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# Evaluation and Optimization of Anti-bacterial and Anti-fungal Activity of Pseudomonas MK-13 Strains.

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## Abstract

**Background:** Antimicrobial resistance is a challenging threat to global health. The resistance developed due to misuse or overuse of antibiotics. Antimicrobial resistance reduces the progress of antibiotics which significantly threaten human health. The study aims to explore antimicrobial activity of Pseudomonas MK-13 as well as optimization of different parameters essential for growth and antimicrobial activity.

**Material and Methods:** Antibacterial and antifungal activity of Pseudomonas MK-13 was checked against MDR bacterial strains (Acinetobacter, MRSA, E.coli and Pseudomonas) and fungi (Alternaria and Fusarium) by agar well diffusion method and dual culture technique respectively. Growth and antibiotic production activity were checked at different parameters i-e temperature, pH, incubation time and aeration.

**Results:** Interpretation of results showed that Pseudomonas MK-13 have significant antibacterial activity against MRSA (17.5 mm) and E. coli (16mm) followed by Acinetobacter (11.7 mm) and Pseudomonas (10 mm). Pseudomonas MK-13 showed potential antifungal activity by significantly reducing growth of Alternaria and Fusarium by 16% and 14% respectively. Pseudomonas MK-13 has significant growth and antibacterial activity at PH 7, Temperature 37°C and at shaking aeration respectively. While negligible growth and activity were observed at PH 3, temperature 4°C and static aeration.

**Conclusion:** Highest growth and antibacterial activity of Pseudomonas MK-13 was found at PH 7, Temperature 37 °C and at shaking aeration. it is concluded that Pseudomonas MK-13 have significant antibacterial activity at PH 7, Temperature 37°C and at shaking aeration as well as potential antifungal activity. It is a beneficial parameter to produce antibacterial compounds to reduce the cost of production of potential compounds.

**Keywords:** Pseudomonas; MDR; MRSA; PH; Enzymes; Antibiotics; Fermentation

## Introduction

Microorganism's screenings to produce innovative antibiotics have been effectively pursued for several years by the researcher. Antibiotics are generally produced by fungi and bacteria are consumed in several fields including farming, veterinary and pharmacological industries [1]. The first antibiotic penicillin, which was produced by fungi, was discovered by Alexander Fleming [2]. Bacteria can also produce a variety of physiologically dynamic secondary metabolites such as antibiotic s, herbicides, insecticides, anti parasitic drugs, and waste treatm ent enzymes like xylenes and cellulose. Antibiotics are the mos t therapeutically and commercially important of these compounds [3]. Bacteria that produce antibiotics include Streptomyces Bacillus spp. and some Pseudomonas spp. Bactericidal activity of antibacterial produce by a microorganism might depend on the bacterial growing phase, and it regularly needs extensive metabolic activity and separation of bacteriological cells. After the screening of antibacterial against a diverse variety of bacteria, vigorous compounds are identified and then produce on a large scale by fermentation, frequently in strongly aerobic environments [4].

Antibiotic resistance is very common nowadays. Although there is large scale production of antibiotics throughout the world, yet there are no effective antibiotics against certain disease causing micro-organisms particularly the disease predominantly in the developing countries [5]. Emergence of resistance is mostly due to evoluonary developments that occurred all along antibiotic therapy. Due to antibiotic usage, bacterial strains with physiologically or genetically improved capability will endure under extreme dosages of antibiotics. In appropriate environment, it might result in healthier development of resistive bacteria, while the development of affected bacteria is prevented with antibiotics [6]. Antibiotics such as erythromycin and penicillin, which were very operative

against numerous bacteriological species and strains, have become less effective, due to the higher resistance of several bacterial strains [7]. Researchers determined that humans annually use about 3 million pounds (weight) of antibiotics. While the animal feed also contains about 3 million pounds (weight) for antibiotics annually. This overuse of antibiotics is one of the main reasons of antibiotic resistance [8].

Microorganisms usually make changes in their targets so new antibiotics with new modes of action are needed [9]. Multi drug resistance (MDR), is a condition enabling disease-causing microorganisms (viruses, bacteria, fungi or parasites) to showed resistance
against antibiotics, fungicidal drugs, antiviral medicines, anti-parasitic medications, chemicals of a wide difference of structures and function targeted for eradication of microorganisms.

Multidruresistant organisms (MDROs) for instance, methicillin resistant Staphylococcus aureus (MRSA), quinolone resistance Enterobacteriaceae, vancomycin-resistant Enterococci (VRE) and some gram-negative bacilli play a significant role in antibiotic resistance [10]. Pseudomonas infections are also crucial to treat due to their intrinsic ability to resist many antibiotics as well as their capability to obtain resistance from other bacteria. This bacterium shows essential developed and modifying mechanisms of antibiotics resistance [11]. Although few new drugs are used to treat Pseudomonal infections but there are some oldest medications such as polymyxins that are still effective against Pseudomonas spp. [12]. Some of the fungal strains such as fusarium and alternaria developed resistance to routinely used antibiotics. The pseudomonas strains produce certain potential compounds that have promising activity against certain fungi.

The study aims to evaluate the antibacterial and antifungal activity of pseudomonas MK-13 strains as well as to optimize the required parameter for growth and antimicrobial activity.

# Materials and Methods

## Sample collection

The present study was conducted on Pseudomonas MK-13 isolated from District Karak, KPK Pakistan and then transferred to the Department of Microbiology, KUST Kohat for the experimental work. This strain was further subjected to antibacterial activity against MDR bacteria and antifungal activity.

Identification of pseudomonas MK13 and MDR bacterial strains

Total four multi-drug resistant (MDR) bacteria (Acinetobacter, E.coli, MRSA, and Pseudomonas) were tested in this research. All bacterial isolates were cultured on nutrient agar plates and after 24 hours of incubation at 37°C all the bacterial isolates were obtained from cultural characteristics and biochemical characteristics such as catalase, oxidase, coagulase DNase, motility indole urease, citrate MR and VP.

## Antibacterial activity of pseudomonas MK-13

### Ager well diusion method

Each MDR isolate was diluted with sterile distilled water in autoclaved test tubes and compared with 0.5 McFarland standards. The lawn of each isolate was made on MHA agar and two wells were bored using a sterile micropipette tip of 1 ml. The first well was filled with 40 µl of supernatant of Pseudomonas culture broth and the negative control well was filled with sterile nutrient broth. The experiment was carried out in triplets.

### Growth optimization and antibacterial activity of pseudomonas MK-13

Optimization of the selected bacterial isolate was performed at different temperatures, pH levels and static and shaking conditions.

### Temperature optimization

The per-cultured Pseudomonas MK-13 (10 µl) was taken and inoculated in 15 ml of the autoclaved nutrient broth followed by incubation at temperatures 4°C, 20°C, 37°C, and 50°C. Subsequently, the OD at 600 nm was read for each temperature and antibacterial activity was determined after 24,48,72 hours of incubation. An experiment was carried out in triplets for each temperature and the zone of inhibition was measured in mm.

### PH optimization

The per-cultured Pseudomonas MK-13 (10 µl) was taken and inoculated in 15 ml of the autoclaved nutrient broth followed by incubation at 37°C and pH of 3,5,7 and 9. Subsequently, the OD at 600nm was read for each pH and antibacterial activity was determined after 24,48,72 hours of incubation. The experiment was carried out in triplets for each pH and zone of inhibition was measured in mm.

### Aeration optimization

The per-cultured Pseudomonas MK-13 (10 µl) was taken and inoculated in 15 ml of the autoclaved nutrient broth. A set of three test tubes were incubated in a shaking incubator while three were incubated in a static incubator for 24 hours at 37°C. OD was read at 600 nm and activity was determined after 24 hours of incubation and the zone of inhibition was measured in mm.

## Antifungal activity of MK-13

### Dual culture techniue

Antagonistic assay was performed according to the dual culture method on PDA medium. Before pouring of media into plates, chloramphenicol (28 mg/L) was added to the media to inhibit bacterial growth. The tested fungal plug was placed at one side of the PDA plate (2 cm away from edge). While on the other side, the well was bored and 50 µl of cell free supernatant of MK-13 was poured into the well. For each fungus, three replicates were prepared. For control, the fungal inoculum was placed in the center of the plate. All the plates were incubated

at 28°C ± 2 for 3-4 days. Percentage (%) of growth inhibition was calculated by:%age inhibition= [(A-B÷A]x100.

## Results

There is cell morphology and biochemical tests of pseudomonas MK-13 (Table 3) and the phylogenetic tree. (Figure: 3).

**Table 1:** Biochemical tests of pseudomonas Mk-13.

| AEB lab ID | Cell morphology | Biochemical test | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Shape | Gram | Spore | Mot | U | Ind | Oxi | Cat | Cit | MR | VP |
| MK-13 | Rod | _ | _ | + | + | _ | + | + | + | _ | _ |

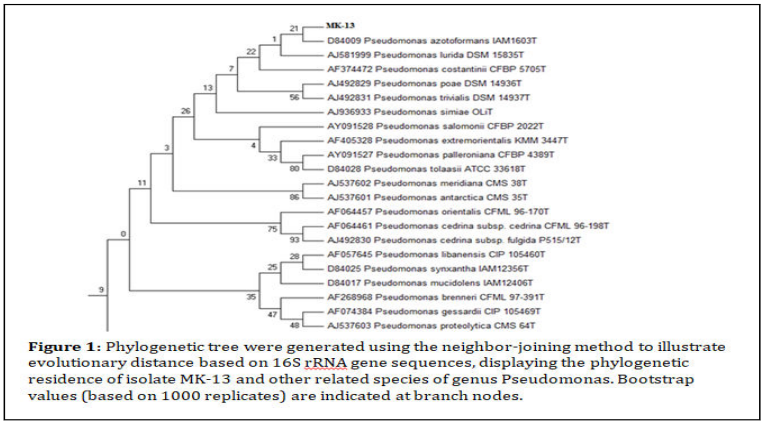


**Figure 1:** Phylogenetic tree were generated using the neighbor-joining method to illustrate evolutionary distance based on 16S rRNA gene sequences, displaying the phylogenetic residence of isolate MK-13 and other related species of genus Pseudomonas. Bootstrap values (based on 1000 replicates) are indicated at branch nodes.

**Table 2:** Identification of tested MDR bacteria.

| Tested MDR Bacteria | Gram staining | Catalase test | Oxidase test |
|---|---|---|---|
| Acinetobacter | Negative | Positive | Negative |
| E.coli | Negative | Positive | Negative |
| MRSA | Positive | Positive | Negative |
| Pseudomonas | Negative | Positive | Positive |

**Table 3:** Antibacterial activity of pseudomonas MK-13 against tested MDR bacteria.

| Tested MDR | Acinetobacter | E.coli | MRSA | Pseudomonas |
|---|---|---|---|---|
| Zone of inhibition (mm) | 11.7 mm | 16 mm | 17.6 mm | M |

**Optimization of culture condition**

**Temperature and incubation time effect on growth and production of antimicrobial metabolites**

The effect of temperature and incubation time on the growth and production of antimicrobial metabolite was checked. Pseudomonas MK-13 exhibited maximum growth and antibacterial activity at 37°C. Whereas no activity was observed at 4°C. Furthermore, little growth and activity were observed at 20°C and 50°C (Figure 3,4,5,6).

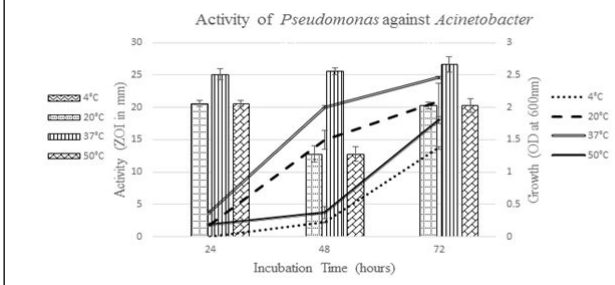


**Figure 2:** Temperature effect on antibacterial activity of Pseudomonas MK-13 against Acinetobacter.

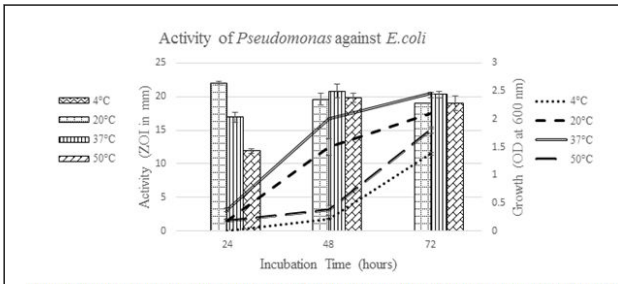


**Figure 3:** Temperature effect on antibacterial activity of Pseudomonas MK-13 against E.coli.

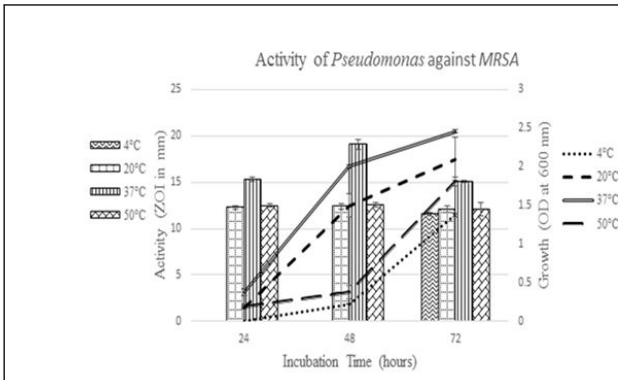


**Figure 4:** Temperature effect on antibacterial activity of Pseudomonas MK-13 against MRSA.

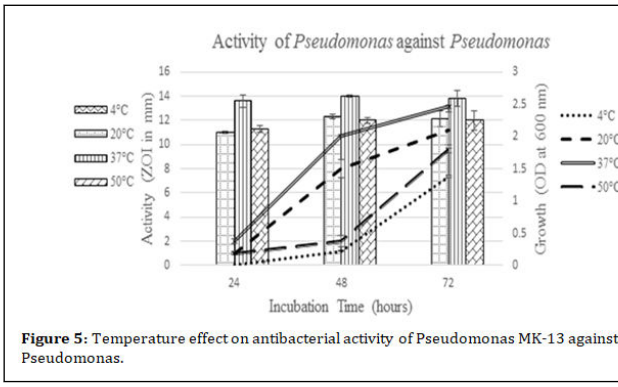


**Figure 5:** Temperature effect on antibacterial activity of Pseudomonas MK-13 against Pseudomonas.

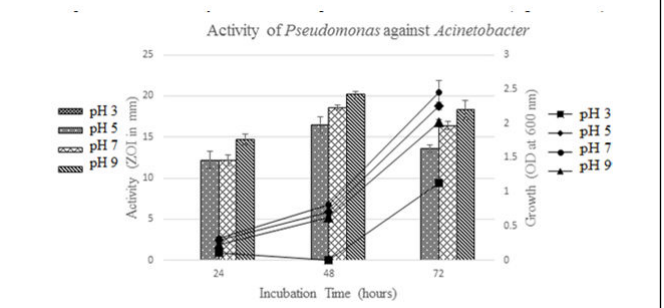


**Figure 6:** pH effect on antibacterial activity of Pseudomonas MK-13 against Acinetobacter.

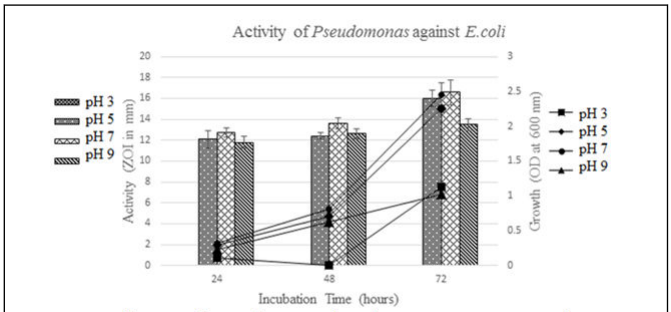


**Figure 7:** pH effect on antibacterial activity of Pseudomonas MK-13 against E.coli.

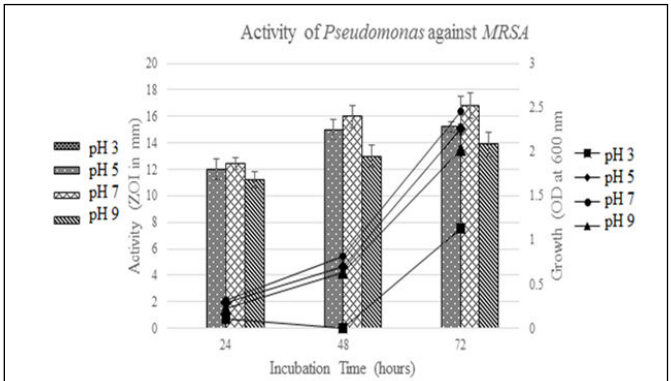


**Figure 8:** pH effect on antibacterial activity of Pseudomonas MK-13 against MRSA.

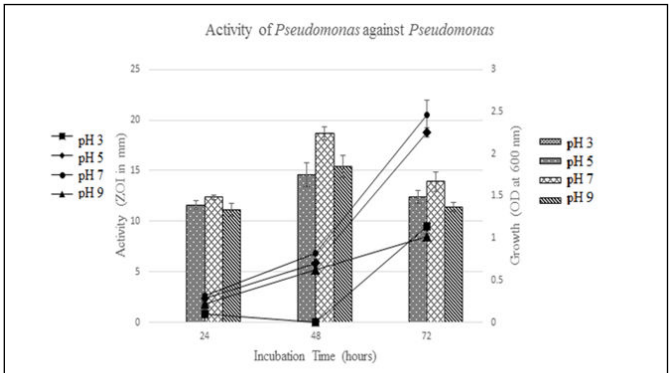


**Figure 9:** pH effect on antibacterial activity of Pseudomonas MK-13 against Pseudomonas.

**PH and incubation time effect on antimicrobial metabolites g rowth and production**

Pseudomonas MK-13 showed significantly increased growth and antimicrobial activity at pH-7, while no activity was observed at pH-3 and even after 72 hours. (Figure 7to10).

**Aeration effect on growth and production of antimicrobial metabolites**

The aeration effect was analyzed based on growth and antibacterial metabolites. Pseudomonas MK-13 was grown both in shaking and statistic incubator for 24 hours. Maximum growth

and activity were observed in the isolate cultured in the shaking incubator, while less activity in the static incubator (Figure 11).

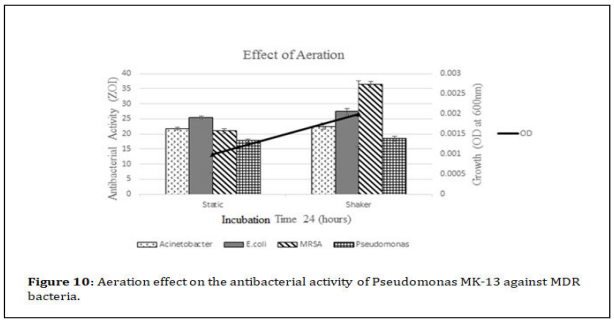


**Figure 10:** Aeration effect on the antibacterial activity of Pseudomonas MK-13 against MDR bacteria.

**Antifungal activity of fungi**

Pseudomonas MK-13 showed promising activity against Alternaria spp. and Fusarium spp.

Zone of inhibition was recoded according to "inhibition percentage" i.e. Pseudomonas MK-13 reduced the growth of Alternaria (16%) and Fusarium (14%). (Table 3.4) (Figure: 3.12).

**Table 4:** Antifungal activity of pseudomonas MK-13 against fusarium and alternaria.

| Fungal strains | Diameter of fungal growth on control (A) | Diameter of fungal growth on activity plate (B) | Percentage inhibition (%) = [(A-B) ÷ A] x 100 |
|---|---|---|---|
| Fusarium | 27 mm | 23 mm | 14% |
| Alternaria | 30 mm | 25 mm | 16% |

**Fungal growth**

Bacteria inhibit fungal growth

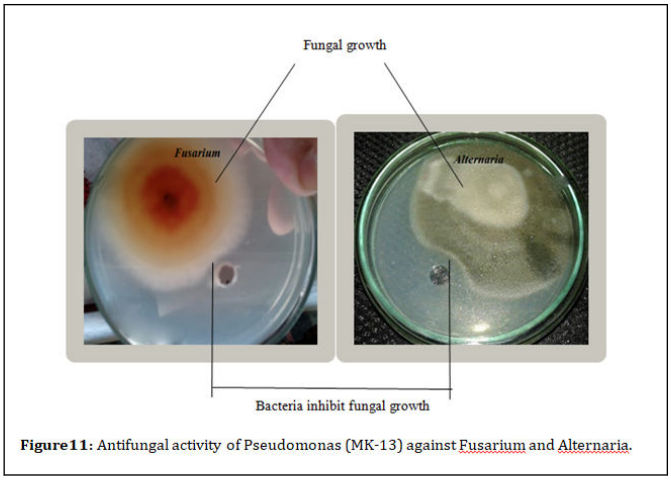


**Figure 11:** Antifungal activity of Pseudomonas (MK-13) against Fusarium and Alternaria.

## Discussion

Rapid involvement of MDR pathogens is one of the leading problems worldwide. Scientists have been continuously in search of new agents being antimicrobial potentials against these MDR bugs. In the present study, Pseudomonas MK-13 was explored for its antimicrobial potential against MDR bacteria and fungi. In the current study checked the antibacterial activity of Pseudomonas MK-13 was evaluated against four different MDR bacteria i.e. Acinetobacter, E.coli, MRSA and Pseudomonas, and were reached to the findings that Pseudomonas MK-13 has an antibacterial potential against all tested MDR bacteria. However the range of activity was different against different bacterial strains like the range of activity was high against MRSA have a zone of inhibition of 17.6 mm followed by E.coli (16mm), Acinetobacter (11.7 mm) and Pseudomonas (10 mm zone of inhibition). Similar results were obtained by 99 [13]. have reported that P. aeruginosa produced compounds that have inhibitory effects against MRSA. On the other hand [14]. have reported that E.coli showed 13 mm zone of inhibition against Pseudomonas spp. While the antibacterial activity of Pseudomonas against several gram-positive bacteria has been reported by several studies [15].

Different species of Pseudomonas produce a variety of bioactive moleules responsible for antibacterial activity. Previous studies reported that P. fluorescens strain CHA0 produces 2, 4 diacetylphloroglucinol and P. Aeruginosa produces phenazine and diketopiperazines [16,17]. Culture conditions including temperature, pH and aeration etc were optimized. Maximum growth was observed at 37 0C, 7 pH and at shaking conditions, while deviation from these conditions had brought a decrease in the concentration of metabolites production. It was noticed that culture conditions including temperature, medium pH and aeration can affect antimicrobial exhibiting bacteria growth as well as production of antimicrobial metabolites. We have observed that the growth and activity were stopped at 40C, 3 pH and static incubator. Our results agree with the results obtained by [18]. Which showed that changes in culture conditions have brought variations in the concentration of antimicrobial metabolites?

We have also checked the activity of Pseudomonas MK-13 against Alternaria and Fusarium fungal strains and observed that it was also able to show activity against these fungal strains. Antifungal activity of Pseudomonas has been reported by several researchers like [19 to 22].

Pseudomonas MK-13 has the potential of synthesizing lipase enzymes. While this is also reported by previous studies like [23]. which have reported the production, purification, characterization, and applications of lipases produced by these bacterial strains.

In the current study, we have observed that Pseudomonas MK-13 has antibacterial as well as antifungal activity against tested bacteria and fungi respectively and can be a suitable choice for inhibiting the growth of these microorganisms [24].

## Conclusion

The highest growth and antibacterial activity of Pseudomonas MK-13 were found at PH 7, Temperature 37 °C and at shaking aeration. it is concluded that Pseudomonas MK-13 have significant antibacterial activity at PH 7, Temperature 37°C and at shaking aeration as well as potential antifungal activity. It is a beneficial parameter to produce antibacterial compounds to reduce the cost of production of potential compounds.

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