International Journal of Applied Chemical and Biological Sciences (2024), 5(4), 1-9
ISSN- 2582-788X

RESEARCH PAPER

Article Identifier: https://identifier.visnav.in/1.0001/ijacbs-24k-27001/

Isolation, Identification, and Evaluation of Antagonistic Activity of Nasal Bacteria against Fungi causing Nasal Infections

Mohammed Ndagidzuru Abdullahi [1], Jibril Mohammed [2*], Abdullateef Abayomi Alabi [2], Abdulafeez Adebayo Sadiq [3]

[1] Department of Medical Microbiology and Parasitology, Faculty of Basic Clinical Sciences, University of Ilorin, Ilorin, Nigeria.
[2] Department of Microbiology, Faculty of Pure and Applied Sciences, Kwara State University, Malete, PMB 1530, Ilorin, Kwara State, Nigeria.
[3] Department of Microbiology, Faculty of Science, University of Ibadan, Ibadan, Nigeria.

* For correspondence: jibrilmohammed2001@gmail.com

Received on: 27 October 2024 *Published on: 21 November 2024*

ABSTRACT

Nasal fungal infections are increasingly challenging to manage due to the side effects associated with conventional antifungal agent. This study aimed to isolate, identify, and evaluate the antagonistic activity of nasal bacteria against these fungal pathogens to explore microbiota-based alternatives for infection control. Fungal pathogens, including *Aspergillus flavus, Mucor mucedo*, and *Fusarium oxysporum*, were collected from the microbiology lab, and nasal swabs were collected from healthy individuals and cultured with the isolation of five bacterial isolates, including *Micrococcus luteus, Bacillus* sp., *Staphylococcus aureus, Staphylococcus epidermidis, and Enterococcus* sp. The antagonistic activity of these bacteria was tested against the fungal pathogens using a plate inhibition assay. *Bacillus* sp. exhibited the highest inhibition with 55%, 65%, and 58%, followed by *Staphylococcus epidermidis* with 42%, 58%, and 48%, and *Micrococcus luteus* with 32%, 40%, and 29% against *Aspergillus flavus, Mucor mucedo*, and *Fusarium oxysporum*, respectively. Lower inhibition was observed in *Staphylococcus aureus* with 28%, 35%, and 25%, and *Enterococcus* sp. with 18%, 25%, and 15% against the same fungal pathogens, respectively. This research suggests that nasal bacteria, especially *Bacillus* sp., have potential as biotherapeutic agents in managing fungal nasal infections, which could provide a sustainable alternative to traditional antifungal drugs.

Keywords: Antagonistic activity, Bacillus sp., *Fungal pathogens, Nasal bacteria.*

1. INTRODUCTION

Nasal infections, particularly those caused by fungi, have become a significant threat due to their potential to

cause chronic conditions such as sinusitis, allergic fungal rhinosinusitis, and even invasive fungal infections, especially in immunocompromised individuals. Although antifungal medications are available, however, their prolonged use often leads to adverse side effects. For example, amphotericin B, a common antifungal agent, can cause severe side effects such as infusion-related toxicity, nausea, vomiting, hypertension, hypoxia, renal insufficiency, hypokalemia, hypomagnesemia, metabolic acidemia, and polyuria [1]. Nystatin, another widely used antifungal, has also been linked to gastrointestinal issues, including diarrhea, nausea, and abdominal pain [2]. Terbinafine, an allylamine, frequently results in central nervous system side effects like headaches, with additional symptoms including rashes, dyspepsia, and upper respiratory inflammation [2]. Similarly, fluconazole is associated with headaches, stomach pain, diarrhea, nausea, and rash [3].

In recent years, increasing attention has been given to the potential of the human microbiota as a natural defense system against pathogenic microorganisms. The human microbiota consists of trillions of microorganisms that reside in various parts of the body that plays a critical role in maintaining health by providing a barrier against infections. Studies have shown that certain bacterial species in the human microbiota can produce antimicrobial compounds, regulate immune responses, and inhibit the growth of harmful pathogens [4].

Specifically, nasal bacteria are an important part of the local defense system in the respiratory tract. The nasal microbiome consists of commensal bacteria that inhibit the growth of opportunistic pathogens, thereby reducing the risk of infections such as sinusitis and bronchitis [5]. Certain nasal bacterial species are known to produce bioactive compounds with antagonistic effects on fungal growth, making them promising candidates for alternative treatments for nasal infections. These bacteria may not only help in preventing infections but could also be used to combat resistant fungal strains, and also open new avenues for microbiota-based therapies.

Given the significant side effects of current antifungal agents, there is a growing need for more sustainable and safer approaches to managing fungal infections. Investigating the potential of nasal bacteria to inhibit fungi responsible for nasal infections would not only deepens our understanding of the nasal microbiome's role in maintaining respiratory health but also holds the potential to develop novel, microbiota-based therapies which could be more effective, with fewer side effects, and possibly reduce the reliance on traditional antifungal drugs.

Therefore, this study aims to isolate, identify, and evaluate the antagonistic activity of nasal bacteria against fungi causing nasal infections, with the goal of exploring potential microbiota-based therapeutic alternatives to conventional antifungal treatments.

2. MATERIALS AND METHODS

2.1. Materials, Media, and Reagents

The materials, media, and reagents used in this study included sterile cotton swabs, nutrient agar (NA), Potato Dextrose Agar (PDA), Gram staining reagents, biochemical test kits, sterile petri dishes, inoculating loops, distilled water, 70% ethanol, and cork borer (7 mm in diameter).

2.2. Washing and Sterilizing Laboratory Bench

Before beginning the experiments, the laboratory bench was washed and sterilized. The surface was first cleaned with detergent and water to remove any debris. Once dry, the bench was wiped with 70% ethanol to disinfect the surface.

2.3. Collection of Fungi Causing Nasal Infections

Fungal isolates known to cause nasal infections including, *Aspergillus flavus, Mucor mucedo, and Fusarium oxysporum*, were collected from the microbiology laboratory. These isolates were stored on Potato Dextrose Agar (PDA) slants and incubated at 25 °C until further use.

2.4. Isolation of Nasal Bacteria

Nasal swabs were collected from healthy individuals using sterile cotton swabs and were streaked onto nutrient agar (NA) plates. The plates were incubated at 37 °C for 24 hours to allow for bacterial growth. Distinct bacterial colonies were sub-cultured by further streaking on fresh agar plates to obtain pure bacterial isolates.

2.5. Morphological Characterization and Gram Staining of Bacterial Isolates

The bacterial isolates were morphologically characterized based on colony shape, size, color, surface texture, elevation, and margin. Gram staining was then performed to determine the Gram's reaction (positive or negative) and cell morphology (shape and arrangement) under a microscope. A fixed smear of the bacterial culture on the slide was treated with crystal violet for 1 minute, and rinsed with distilled water. Lugol's iodine was then applied for 1 minute, and after another rinse, the smear was decolorised with acetone alcohol for 10 seconds. Safranin was applied for 30 seconds, then the slide was washed again with distilled water. The smear was examined using an oil immersion objective lens under the microscope. Gram-positive bacteria stained purple, while Gram-negative bacteria appeared red [6].

2.6. Biochemical Identification of Bacterial Isolates

The biochemical characteristics of the bacterial isolates were assessed using several tests, including catalase, oxidase, indole, methyl red, voges-proskauer, citrate, urease, coagulase, and motility tests.

2.6.1. Catalase Test

This test was conducted to detect the presence of the catalase enzyme in the bacterial isolates. Small samples from the isolates were placed on microscope slides using sterile inoculating loops. A drop of 3% hydrogen peroxide (H_2O_2) was added using a dropper. The formation of bubbles indicated a positive catalase result [7].

2.6.2. Oxidase Test

This test was conducted to detect the presence of the cytochrome oxidase enzyme in the bacterial isolates. Filter paper strips soaked in a 1% solution of tetra-methyl-phenylene diamine dihydrochloride (oxidase reagent) and dried were placed on clean slides. Fresh cultures of the isolates were transferred using sterile glass rods and smeared on the filter paper. The development of a blue-purple colour within 5–10 seconds indicated a positive result [8].

2.6.3. Indole Test

This test was conducted to detect whether the bacterial isolates could degrade tryptophan to produce indole. The isolates were cultured in peptone water containing tryptophan and incubated at 37 °C for 24 hours. After incubation, 1 mL of Kovac's reagent was carefully added along the side of the test tubes. A pink colour appearing

in the reagent layer within one minute indicated a positive indole reaction [9].

2.6.4. Methyl Red Test

This test was conducted to determine whether the bacterial isolates could ferment glucose and produce acidic byproducts. The isolates were inoculated into glucose-phosphate broth and incubated at 37 °C for 24 hours. After incubation, two drops of methyl red reagent were added and mixed. A bright red colour indicated a positive result, while a yellowish or orange colour signified a negative result [10].

2.6.5. Voges-Proskauer Test

This test was conducted to determine the ability of the bacterial isolates to ferment glucose and produce neutral end products such as acetoin. The isolates were inoculated in glucose phosphate broth and incubated at 37 °C for 24 hours. After incubation, 0.2 mL of 40 % potassium hydroxide (KOH) and 1.6 mL of 5 % alpha-naphthol solution were added to the cultures and shaken. A cherry red colour indicated a positive result, while a yellowish-orange colour signified a negative result [10].

2.6.6. Citrate Test

This test was conducted to determine whether the bacterial isolates could utilise citrate as the sole source of carbon. The isolates were streaked onto citrate agar slants and incubated at 37 °C for 48 hours. A colour change from green to blue indicated a positive result, while no colour change or growth indicated a negative result [11].

2.6.7. Urease Test

This test was conducted to detect the ability of the bacterial isolates to hydrolyse urea into ammonia and carbon dioxide. The isolates were streaked onto the surface of urea agar slants and incubated at 37 °C for 48 hours. A pink colour change indicated a positive reaction [12].

2.6.8. Coagulase Test

This test was conducted to detect the production of the coagulase enzyme by the bacterial isolates. Small portions of the isolates were emulsified in 0.5 mL of rabbit plasma in sterile test tubes. After gentle shaking, the tubes were incubated at 37 °C for 4 hours. The formation of a clot indicated a positive coagulase result, while the absence of clotting signified a negative result [13].

2.6.9. Motility Test

This test was conducted to determine the motility of the bacterial isolates. Sterile inoculating needles were used to stab the centre of tubes containing semi-solid motility agar, which were then incubated at 37 °C for 24 hours. Diffuse growth spreading away from the stab line indicated motility, while growth restricted to the stab line indicated non-motility [14].

2.7. Antagonistic Activity of Bacterial Isolates Against Fungal Pathogens

The antagonistic activity of nasal bacteria against fungal pathogens was evaluated using Fokkema's method with modifications [15]. In this study, fungal discs were used instead of fungal spore suspensions, and the incubation period was reduced to 5 days to prevent fungal overgrowth. Bacterial isolates were streaked on freshly prepared Potato Dextrose Agar (PDA) plates (90 mm) using a sterile inoculating loop to create a bacterial streak approximately 40 mm long, and positioned 35 mm away from the center of the plate. A 7 mm diameter fungal disc were cut from the actively growing edge of

the collected fungal colony, and aseptically placed in the center of the plate using a sterile cork borer. The plates were incubated at 25 °C for 5 days, and observed the interaction between the bacterial streak and fungal growth daily. The percentage of inhibition (I) of fungal radial growth was calculated using the following formula:

$$I = \frac{r1 - r2}{r1} \times 100$$

where $r1$ represents the radius of fungal growth away from the bacterial streak, and $r2$ is the radius of fungal growth towards the bacterial streak..

3. RESULTS AND DISCUSSION

3.1. *Morphological Characteristics and Gram's Reaction of the Bacterial Isolates*

The colonial characteristics of the bacterial isolates, including color, shape, size, texture, and elevation, are summarized in Table 1. All five isolates were identified as Gram-positive bacteria. Four of the isolates exhibited cocci (round) morphology, with one classified as bacilli (rod-shaped). The Gram-staining confirmed the Gram-positive nature of the isolates, as they retained the crystal violet stain and appeared purple under the microscope. This outcome indicates the thick peptidoglycan layer in their cell walls, which is characteristic of Gram-positive bacteria. The results are consistent with the previous findings, where Gram-positive bacteria are frequently observed in the human nasal cavity [16].

3.2. *Biochemical Characteristics and Identification of the Bacterial Isolates*

Table 2 showed the biochemical tests results of the bacterial isolates. The bacterial isolates were identified as *Micrococcus luteus, Bacillus* sp., *Staphylococcus aureus, Staphylococcus epidermidis*, and *Enterococcus* sp. These bacteria genus have all been previously isolated from nasal swabs. *Staphylococcus aureus*, and *Staphylococcus epidermidis* are one of the most well-known nasal colonizers. Their isolation is supported by the work of Zhou [16], who isolated *Staphylococcus* species from the nasal cavity of preclinical medical students. *Micrococcus luteus* is commonly found in the nasal cavity. Their isolation in this study is also supported by the work of Zhou [16]. *Bacillus* sp., although primarily associated with soil, has occasionally been isolated from human nasal passages, likely due to environmental exposure. The isolation of *Bacillus* sp. is supported by the work of Mutua et al. [17]. *Enterococcus* species, while typically found in the gastrointestinal tract, have also been isolated from nasal swabs [18].

3.3. *Antagonistic Activity of Bacterial Isolates Against Fungal Pathogens*

Table 1. Morphological Characteristics and Gram's Reaction of Bacterial Isolates

Isolates	Shape	Size	Color	Texture	Elevation	Margin	Cell's Shape	Gram's Reaction
1	Circular	Small	Yellow	Smooth	Convex	Entire	Cocci	Positive
2	Circular	Large	Cream	Smooth	Convex	Entire	Bacilli	Positive
3	Circular	Medium	Golden-yellow	Smooth	Convex	Entire	Cocci	Positive
4	Circular	Medium	White	Smooth	Raised	Entire	Cocci	Positive
5	Circular	Medium	Cream	Smooth	Convex	Entire	Cocci	Positive

Table 2. Biochemical Characteristics of Bacterial Isolates

Isolates	CAT	OX	IND	MR	VP	CIT	UR	CO	MOT	Organism
1	+	+	-	+	-	-	+	-	-	*Micrococcus luteus*
2	+	+	-	-	+	+	-	-	+	*Bacillus* sp.
3	+	-	-	-	+	+	+	+	-	*Staphylococcus aureus*
4	+	-	-	-	+	-	+	-	-	*Staphylococcus epidermidis*
5	-	-	-	-	+	-	-	-	-	*Enterococcus* sp.

KEY: CAT= catalase, OX= oxidase, IND= indole, MR= methyl red, VR= voges proskauer, CIT=citrate, UR=urease, CO = coagulase, MOT = motility, + = positive, - = negative

Table 3 displays the antagonistic activity of the bacterial isolates against the fungal pathogens. The results demonstrated that various bacterial isolates from the nasal cavity exhibit varying percentage of antifungal activity, with *Bacillus* sp. emerging as the most potent antagonist across all tested fungi. *Mucor mucedo*, a mold known for causing mucormycosis, appears to be more susceptible to bacterial isolates, while the inhibition percentages against *Fusarium oxysporum* were generally lower across all bacterial isolates. The variation observed in antagonistic activity against the fungal pathogen could be explained by the differences in fungal morphology and physiology. The significant inhibitory effect of *Bacillus* sp. against *Mucor mucedo* (65%) and *Fusarium oxysporum* (58%) is supported by the existing literature that highlights the genus *Bacillus* as a prolific producer of antifungal lipopeptides, such as iturins and fengycins, which disrupt fungal cell membranes and inhibit spore germination [19]. The high inhibition percentages suggest that *Bacillus* sp. produces these or other bioactive compounds in quantities sufficient to significantly inhibit fungal growth. Furthermore, the high antagonistic activity of *Bacillus* sp. may be due to its ability to produce diverse antimicrobial agents, which can target different fungal species through multiple mechanisms. These findings showed the potential of *Bacillus* sp. as a biotherapeutic candidate for treating fungal nasal infections, especially given the increasing resistance to conventional antifungal agents. *Staphylococcus epidermidis* also displayed strong antifungal activity, specifically against *Mucor mucedo* (58%) and *Fusarium oxysporum* (48%). Previous studies have shown that *Staphylococcus epidermidis* can produce bacteriocins, which may have a broader range of antimicrobial activity [20]. The slightly lower activity observed in *Staphylococcus epidermidis* could be due to the specificity of its bacteriocins, which may not be as effective against the structural composition of fungal cell walls compared to bacterial membranes. However, its significant inhibition percentages still suggest that *Staphylococcus epidermidis* can be a useful agent in combating certain fungal infections. *Micrococcus luteus* demonstrated moderate antagonistic activity, especially against *Mucor mucedo* (40%) and *Aspergillus flavus* (32%). This is consistent with reports by Majeed [21], who found that carotenoid pigments from *Micrococcus luteus* exhibit antifungal properties against fungal pathogens. However, its efficacy is lower than that of *Bacillus* sp. and *Staphylococcus epidermis*, which might possibly due to the nature of the antimicrobial compounds it produces, and may be less effective against the specific fungi tested in this study. Additionally, the environmental conditions under which *Micrococcus luteus* grows might influence its ability to produce sufficient quantities of antifungal compounds, which could explain the lower inhibition percentages.

Table 3. Antagonistic Activity of Nasal Bacteria against Fungal Pathogens

Bacterial Isolates	Fungal Pathogen		
	***Aspergillus flavus* (%)**	***Mucor mucedo* (%)**	***Fusarium oxysporum* (%)**
Micrococcus luteus	32	40	29
Bacillus sp.	55	65	58
Staphylococcus aureus	28	35	25
Staphylococcus epidermidis	42	58	48
Enterococcus sp.	18	25	15

Staphylococcus aureus, and *Enterococcus* sp. displayed the lowest antagonistic activity against all fungal species tested, particularly against *Fusarium oxysporum* (25%, and 15%, respectively). The relatively weak performance of *Staphylococcus aureus* may be attributed to the fact that their primary antimicrobial mechanisms, such as the production of bacteriocins [22] are more effective against bacterial pathogens rather than fungi. Similarly, *Enterococcus* sp., which is known for its production of enterocins, may not produce sufficient antifungal compounds to inhibit the fungal pathogens effectively. This suggests the potential limitations of using certain bacterial species in antifungal therapies, as their antimicrobial activity may be highly specific to bacterial targets rather than fungal pathogens.

4. CONCLUSION

This study highlights the potential of nasal bacteria as promising antagonists against fungi responsible for nasal infections, particularly *Aspergillus flavus, Mucor mucedo*, and *Fusarium oxysporum*. Among the isolated bacteria, *Bacillus* sp. exhibited the strongest inhibitory activity, indicating its significant antifungal potential, especially against *Mucor mucedo* and *Fusarium oxysporum*. Other bacterial species such as *Micrococcus luteus* and *Staphylococcus epidermidis* also showed moderate antagonistic effects. These findings suggest that nasal bacteria, particularly *Bacillus* sp. could serve as alternative or complementary biotherapeutic agents to conventional antifungal treatments, which can potentially reduce the reliance on traditional antifungals, which often cause side effects.

5. RECOMMENDATIONS

It could be recommended from this study that future research should focus on isolating and characterizing the specific antimicrobial compounds produced by nasal bacteria, especially those secreted by Bacillus sp., to better understand their mechanisms of action. Additionally, in vivo. Studies are necessary to confirm the clinical efficacy and safety of using these bacteria or their metabolites as antifungal agents.

6. ACKNOWLEDGEMENT

NA

7. CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

8. SOURCE/S OF FUNDING

NA

9. REFERENCES

1. Laniado-Laborin R, Cabrales-Vargas MN, (2009), Amphotericin B: Side Effects and Toxicity, Revista Iberoamericana de Micologia; 26(4): 223-227.

2. McKeny PT, Nessel TA, Zito PM (2023, March 7). Antifungal Antibiotics, In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; Retrieved from: https://www.ncbi.nlm.nih.gov/books/NBK538168/.
3. National Health Service UK (2023, March 9). Side Effects of Fluconazole. Retrieved from: https://www.nhs.uk/medicines/fluconazole/side-effects-of-fluconazole.
4. Al-Judaibi AA (2021), Microbiota and their Influence in the Human Body, J Pure Appl Microbiol; 15(1): 42-52. https://doi.org/10.22207/JPAM.15.1.27
5. Kang HM, Kang JH (2021), Effects of Nasopharyngeal Microbiota in Respiratory Infections and Allergies, Clin Exp Pediatr; 63:543-51. https://doi.org/10.3345/cep. 2020.0145.
6. Willey J., Sherwood L., Woolverton C., 2014, Microscopy, Prescott's Microbiology: 9th Edition, McGraw Hill, New York: 32-33.
7. Reiner K (2010), Catalase Test Protocol, American Society for Microbiology; 1-6.
8. Shields P, Cathcart L (2010), Oxidase Test Protocol, American Society for Microbiology; 1-9.
9. MacWilliams MP (2012), Indole Test Protocol, American Society for Microbiology; Washington, DC; 1-6.
10. McDevitt S (2009), Methyl Red and Voges Proskauer Test Protocol, American Society for Microbiology; 1-6.
11. Hamza TA, Alebejo AL (2017), Isolation and Characterization of Rhizobia from Rhizospher and Root Nodule of Cowpea, Elephant and Lab Lab Plants, Int. J. Nov. Res. Interdiscp. Stud.; 4(4):1-7.
12. Brink B (2010), Urease Test Protocol, American Society for Microbiology; 1-4.
13. Sue Katz D (2010), Coagulase Test Protocol, American Society for Microbiology; 1-12.
14. Shields P, Cathcart L (2011), Motility Test Medium Protocol, American Society for Microbiology; 1-10.
15. Fokkema NJ (1973), The Role of Saprophytic Fungi in Antagonism Against Drechslera Sorokiniana (Helminthosporium Sativum) on Agar Plates and on Rye Leaves with Pollen, Physiological Plant Pathology; 3: 195-205.
16. Zhou K, Sun F, Xu X, Hao X, Liu J (2020), Prevalences and Characteristics of Cultivable Nasal Bacteria Isolated from Preclinical Medical Students, Journal of International Medical Research; 48(10): 1–10. DOI: 10.1177/0300060520961716.
17. Mutua JM, Gitao CG, Bebora LC, Mutua FK (2017), Antimicrobial Resistance Profiles of Bacteria Isolated from the Nasal Cavity of Camels in Samburu, Nakuru, and Isiolo Counties of Kenya, Journal of Veterinary Medicine; 1216283: 1-6.
18. Yameen MA, Iram S, Mannan A, Khan SA,Naeem Akhtar (2013), Nasal and Perirectal Colonization of Vancomycin Sensitive and Resistant Enterococci In Patients of Paediatrics ICU (PICU) of Tertiary Health Care Facilities, BMC Infectious Diseases; 13(156): 1-10. http://www.biomedcentral.com/1471-2334/13/156
19. Ongena M, Jacques P (2008). Bacillus lipopeptides: Versatile Weapons for Plant Disease Biocontrol, Trends in Microbiology, 16(3): 115-125. https://doi.org/10.1016/j.tim.2007.12.009.
20. Luti KJK (2020), Bacteriocin Production by Staphylococcus epidermidis the Normal Flora of Outer Ear: A Potential Probiotic Against Outer Ear Infections, Journal of Biotech Research; 11: 13-22.
21. Majeed HZ (2017), Antimicrobial Activity of Micrococcus luteus Cartenoid Pigment, Al-

Mustansiriyah Journal of Science; 28(1): 64-69. DOI: http: //doi.org/10.23851/mjs.v28i1.314.

22. Fernández-Fernández R, Lozano C, Eguizábal P, Ruiz-Ripa L, Martínez-Álvarez S, Abdullahi IN, et al. (2022), Bacteriocin-Like Inhibitory Substances in Staphylococci of Different Origins and Species with Activity Against Relevant Pathogens, Front. Microbiol.; 13(870510): 1-13. doi: 10.3389/fmicb.2022.870510.