RESEARCH PAPER

# Identification and Characterization of Antimycobacterial Peptides from Marine Origin Bacillus Species with Relation to Tuberculosis

*Pauline Deborah[1], Bini Balakrishnan[2], S. Anthony Ammal[3], G. Priyanka[4*], P. R. Devaki[5], D. Saravanan[6]*

[1] IIIrd Year MBBS Student, Sree Balaji Medical College and Hospital, Chrompet, Chennai, Tamil Nadu, India
[2] Assistant Professor, Department of Physiology, Krishnanagar Institute of Medical Sciences, Krishnanagar, Nadia, West Bengal, India
[3] Associate Professor, Department of Anatomy, Karpaga Vinayaga Institute of Medical Sciences, Chengalpattu, Tamil Nadu, India
[4*] Assistant Professor, Department of Physiology, Sree Balaji Medical College and Hospital, Chrompet, Chennai, Tamil Nadu, India (Corresponding Author)
[5] Department of Physiology, Sree Balaji Medical College and Hospital, Chrompet, Chennai, Tamil Nadu, India
[6] Centre for Laboratory Animal Technology and Research, Sathyabama Institute of Science and Technology, Chennai, Tamil Nadu, India

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## ABSTRACT

### Background and Objectives

Tuberculosis remains a major global health burden, exacerbated by the emergence of multidrug-resistant and extensively drug-resistant strains of Mycobacterium tuberculosis. Marine microorganisms, particularly species of the genus Bacillus, are recognized sources of antimicrobial peptides with broad biological activity. This study aimed to isolate Bacillus species from marine sediments and evaluate their antibacterial and antimycobacterial potential with reference to tuberculosis.

### Materials and Methods

Marine sediment samples were collected from five coastal locations along the East Coast Road, Chennai, India. Bacterial isolates were obtained by serial dilution and culture on nutrient agar, followed by morphological, cultural and biochemical characterization. Antimicrobial activity was screened using well diffusion, agar plug and direct spot assays against standard bacterial pathogens. Optimization of bioactive metabolite production was performed by varying pH, temperature, incubation period and nutrient sources. Bioactive compounds were extracted using organic solvents and partially purified by thin-layer chromatography. Antimycobacterial activity was assessed using the luciferase reporter phage assay.

### Results

Forty-three bacterial isolates were obtained, with counts ranging from $1.2 \times 10^3$ to $1.5 \times 10^5$ CFU/g of soil. Gram-positive isolates accounted for 53.48%, while 37.2% of colonies were pigmented. Among 17 Bacillus isolates screened, 7 (40.17%) exhibited antibacterial activity. Isolate KP3 showed broad-spectrum antibacterial activity with inhibition zones of 24 mm against Bacillus subtilis, 21 mm against Acinetobacter baumannii, 20 mm against Staphylococcus aureus, 18 mm against Escherichia coli and 16 mm against Klebsiella pneumoniae and Pseudomonas aeruginosa. Ethyl acetate extraction yielded 180 mg of crude compound per litre and the active TLC fraction showed an Rf value of 0.7 with inhibition zones up to 28 mm. No isolate demonstrated antimycobacterial activity.

### Conclusions

Marine-origin Bacillus isolate KP3 produces potent antibacterial compounds with broad-spectrum activity, supporting further investigation of marine Bacillus-derived peptides as antibacterial agents.

**Keywords:** Bacillus; antimicrobial peptides; marine bacteria; tuberculosis; antibacterial activity

**Abbreviations:** AMP, antimicrobial peptide; MDR, multidrug-resistant; XDR, extensively drug-resistant; CFU, colony-forming units; LRP, luciferase reporter phage

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## INTRODUCTION

Tuberculosis remains one of the most persistent infectious diseases worldwide, caused by *Mycobacterium tuberculosis*. Despite the availability of standardized treatment regimens, tuberculosis continues to pose a significant public health challenge, particularly in low- and middle-income countries [1]. The burden of disease is compounded by the emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains, which compromise the effectiveness of first- and second-line antitubercular drugs [2]. Inadequate treatment adherence, prolonged therapy duration, drug toxicity and inconsistent healthcare access have further contributed to the persistence of tuberculosis as a global health concern [3].

India accounts for a substantial proportion of global tuberculosis cases, with a parallel rise in drug-resistant forms of the disease [4]. Conventional antitubercular drugs primarily target cell wall synthesis, transcription, or protein synthesis in *M. tuberculosis*. However, resistance mechanisms such as target modification, efflux pumps and enzymatic drug inactivation have reduced therapeutic efficacy [5]. These challenges necessitate the exploration of alternative antimicrobial agents with novel mechanisms of action.

Antimicrobial peptides (AMPs) have emerged as promising candidates in this context. AMPs are short, cationic peptides produced by a wide range of organisms, including bacteria, fungi, plants and animals, as part of their innate defense systems [6]. Unlike conventional antibiotics, AMPs often exert their activity by disrupting microbial membranes, reducing the likelihood of resistance development [7]. Several studies have demonstrated the efficacy of AMPs against drug-resistant bacterial pathogens, highlighting their therapeutic potential [8].

Marine environments are increasingly recognized as rich sources of bioactive microorganisms due to their ecological diversity and exposure to extreme physicochemical conditions [9]. Marine bacteria, in particular, have evolved unique metabolic pathways that enable the production of structurally novel secondary metabolites [10]. Among marine bacteria, species of the genus *Bacillus* are of particular interest due to their capacity to produce a wide array of antimicrobial compounds, including bacteriocins, lipopeptides and cyclic peptides [11].

Previous studies have reported antimicrobial and antimycobacterial activities of *Bacillus*-derived metabolites isolated from terrestrial and marine sources [12,13]. These compounds exhibit diverse chemical structures and biological activities, making them attractive candidates for drug discovery. However, systematic investigations focusing on marine-origin *Bacillus* species and their potential activity against mycobacterial pathogens remain limited.

The present study was designed to address this gap by isolating *Bacillus* species from marine sediments collected along the East Coast Road, Chennai, India. The study aimed to evaluate their antibacterial activity, optimize bioactive metabolite production, characterize active fractions and assess their antimycobacterial potential using a luciferase-based assay.

## MATERIALS AND METHODS

### Collection of Marine Sediment Samples

Marine sediment samples were collected from five coastal locations along the East Coast Road, Chennai, Tamil Nadu, India, namely Ennore Beach, Marina Beach, Kovalam Beach, Mamallapuram Beach and Kalpakkam Beach. Samples were collected using sterile polythene bags from the upper sediment layer and transported to the laboratory under aseptic conditions for further processing. Details of the sampling locations and corresponding sample codes are summarized (Table 1).

### Isolation and Maintenance of Bacterial Isolates

Sediment samples were serially diluted using sterile saline. Aliquots from appropriate dilutions were spread-plated on nutrient agar supplemented with nystatin to inhibit fungal growth. Plates were incubated at 28°C for 48 hours. Distinct colonies based on morphological differences were selected, purified by repeated subculturing and maintained on nutrient agar slants at 4°C.

### Morphological and Biochemical Characterization

Isolates were characterized based on colony morphology, pigmentation, Gram staining, motility, spore staining and capsule staining. Biochemical tests were performed following standard protocols and identification was carried out using Bergey's Manual of Systematic Bacteriology [14].

### Screening for Antimicrobial Activity

Antimicrobial activity was evaluated using well diffusion, agar plug and direct spot methods against standard bacterial pathogens, including *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Acinetobacter baumannii*. Zones of inhibition were measured after incubation at 37°C for 24 hours.

### Optimization of Bioactive Metabolite Production

Optimization studies were conducted by varying pH, temperature, incubation period, carbon sources and nitrogen sources to enhance bioactive compound production. Antimicrobial activity under different conditions was assessed using the well diffusion method. The influence of carbon source, nitrogen

source, pH, temperature and incubation period on antibacterial metabolite production by isolate KP3 was evaluated and is illustrated graphically (Figure 4).

**Extraction and Partial Purification of Bioactive Compounds**

Submerged fermentation was employed for large-scale production of bioactive metabolites. Crude extracts were obtained using different organic solvents and antibacterial activity was evaluated. Thin-layer chromatography was used for partial purification of active fractions.

**Antimycobacterial Activity**

Antimycobacterial activity was assessed using the Luciferase Reporter Phage assay against *Mycobacterium* species following standard protocols [15].

## RESULTS

A total of forty-three morphologically distinct bacterial isolates were recovered from marine sediment samples (Table 2). Gram-positive bacteria constituted the majority of isolates, with *Bacillus* species being predominant. Among all isolates screened, KP3 demonstrated consistent antibacterial activity across multiple assay methods (Table 3).

Optimization studies revealed that starch and yeast extract supported maximal bioactive metabolite production at pH 7 and 30°C after 72 hours of incubation (Table 4, Figure 4). Ethyl acetate was identified as the most effective solvent for extracting antibacterial compounds. Thin-layer chromatography resolved two fractions, of which KP3-A exhibited significant antibacterial activity (Figure 5).

Despite robust antibacterial activity, none of the isolates demonstrated detectable antimycobacterial activity in the Luciferase Reporter Phage assay.

## DISCUSSION

The present study investigated marine-origin *Bacillus* species as potential producers of antimicrobial and antimycobacterial peptides, motivated by the increasing burden of drug-resistant bacterial infections and tuberculosis. The results demonstrate that marine sediments along the East Coast Road harbor a diverse bacterial population, with a predominance of Gram-positive *Bacillus* species. This observation aligns with earlier reports indicating that *Bacillus* species are well adapted to marine and coastal environments due to their spore-forming ability and metabolic versatility [16,17].

A key finding of this study was the consistent and broad-spectrum antibacterial activity exhibited by the isolate designated KP3. The ability of KP3 to inhibit both Gram-positive and Gram-negative bacteria supports previous studies describing *Bacillus*-derived antimicrobial peptides as having wide inhibitory spectra [18,19]. Such broad activity is often attributed to membrane-targeting mechanisms, which are less dependent on specific intracellular targets and therefore less susceptible to classical resistance pathways [20]. The observed inhibition of clinically relevant pathogens such as *Staphylococcus aureus*, *Acinetobacter baumannii* and *Escherichia coli* reinforces the potential clinical relevance of the bioactive compounds produced by KP3.

Optimization experiments demonstrated that carbon and nitrogen sources, pH, temperature and incubation period significantly influenced bioactive metabolite production. Maximum antibacterial activity was observed under neutral pH conditions with starch and yeast extract as nutrient sources. These findings are consistent with earlier reports indicating that secondary metabolite biosynthesis in *Bacillus* species is strongly regulated by nutritional and environmental parameters [21,22]. Starch has been shown to act as a favorable carbon source for peptide antibiotic production, possibly by supporting sustained growth and delayed catabolite repression [23]. Similarly, yeast extract provides essential amino acids and growth factors that may enhance peptide biosynthesis [24].

Ethyl acetate emerged as the most effective solvent for extracting antibacterial compounds from the KP3 isolate. This suggests that the active metabolites are moderately polar in nature, a characteristic commonly reported for *Bacillus*-derived lipopeptides and cyclic peptides [25]. The failure of other solvents to extract active compounds has been noted in earlier studies and highlights the importance of solvent selection during natural product screening [26]. The inhibition zones obtained with ethyl acetate extracts in the present study were comparable to those reported for other marine-derived *Bacillus* metabolites, supporting the robustness of the antibacterial activity observed [27].

Partial purification by thin-layer chromatography resulted in the separation of two fractions, of which only one (KP3-A) demonstrated antibacterial activity. This observation suggests that the crude extract contains a mixture of compounds, not all of which contribute to antimicrobial effects. Similar findings have been reported in studies where bioactivity was localized to specific fractions following chromatographic separation [28]. Early identification of active fractions is advantageous, as it reduces the time and resources required for downstream purification and structural elucidation [29].

Despite strong antibacterial activity, none of the isolates demonstrated detectable antimycobacterial

activity in the luciferase reporter phage assay. This finding contrasts with some earlier reports describing antimycobacterial compounds from *Bacillus* species [30,31]. Several factors may account for this discrepancy. First, antimycobacterial activity is often strain-specific and dependent on the precise chemical nature of the compound produced [32]. Second, the unique cell wall structure of *Mycobacterium* species, rich in mycolic acids, presents a formidable permeability barrier that limits the efficacy of many antimicrobial agents [33]. Third, it is possible that the concentration of active compounds obtained in this study was insufficient to exert detectable effects against mycobacteria under the assay conditions used.

The absence of antimycobacterial activity does not negate the relevance of the findings. Instead, it highlights the complexity of discovering compounds effective against *Mycobacterium tuberculosis* and underscores the need for expanded screening approaches. Studies have shown that antimycobacterial activity may only become apparent after further purification, structural modification, or synergistic evaluation with existing antitubercular drugs [34,35]. Additionally, alternative assays and longer exposure periods may be required to detect subtle inhibitory effects [36].

From a broader perspective, the results of this study reinforce the value of marine ecosystems as reservoirs of antibacterial compounds, particularly against non-mycobacterial pathogens. Given the global rise in infections caused by multidrug-resistant bacteria such as *A. baumannii* and methicillin-resistant *S. aureus*, the identification of new antibacterial agents remains a priority [37]. Marine-derived *Bacillus* metabolites, such as those produced by KP3, could contribute to this effort either as standalone agents or as templates for synthetic modification [38].

Future research should focus on detailed chemical characterization of the active KP3-A fraction using spectroscopic techniques such as mass spectrometry and nuclear magnetic resonance. Genomic analysis of the KP3 isolate may also reveal biosynthetic gene clusters responsible for antimicrobial peptide production, enabling targeted manipulation to enhance yield or activity [39]. Expanding the screening to include additional marine sites and employing combinatorial or synergistic assays may improve the likelihood of identifying antimycobacterial compounds [40].

## CONCLUSION

In summary, while the working hypothesis regarding antimycobacterial activity was not supported under the conditions tested, the study provides clear evidence that marine-origin *Bacillus* species are valuable sources of antibacterial compounds. These findings contribute to the growing body of evidence supporting marine bacteria as promising candidates in the search for novel antimicrobial agents and provide a rational foundation for future investigations targeting drug-resistant pathogens.

## CONFLICT OF INTEREST

Nil

## AUTHOR'S CONTRIBUTIONS

Conceptualization, first author.;
methodology, second author.;
software, third author
validation, fourth author.;
formal analysis, fifth author.;
investigation, sixth author.;
resources, first author.;
data curation, second author.;
writing—original draft preparation, third author.;
writing—review and editing, fourth author.;
visualization, fifth author.;
supervision, sixth author.;
project administration, first author.;
funding acquisition, second author.
All authors have read and agreed to the published version of the manuscript".

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Nil

## REFERENCES


1. World Health Organization. Global tuberculosis report 2017. Geneva: World Health Organization; 2017.
2. Zumla A, Raviglione M, Hafner R, Fordham von Reyn C. Tuberculosis. N Engl J Med. 2013;368(8):745-55. doi:10.1056/NEJMra1200894.
3. Paul S, Akter R, Aung MS, Ahmed S. Factors contributing to tuberculosis drug resistance. Int J Tuberc Lung Dis. 2007;11(12):1334-40.
4. Zheng Y, Li J, Liu Y, Li H, Wang L. Tuberculosis burden in Asia: a systematic analysis. BMC Public Health. 2017;17(1):1-9. doi:10.1186/s12889-017-4521-3.
5. Zhang Y, Yew WW. Mechanisms of drug resistance in Mycobacterium tuberculosis. Int J Tuberc Lung Dis. 2009;13(11):1320-30.
6. Hancock REW, Sahl HG. Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. Nat Biotechnol. 2006;24(12):1551-7. doi:10.1038/nbt1267.
7. Zasloff M. Antimicrobial peptides of multicellular organisms. Nature. 2002;415(6870):389-95. doi:10.1038/415389a.
8. Sumi CD, Yang BW, Yeo IC, Hahm YT. Antimicrobial peptides of the genus Bacillus: a new era for antibiotics. Can J Microbiol. 2015;61(2):93-103. doi:10.1139/cjm-2014-0613.
9. Berdy J. Thoughts and facts about antibiotics: where we are now and where we are heading. J Antibiot. 2012;65(8):385-95. doi:10.1038/ja.2012.27.
10. Bull AT, Stach JEM, Ward AC, Goodfellow M. Marine actinobacteria: perspectives, challenges, future directions. Antonie Van Leeuwenhoek. 2005;87(1):65-79. doi:10.1007/s10482-004-6549-5.
11. Baindara P, Mandal SM, Chawla N, Singh PK, Pinnaka AK, Korpole S. Characterization of two antimicrobial peptides produced by a halotolerant Bacillus subtilis strain SK.DU.4. Appl Microbiol Biotechnol. 2013;97(6):2591-601. doi:10.1007/s00253-012-4396-2.
12. Bizani D, Motta AS, Morrissy JA, Terra RM, Souto AA, Brandelli A. Antibacterial activity of cerein 8A, a bacteriocin-like peptide produced by Bacillus cereus. Int Microbiol. 2005;8(2):125-31.
13. Anand TP, Bhat AW, Shouche YS, Roy U, Siddharth J, Sarma SP. Antimicrobial activity of marine bacteria associated with sponges from the waters off the coast of South East India. Microbiol Res. 2006;161(3):252-62. doi:10.1016/j.micres.2005.09.002.
14. Sharpe ME. Identification of the lactic acid bacteria. In: Skinner FA, Lovelock DW, editors. Identification methods for microbiologists. London: Academic Press; 1979. p. 233-59.
15. Manzour HH, Nora G, Beatriz EF, Ana LR, Luz AL, Rafael T, et al. Photographic and luminometric detection of luciferase reporter phages for drug susceptibility testing of clinical Mycobacterium tuberculosis isolates. J Clin Microbiol. 2003;41(10):4865-9. doi:10.1128/JCM.41.10.4865-4869.2003.
16. Guendouzi S, Hassi M, Kihal M, Kihal N. Inhibitory activity of Bacillus strains isolated from Moroccan biotopes against mycobacteria. Afr J Microbiol Res. 2011;5(15):2127-33.
17. Hassi M, Guendouzi S, Kihal M, Kihal N. Antimycobacterial activity of a Bacillus pumilus strain isolated from soil. Afr J Biotechnol. 2012;11(13):2999-3004.
18. Shimei L, Xia Z, Liang Y, Zhong J. Characterization of antimicrobial peptides produced by Bacillus subtilis JM4. J Appl Microbiol. 2005;99(2):238-44. doi:10.1111/j.1365-2672.2005.02618.x.
19. Youcef-Ali M, Kihal M, Henni DE. Antifungal activity of Bacillus mojavensis and Bacillus subtilis isolated from palm trees. Afr J Microbiol Res. 2014;8(3):244-51.
20. Awais M, Pervez A, Yaqub A, Shah MM. Production of antimicrobial metabolites by Bacillus subtilis immobilized in agar. J Ind Microbiol Biotechnol. 2010;37(5):1-7. doi:10.1007/s10295-010-0717-3.
21. Arora DS, Kaur H, Onsare JG, Sharma V. Production, optimization and characterization of antimicrobial compounds from Aspergillus species. J Appl Pharm Sci. 2014;4(01):157-71.
22. Usha KM, Vijayalakshmi M, Poda S, Agasar D. Optimization of cultural parameters for improved production of antimicrobial metabolites by Streptomyces gulbargensis. Br J Pharm Res. 2014;4(9):1130-45.
23. Hobbs G, Frazer CM, Gardner DCJ, Flett F, Oliver SG. Dispersed growth of Streptomyces in liquid culture. Appl Microbiol Biotechnol. 1990;31(3):272-7.
24. Srividhya S, Saravanan D, Radhakrishnan M, Balagurunathan R. Submerged fermentation

for antibiotic production. J Appl Sci Res. 2009;5(7):1008-14.

25. Das K, Tiwari RKS, Shrivastava DK. Techniques for evaluation of medicinal plant products as antimicrobial agent. J Med Plants Res. 2010;4(2):104-11.
26. Selvaraj R, Jansirani S, Saravanan D. Antimicrobial and phytochemical study of Trigonella foenum-graecum against diarrheal pathogens. J Chem Pharm Res. 2015;7(2):994-8.

## TABLES

**Table 1.** Sampling locations and sample codes of marine sediment samples

| S. No. | Sampling location | Geographic region | Sample code |
|---|---|---|---|
| 1 | Ennore Beach | Chennai, Tamil Nadu | EB |
| 2 | Marina Beach | Chennai, Tamil Nadu | MB |
| 3 | Kovalam Beach | Chennai, Tamil Nadu | KB |
| 4 | Mamallapuram Beach | Tamil Nadu | MP |
| 5 | Kalpakkam Beach | Tamil Nadu | KP |

**Table 2. Morphological and cultural characteristics of bacterial isolates from marine sediments**

| Characteristic | Observation |
|---|---|
| Total isolates | 43 |
| Gram-positive isolates | 53.48% |
| Gram-negative isolates | 46.52% |
| Predominant morphology | Rod-shaped |
| Pigmented colonies | Yellow, orange, red, white |
| Non-pigmented colonies | Predominant |

**Table 3. Antibacterial activity of selected *Bacillus* isolates by different screening methods**

| Isolate code | Well diffusion | Agar plug method | Direct spot method | Overall activity |
|---|---|---|---|---|
| EB series | Low | Low | Variable | Limited |
| KB7 | Moderate | Moderate | Positive | Moderate |
| KP3 | High | High | High | Broad-spectrum |

**Table 4. Effect of cultural parameters on antibacterial metabolite production by isolate KP3**

| Parameter | Optimal condition |
|---|---|
| Carbon source | Starch |
| Nitrogen source | Yeast extract |
| pH | 7 |
| Temperature | 30°C |
| Incubation period | 72 hours |

**Table 5. Antibacterial activity of ethyl acetate extract of KP3 isolate by disc diffusion assay**

| Test organism | Zone of inhibition (mm) |
|---|---|
| Bacillus subtilis | 24 |
| Acinetobacter baumannii | 21 |
| Staphylococcus aureus | 20 |
| Escherichia coli | 18 |
| Klebsiella pneumoniae | 16 |
| Pseudomonas aeruginosa | 16 |

**Table 2.** Table Title – A brief descriptive title of the table

| **Title 1** | **Title 2** | **Title 3** |
|---|---|---|
| entry 1 | data | data |
| entry 2 | data | data [1] |

[1] Tables may have a footer.

**FIGURES**

Figure 1. Antimicrobial activity of the bacterial isolates

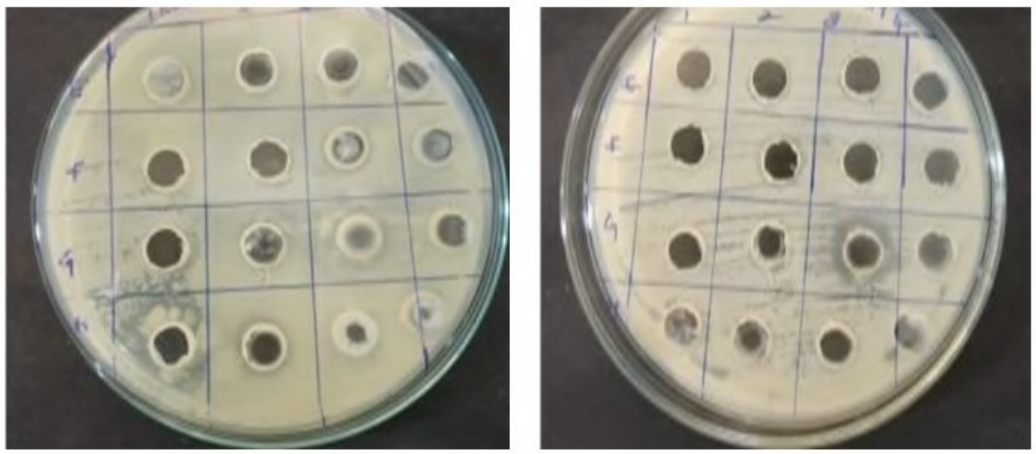

Figure 2. Antimicrobial activity by direct spot method.

**Figure 3. Production of antimicrobial compound by submerged fermentation**

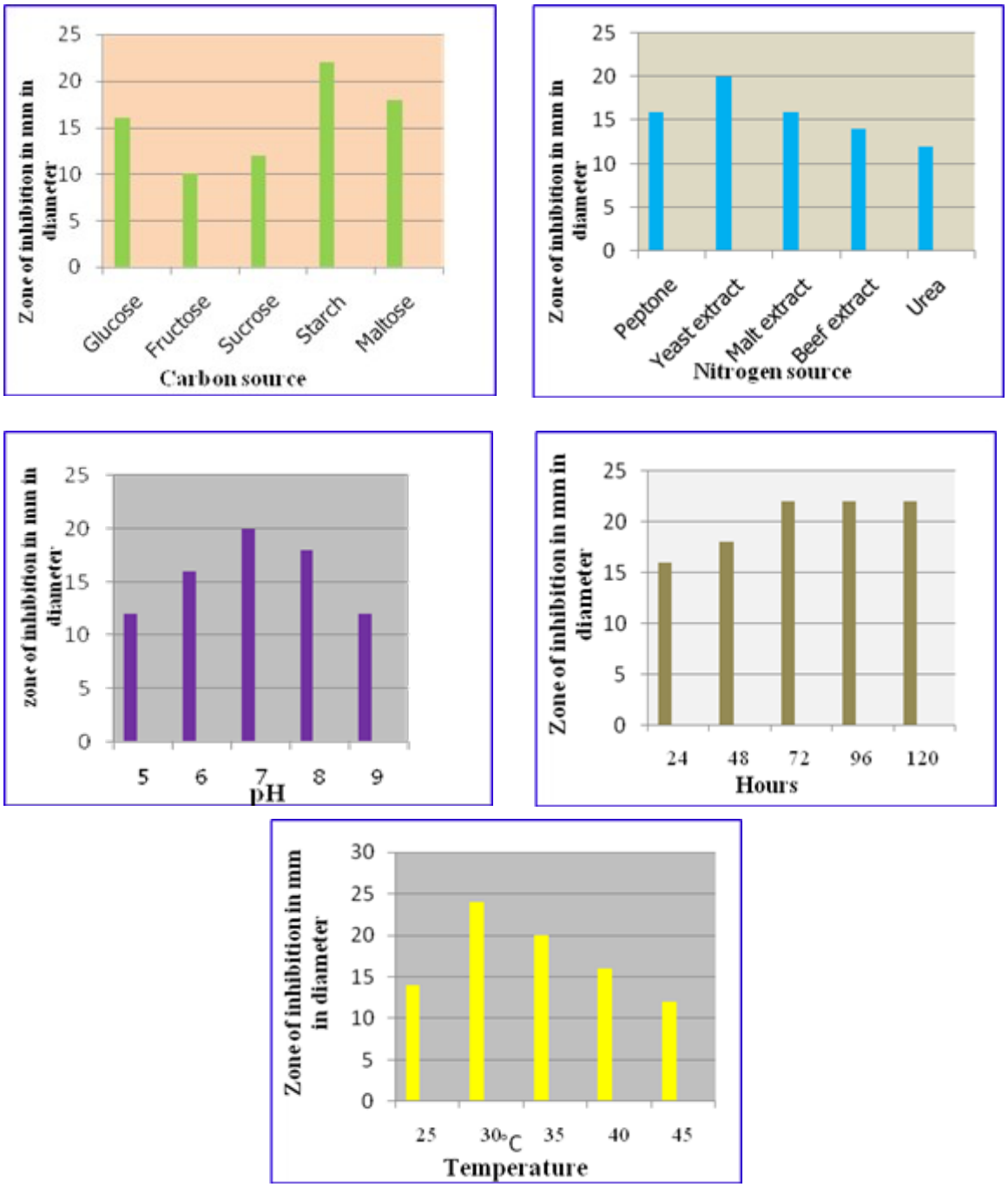


**Figure 4. Graphical representations of optimization of compound produced from the bacterial isolate from Kalpakkam samples - KP3**