2mm) and *S.aureus* (14.3±0.2mm) with the highest inhibition zone of 23.4±0.4mm was observed against *Salmonella enterica*.

Overall, based on the zones of inhibition, *Lysinibacillus fusiformis* appears to be the most effective isolate against most of the test organisms, followed by *Bacillus oshimensis* and then *Bacillus paramycoides*.

3.2.4 Effect of salt concentration on metabolite production

Sodium chloride (NaCl) concentration plays a crucial role in modulating bacterial growth and secondary metabolite production, especially in marine

microorganisms **[34]**. Antibiotic susceptibility testing revealed that the biosynthesis of antimicrobial compounds was differentially influenced by varying salt concentrations **[35, 36]**.

Figure 4 displays the zone of inhibition in mm by *Bacillus paramycoides*, *Lysinibacillus fusiformis* and *Bacillus oshimensis* at different concentrations of salt (1%, 2%, 3% and 4%).

At 1% concentration, the isolates showed relatively low zones of inhibition against most test cultures. Zones were not produced for *E. coli*, *P.vulgaris*, *S.aureus* by these three isolates at 1% salt concentration. At 2% salt concentration all three isolates produced inhibition zones against most of the test pathogens, the highest inhibition zone was observed against *S. aureus* (24.9±0.4mm) by both *Bacillus paramycoides* and *Bacillus oshimensis*, while *Lysinibacillus fusiformis* exhibited the highest inhibition zone against *S. mutans* (21.2±0.6mm), indicating high sensitivity.

At 3% and 4% of NaCl the isolates exhibited zones of inhibition against most of the test cultures, indicating their antimicrobial potency at higher concentrations also with the highest being 20.4±0.6mm by *Bacillus paramycoides* against *S.aureus* but less inhibitory activity was displayed comparing with 2% concentration.

3.2.5 Effect of carbon sources on metabolite production

Among the five carbon sources, ribose favoured the highest antimicrobial activity by *B. paramycoides* with peak inhibition zones of 10±0.5mm against *S. mutans* and 9.6±0.5mm against *A. protophormiae*; *L. fusiformis* showed the peak inhibition zones of 13.1±0.6 mm for both *S. enterica* and *L. acidophilus* where as *B.oshimensis* produced highest zone of 13.4±0.2mm against *S.mutans*. Glucose also demonstrated consistently strong activity, particularly effective against *S.mutans* (9.2±0.2mm) and *R.rhodochrous* (8.4±0.2mm) by *B.paramycoides*. *L.fusiformis* displayed highest zone of 10.2±0.1mm towards *S.enterica* and peak activity of 9mm against *S. aureus* was observed by *B.oshimensis* with glucose as carbon source. Fructose, starch and lactose also displayed variable but generally moderate antimicrobial effects on most other test organisms compared to glucose and ribose **(Figure 5)**. The findings by Mysoon Al-Ansari *et al*., 2020 **[37]** identified that glucose served as the optimal carbon source at 31°C and pH 7.3 for marine *Streptomyces* AS11, enabling this bacteria to produce secondary metabolites with antibacterial and antifungal activities.

3.2.6 Effect of nitrogen sources on metabolite production

Among the 5 nitrogen sources, strong activity was demonstrated with sodium nitrate followed by yeast extract for *B. paramycoides* and *B.oshimensis* whereas for *L.fusiformis* the strong activity was displayed with sodium nitrate followed by ammonium hydroxide. *B. paramycoides* produced a highest zone of 14.5±0.5 mm with sodium nitrate and 11.4±0.4 mm with yeast extract against *R. rhodochrous*; *L.fusiformis* produced a zone of 22±0.4mm against *A. protophormiae* whereas moderate activity was observed against *E. faecalis* (12±0.3mm) by *B.oshimensis* with sodium nitrate as nitrogen source. *B. paramycoides* displayed variable but moderate activity against *A. protophormiae* (9.3±0.3 mm), *S.aureus* (9.2±0.2 mm), whereas highest activity by *L.fusiformis* was observed against *A. protophormiae* (16.5±0.2 mm) and *B.oshimensis* (9.6±0.5mm) against *S.mutans* with urea as nitrogen source. Ammonium chloride, ammonium hydroxide consistently showed the weakest antimicrobial activity across most test organisms by *B. paramycoides* and *B.oshimensis*. *L. fusiformis* displayed good activity with highest zones by ammonium chloride towards *R.rhodochrous* (20±0.3 mm) and ammonium hydroxide against *A.protophormiae* (20.8 ±0.8 mm) performing much better than *B. paramycoides* and *B.oshimensis*. **(Figure 6).** Mysoon Al-Ansari *et al*., 2020 reported that peptone followed by yeast extract served as optimal nitrogen source among the ammonium chloride, sodium nitrate, yeast extract and peptone at 31°C and pH 7.3 for maximum metabolite production by marine *Streptomyces* AS11 isolated from Saudi Arabian coastal water.

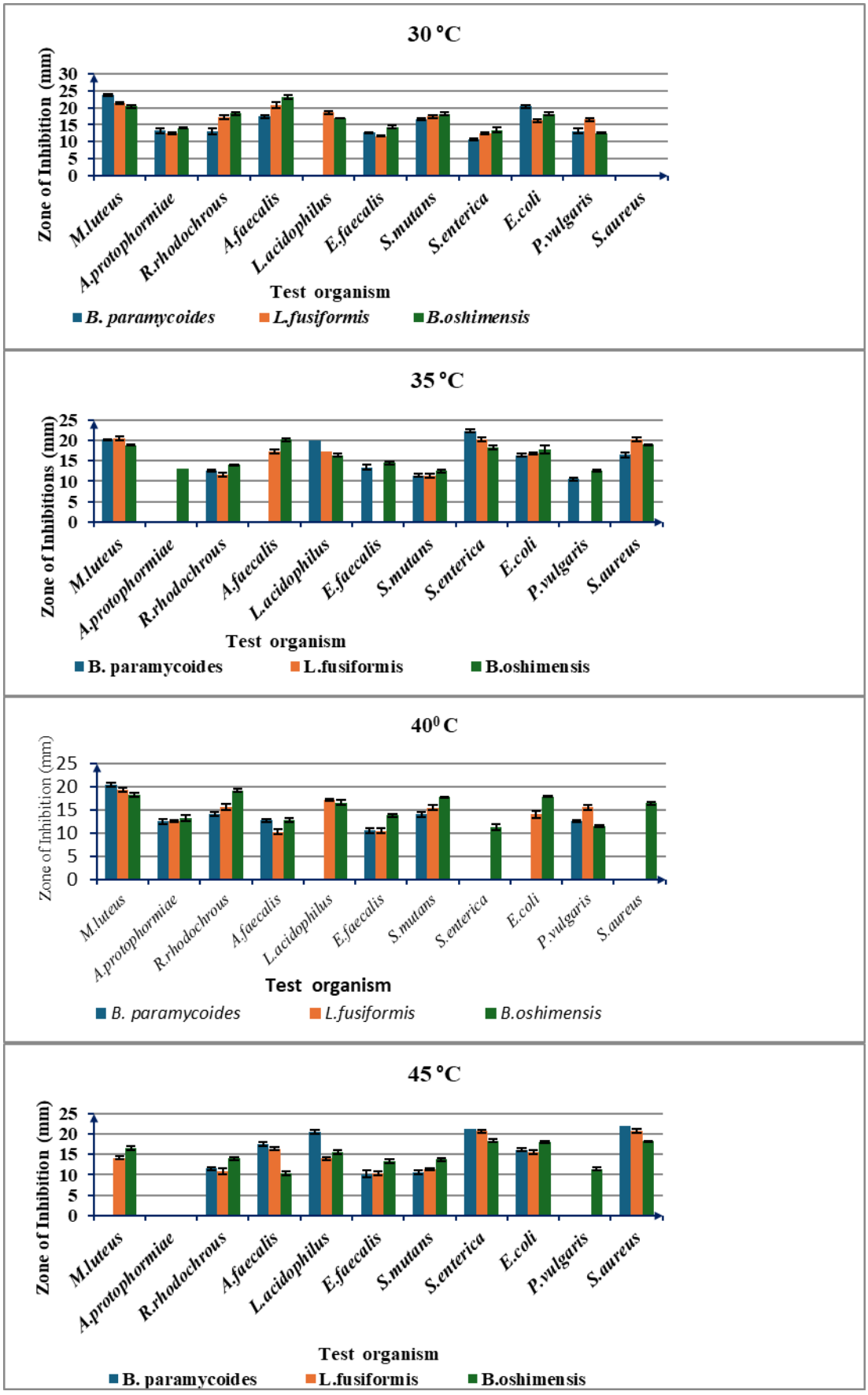


Figure 1: Effect of Temperature on metabolite production

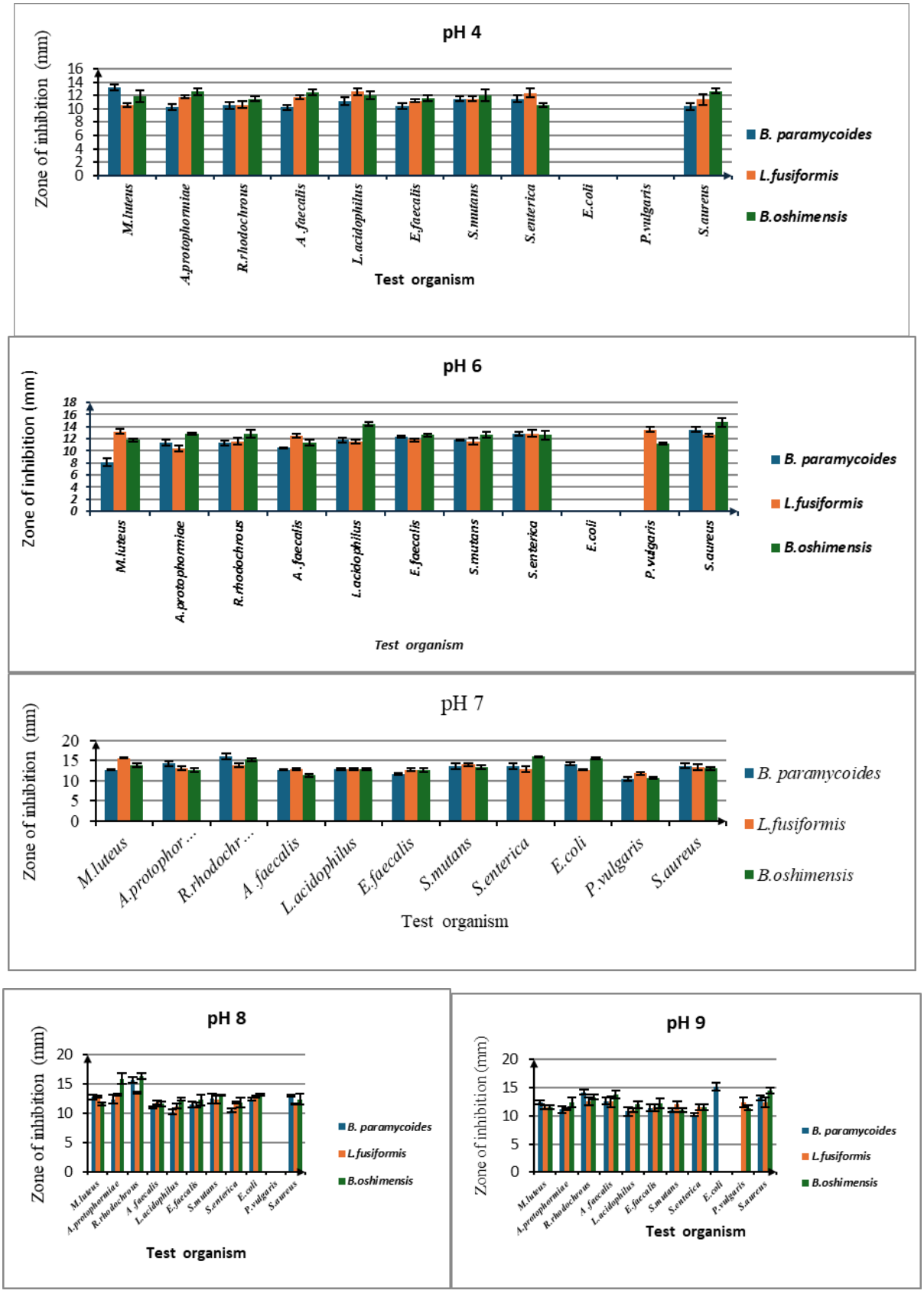


Figure 2: Impact of pH on metabolite production

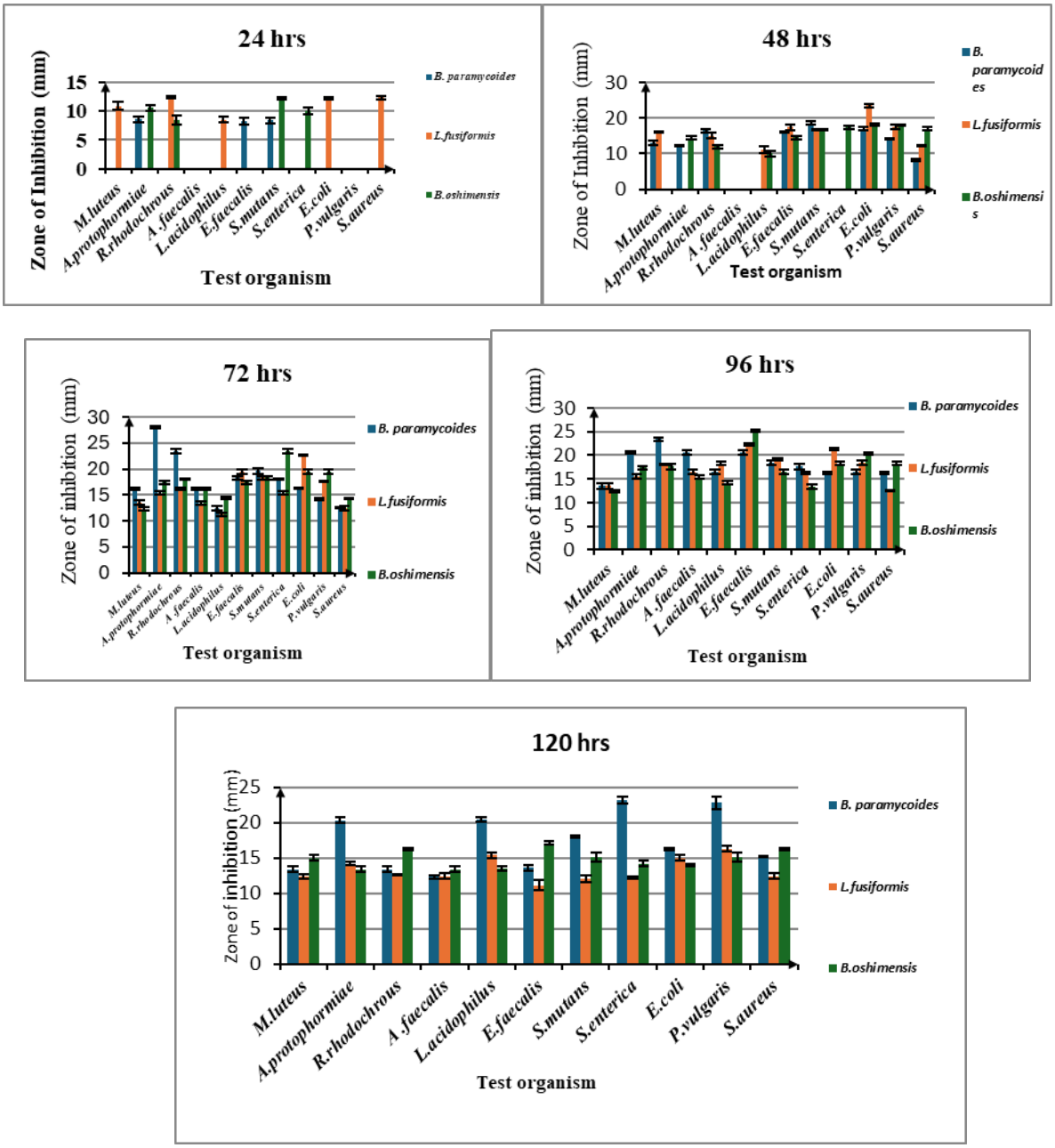


Figure 3: Impact of incubation period (hrs) on metabolite production

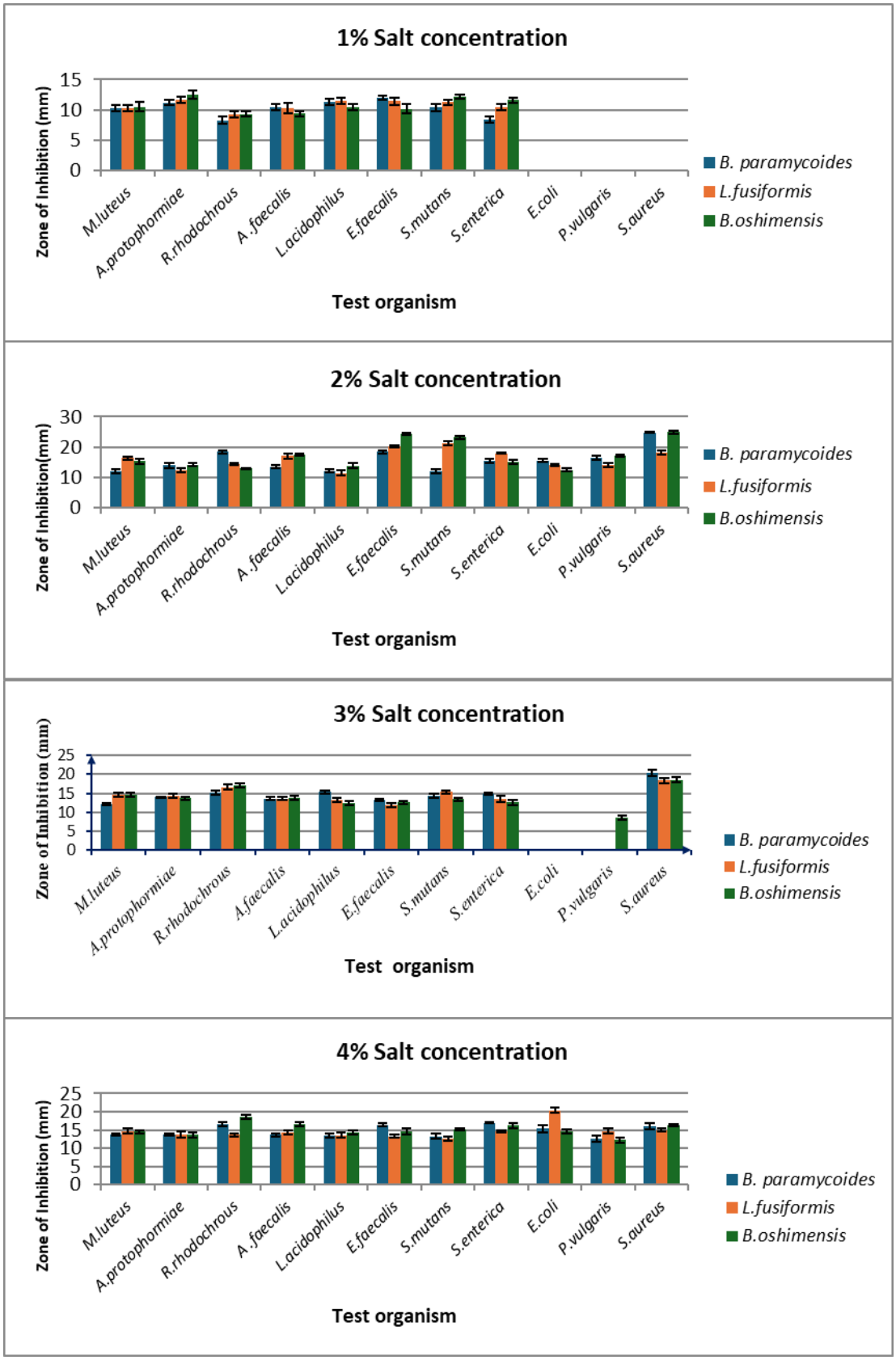


Figure 4: Effect of Salt concentration on metabolite production

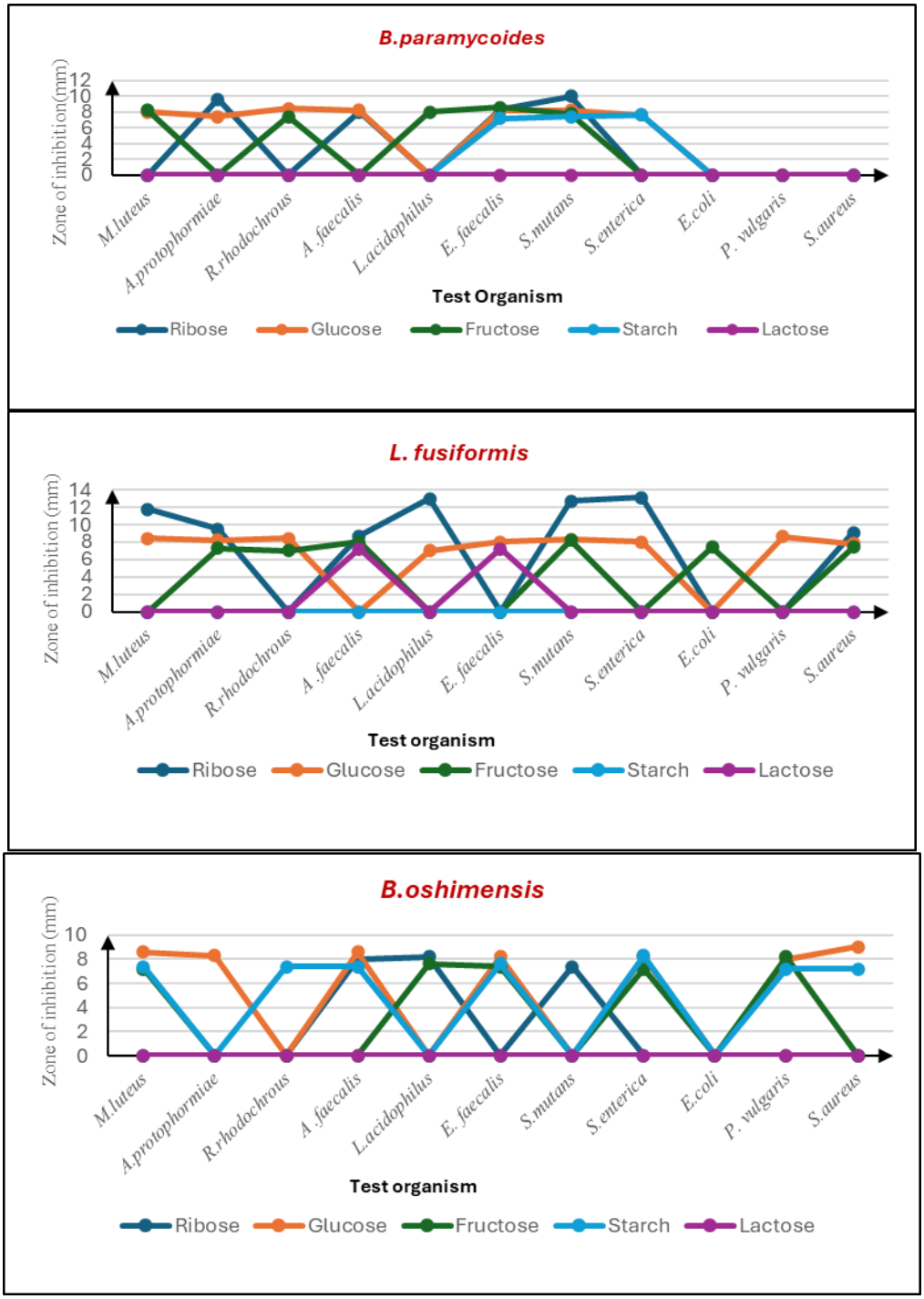


Figure 5: Effect of carbon sources on metabolite production

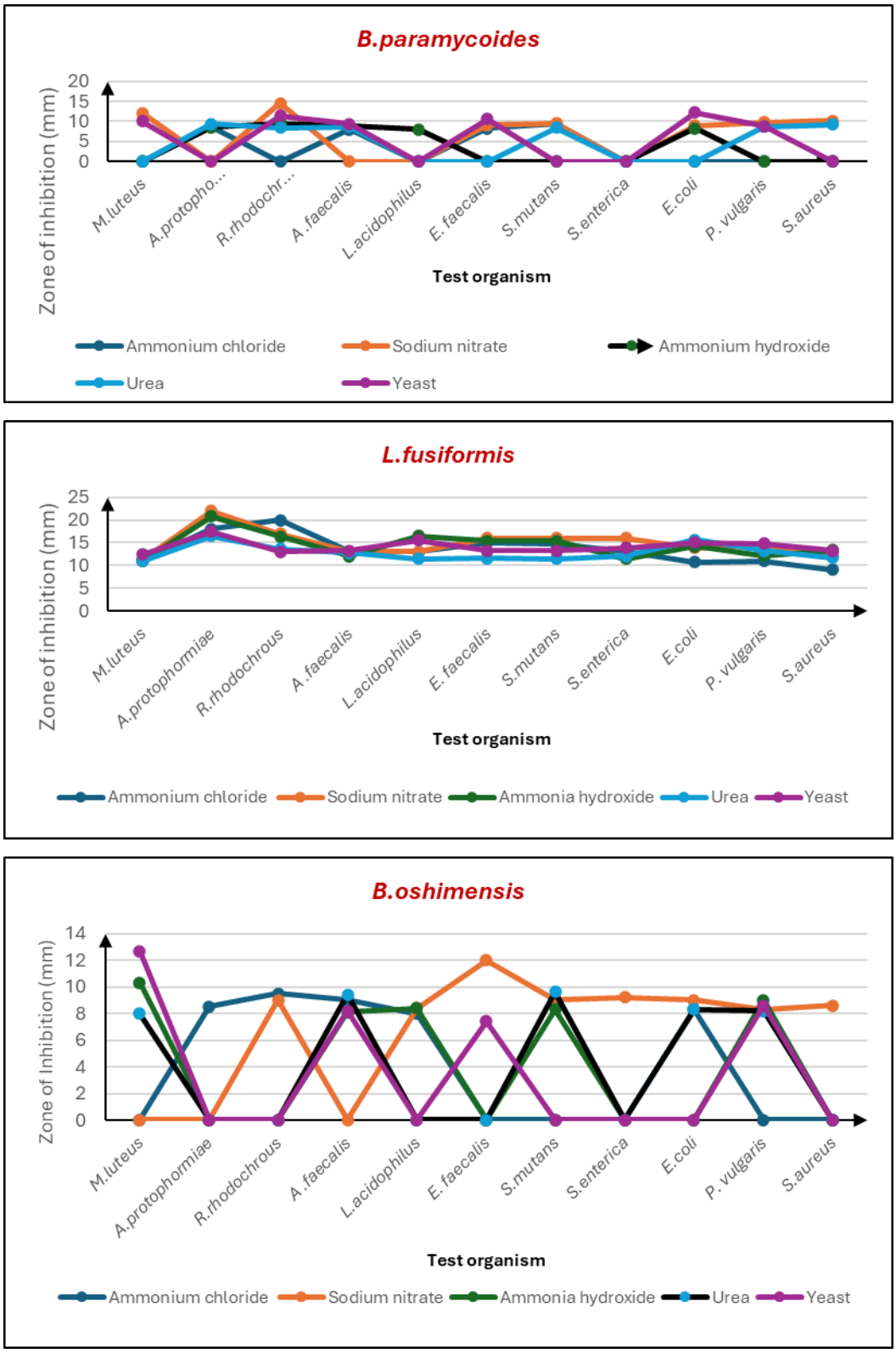


Figure 6: Effect of nitrogen sources on metabolite production

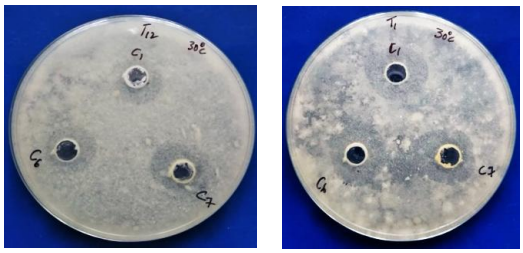
These days, there is a major risk of emerging drug-resistant diseases anywhere in the world. MRSA is a significant drug-resistant bacteria that is frequently seen in nosocomial infections in the human population **[38]**. The results demonstrated that the *Bacillus paramycoides* (*Bp*), *Lysinibacillus fusiformis* (*Lf*) and *Bacillus oshimensis* (*Bo*) exhibited antimicrobial activity against various test organisms with the varying degrees of inhibition based on the environmental conditions. These findings are in consistent with numerous studies that have explored the antimicrobial properties of natural products or microbial isolates **[39, 40]**.

The differential susceptibility among the test organisms could be attributed to the variations in the cell wall composition, membrane permeability or resistance mechanisms among different bacterial species **[41]**. The effects of different incubation times, temperatures, pH, and salt concentrations can significantly influence the growth and susceptibility of microorganisms, as well as the stability and activity of antimicrobial agents **[42]**.

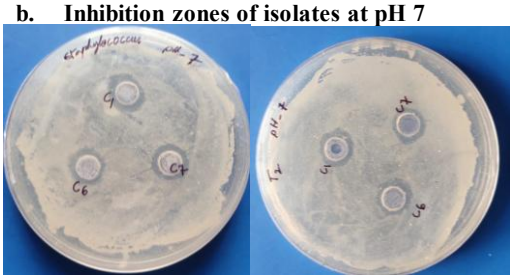
4. CONCLUSION

At the end, our findings showed that by optimizing the various environmental factors and nutritional factors the isolates *Bacillus paramycoides*, *Lysinibacillus fusiformis* and *Bacillus oshimensis* demonstrated good antibacterial metabolite synthesis. However, further investigation into the characterization of the bioactive compounds is recommended to gain a comprehensive understanding of their antimicrobial properties and potential applications.

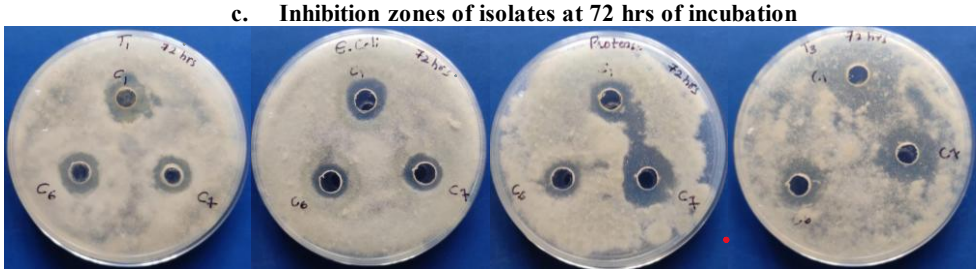
a. Inhibition zones of isolates at 30°c



b. Inhibition zones of isolates at pH 7



c. Inhibition zones of isolates at 72 hrs of incubation



d. Inhibition zones of isolates at salt concentration 2%

*C1 - *Bacillus paramycoides* *C6 - *Lysinibacillus fusiformis* *C7- *Bacillus oshimensis*

Figure 7: Images of antibacterial activity by *Bacillus paramycoides* (C1), *Lysinibacillus fusiformis paramycoides* (C6) and *Bacillus oshimensis* (C7) against test pathogens

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REFERENCES

[1] Demain, A.L: Antibiotics. (2009). natural products essential to human health. Medicinal Research Reviews, 29(6), pp.821-842.

[2] Shank, E.A. and Kolter, R. (2009). New developments in microbial interspecies signaling. Current Opinion in Microbiology, 12(2), pp.205-214.

[3] O'Brien, J. and Wright, G.D. (2011). An ecological perspective of microbial secondary metabolism. Current Opinion in Biotechnology, 22(4), pp.552-558.

[4] Tyc, O., Song, C., Dickschat, J.S., Vos, M. and Garbeva, P. (2017). The ecological role of volatile and soluble secondary metabolites produced by soil bacteria. Trends in Microbiology, 25(4), pp.280-292.

[5] Bibb, M.J. (2005). Regulation of secondary metabolism in Streptomycetes, Current Opinion in Microbiology, 8(2), pp.208-215.

[6] Netzker, T., Fischer, J., Weber, J., Mattern, D.J., König, C.C., Valiante, V., Schroeckh, V. and Brakhage, A.A. (2015). Microbial communication leading to the activation of silent fungal secondary metabolite gene clusters. Frontiers in Microbiology, 6, p.299.

[7] Newman, D.J. and Cragg, G.M. (2020). Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. Journal of Natural Products, 83(3), pp.770-803.

[8] Berdy, J. (2005) Bioactive microbial metabolites, The Journal of Antibiotics, 58(1),1-26

[9] Malla, S., Niraula, N.P., Singh, B., Liou, K. and Sohng, J.K. (2010) .Limitations in doxorubicin production from Streptomyces peucetius. Microbiological Research, 165(5), pp.427-435.

[10] Borel, J.F. (2002). History of the discovery of cyclosporin and of its early pharmacological development, Wiener Klinische Wochenschrift, 114(12), pp.433-437.

[11] Demain, A.L. and Fang, A. (2000). The natural functions of secondary metabolites. Advances in Biochemical Engineering/Biotechnology, 69, pp.1-39.

[12] Feling, R.H., Buchanan, G.O., Mincer, T.J., Kauffman, C.A., Jensen, P.R. and Fenical, W (2003). Salinosporamide A. a highly cytotoxic proteasome inhibitor from a novel microbial source, a marine bacterium of the new genus Salinospora. Angewandte Chemie International Edition, 42(3), pp.355-357.

[13] Potts, B.C., Albitar, M.X., Anderson, K.C., Baritaki, S., Berkers, C., Bonavida, B., Chandra, J., Chauhan, D., Cusack Jr, J.C., Fenical, W. and Ghobrial, I.M. (2011). Marizomib, a proteasome inhibitor for all seasons: preclinical profile and a framework for clinical trials. Current Cancer Drug Targets, 11(3), pp.254-284.

[14] Luesch, H., Moore, R.E., Paul, V.J., Mooberry, S.L. and Corbett, T.H. (2001). Isolation of dolastatin 10 from the marine cyanobacterium Symploca species VP642 and total stereochemistry and biological evaluation of its analogue symplostatin 1. Journal of Natural Products, 64(7), pp.907-910.

[15] Hamann, M.T., Otto, C.S., Scheuer, P.J. and Dunbar, D.C: Kahalalides.(1996). Bioactive peptides from a marine mollusk Elysia rufescens and its algal diet Bryopsis sp. The Journal of Organic Chemistry, 61(19), pp.6594-6600.

[16] Hong, J. and Luesch, H: Largazole. (2012). from discovery

to broad-spectrum therapy. Natural Product Reports, 29(4), pp.449-456.

[17] Sánchez, S., Chávez, A., Forero, A., García-Huante, Y., Romero, A., Sánchez, M., Rocha, D., Sánchez, B., Ávalos, M., Guzmán-Trampe, S., Rodríguez-Sanoja, R., Langley, E., & Ruiz, B. (2010). Carbon source regulation of antibiotic production. *The Journal of Antibiotics*, 63(8), 442-459. https://doi.org/10.1038/ja.2010.78

[18] Yan, L., Boyd, K.G., Adams, D.R. and Burgess, J.G. (2003).Biofilm-specific cross-species induction of antimicrobial compounds in bacilli. Applied and Environmental Microbiology, 69(7), pp.3719-3727.

[19] Singh, V., Haque, S., Niwas, R., Srivastava, A., Pasupuleti, M. and Tripathi, C.K.M. (2017). Strategies for fermentation medium optimization: an in-depth review. Frontiers in Microbiology, 7, p.2087.

[20] Liu, R.; Huang, Z.B.; Dong, C.M.; Shao, Z.Z. (2019). *Lottiidibacillus patelloidae* Gen. Nov., Sp. Nov., isolated from the intestinal tract of a Marine Limpet and Reclassification of *Bacillus taeanensis* as *Maribacillus taeanensis* gen. nov., comb. nov. Antonie Leeuwenhoek , 112, 797–807.

[21] Ramachandran, R., Chakraborty, K., & Thankappan, R. (2022). Antioxidant and cytotoxic potential of marine Bacillus species isolated from the Bay of Bengal sediments. Biotechnology Reports, 34, e00698.

[22] Jeong, S.Y., Ishida, K., Ito, Y., Okada, S. and Murakami, M. (2003). Bacillamide, a novel algicide from the marine bacterium, Bacillus sp. SY-1, against the harmful dinoflagellate, Cochlodinium polykrikoides. Tetrahedron Letters, 44(43), pp.8005-8007.

[23] Cladera-Olivera F, Caron GR, Brandelli A. (2004). Bacteriocin-like peptide production by *Bacillus licheniformis* strain P40, Letters in Applied Microbiology, 38, 251–256.

[24] Jaruchoktaweechai C, Suwanborirux K, Anasupawatt S, Kittakoop P, Menasveta, P. (2000). New macrolactins from a marine Bacillus sp. Sc026, Journal of natural products , 63, 984–986.

[25] Nagai K, Kamigiri K, Arao N, Suzumura K, Kawano Y, Yamaoka M, Zhang H, Watanabe

M, Suzuki K, YM- 266183 and YM-266184. (2003).A novel thiopeptide antibiotics produced by Bacillus cereus isolated from a marine sponge, The Journal of Antibiotics, 56(2), 123–128.

[26] Kreig NR, Sneath PHA, Mair NS, Sharpe EM, Holt JG, Williams & Wilkins, Baltimore.(1984). Bergey's manual of systematic Bacteriology, 911–1504.

[27] Song Q, Huang Y, Yang H. (2012). Optimization of fermentation conditions for antibiotic production by Actinomycetes YJ1 strain against Sclerotinia sclerotiorum, Journal of Agricultural Science, 4(7), 95–102.

[28] Devavani K, Rao VU. (2024). Isolation, Screening and Identification of Marine bacteria for their Potential Bioactivity. Asian J Biol Life Sci;13(2):551-60.

[29] Adinarayana, K., Ellaiah, P., Srinivasulu, B., Devi, R. B., & Adinarayana, G. (2003). Response surface methodological approach to optimize the nutritional parameters for neomycin production by *Streptomyces marinensis* under solid-state fermentation. *Process Biochemistry*, 38(11), 1565-1572. https://doi.org/10.1016/S0032-9592(03)00057-8

[30] Jin, X., & Kirk, M.F. (2018). pH as a primary control in environmental microbiology: 1. Thermodynamic perspective. *Frontiers in Environmental Science*, 6, 21. DOI: 10.3389/fenvs.2018.00021.

[31] Smith, J., Garcia, R., & Patel, N. (2020). Temporal dynamics of antibacterial metabolite production in microbial isolates. FEMS Microbiology Letters, 367(8), fnaa052.

[32] Johnson, M., & Lee, K. (2019).Optimizing fermentation conditions for secondary metabolite production. Biotechnology Advances, 37(3), 456-470.

[33] Brown, A. (2018). Statistical methods in microbiology, Journal of Applied Microbiology, 45(2), 78-92

[34] Zhang, Y., Li, Q., & Chen, W. (2018).Effects of salinity on growth and secondary metabolite production in marine-derived microorganisms. Frontiers in Microbiology, 9, 1709.

[35] Lee, S.H., & Kim, J.Y. (20210. Salt-dependent regulation of

antimicrobial metabolite production in marine bacteria. Marine Drugs, 19(4), 215-230 Cheng, L., Ren, J., Wang, Y., Zhang, Y., & Lin, H. (2019). Antimicrobial activity of cinnamaldehyde and citral combined with amino-functionalized mesoporous silica nanoparticles: A study of antimicrobial effects. LWT, 101, 576-584.

[36] Ng, Y. K., Hodson, M. P., Hewavitharana, A. K., Bose, U., Shaw, P. N., & Fuerst, J. A. (2014). Effects of salinity on antibiotic production in sponge-derived Salinispora actinobacteria. *Journal of applied microbiology*, *117*(1), 109–125. https://doi.org/10.1111/jam.12507.

[37] Mysoon Al-Ansari, Mani Kalaiyaras, Mohammed, A. Almalki, Ponnuswamy Vijayaraghavan. (2020). Optimization of medium components for the production of antimicrobial and anticancer secondary metabolites from *Streptomyces* sp. AS11 isolated from the marine environment. *Saudi Journal of Biological Sciences*, 27(12), 3624-3635. DOI: 10.1016/j.sjbs.2020.02.019.

[38] Saritha Valsalam, Paul Agastian, Mariadhas Valan Arasu, Naif Abdullah Al-Dhabi, Abdul-Kareem Mohammed Ghilan, K. Kaviyarasu, Balasubramani Ravindran, Soon Woong Chang, S. Arokiyaraj (2019). Rapid biosynthesis and characterization of silver nanoparticles from the leaf extract of Tropaeolum majus L. and its enhanced in-vitro antibacterial, antifungal, antioxidant and anticancer properties, Journal of Photochemistry and Photobiology B: Biology, Volume 191, Pages 65-74, ISSN 1011-1344 .https://doi.org/10.1016/j.jphotobiol.2018.12.010.

[39] Ganesan, P., Pradeepa, P., & Pugalenthi, V. (2017). Antimicrobial activity of extracts from marine organisms. Journal of Applied Pharmaceutical Science, 7(12), 227-232.

[40] Salehi, B., Sharifi-Rad, J., Quispe, C., Llaiqui, N., Villaroel, C., Ayala, N & Shaheen, S. (2019). Antimicrobial activity of nanoparticles: A review from a clinical and environmental perspective. Nanomaterials, 9(11), 1596.

[41] Ríos, J. L., & Recio, M. C. (2005). Medicinal plants and antimicrobial activity. Journal of Ethnopharmacology, 100(1-2), 80-84.

[42] Yoon, J. Y., *et al*. (2022). Effects of temperature, pH and sodium chloride on antimicrobial activity of magnesium oxide nanoparticles against *E.coli* O157:H7. Letters in Applied Microbiology, 75(5), 1202-1210. https://doi.org/10.1111/lam.13763