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# Significant Antifungal Activity of *Streptomyce plicatus* NM3 Against Clinically Relevant *Candida* Species Collected from Jazan Hospital

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**Abstract**

**Objective:** The purpose of this work was the biocontrol of *Candida* species from Jazan hospitals using non polyenic antifungal agent from actinomycetes.

**Methods:** Different isolates of *Candida* were collected, identified by Vitek 2, their characterized growth was recorded on chromoagar medium and their identification was confirmed using molecular method. Some isolated actinomycetes were tested for their biocontrol activity of *Candida* isolates.

**Results:** Twenty-four isolates of *Candida* species obtained from Jazan hospital were identified and most of them were belong to *Candida albican, Candida auris, Candida parapsilosis, Candida tropicalis, Candida glabrata, and Candida ciferrii*. Out of 39 actinomycete isolates, and using agar well diffusion method, the isolate NM3 which was morphologically, physiologically and molecually identified as *Streptomyce plicatus* NM3, inhibited many *Candida* isolates, specially *C. parapsilosis, C. auris and C. tropicalis* with inhibition zone diameter ranged from 19-21 mm. Excellent antifungal activities were recorded for the ethyl acetate extract and the presence of ergesterol in the cultivation medium decreased the antifungal activity. The UV spectra of the isolate NM3 extract showed non-polyenic antifungal structure.

**Conclusions:** *Streptomyce plicatus* NM3 showed excellent activity against resistant *Candida* isolates and produce secondary products with non polyenic antifungal structure, thus it can be used for further studies.

**Keywords:** *Streptomyce plicatus, candida*, Jazan, non polyenis

## Introduction

Fungal pathogens represent a significant menace, leading to approximately 1.5 million deaths worldwide each year.[1,2] The term *Candida* originates from the Latin word candid, meaning white. The *Candida* species are opportunistic pathogenic organisms, but they may also develop superficial and systemic infections in the presence of predisposing factors. High mortality rates can be attributed to the increasing occurrence of invasive systemic infections and cases of septicaemia, especially in immunocompromised patients.[3,4] Currently, *Candida* systemic diseases are the fourth leading cause of nosocomial bloodstream infections.[4,5] Of all invasive infections, 90% are caused by opportunistic *Candida*.[3] In 2020, an estimated 1.7 million fatalities from fungal infections were reported.[6] Fungal infections are increasingly becoming a global health problem that is associated with high morbidity and mortality rates as well as devastating consequences socioeconomic.[7] Infections caused by the genus *Candida* are the main cause of nosocomial fungal infections especially in tertiary care hospitals.[8] In the USA, it was reported that sepsis caused by Candida albicans has a mortality rate of approximately 40%, which is higher than any other sepsis caused by bacteria or fungi.[9] Non-albicans Candida species are also known to have decreasing susceptibility to antifungal agents. They are responsible for more than 80% of yeast infections in humans.[10] Candidiasis is an opportunistic infection caused by yeasts of the genus *Candida* and accounts for 75 to 88% of fungal infections.[11] The pathogenicity of Candida species is attributed to certain virulence factors, such as the ability to evade host defences, adhesion and biofilm formation (on host tissues and or on medical devices), and the production of tissue-damaging hydrolytic enzymes, such as proteases, phospholipases and hemolysins.[12]

In recent years there has been an increased interest in infections caused by the opportunistic pathogen *Candida*. Identification of infecting strains of *Candida* is important because isolates of *Candida* species differ widely, both in their ability to cause infection and also in their susceptibility to antifungal agents. The antifungals mainly used to treat infections caused by *Candida* species are the azoles, echinocandins, and polyenes; however, these drugs tend to not be successful when the infection involves the *C. albicans* biofilm.[13] Unfortunately, these treatment options have become unsatisfactory due to the increased development of resistance. Antifungal susceptibility testing is a crucial requirement to find the optimal treatment option for a patient as well as for the detection of antifungal resistance.[14] Natural products from microbes are useful sources of new drugs. Bacteria are well known for the production of important commercial metabolites. Among bacteria, actinomycetes are well-documented group for the production of a large number of antibiotics.[15] The increase in antifungal resistance, the emergence of multidrug-resistant species, and the grim mortality rates of fungal infections all amount to the continuous need for novel antifungal compounds.[16] The aim of this study was to control different resistant *Candida* isolates from Jazan Hospitals by actinomycetes that produce non polyenic antifungal agents.

## Materials and Methods

### Collection and Identification of Candida Isolates

*Candida* isolates of were collected from Jazan hospitals, Jazan, Saudi Arabia from 24/1 to 23/2/2024. The isolates were obtained from swabs from body fluids like urine, genital vaginal and blood). All samples were inoculated to the Sabouraud Dextrose Agar (SDA) plates and incubated at 37°C for 48 hours. Then, all isolates examined under microscope after stained with crystal violet. Cells illustrated in spherical to subspherical budding blastoconidia, blue color with a different format of *Candida* cells.

**Germ-tube Test:** The cells of *Candida* incubated in serum at 37°C for 2 to 4 hours, they produce short and slender tubes. Aliquots were removed for. A germ tube examined under microscope, it is a short lateral extension of a yeast cell and does not have a constricted region.

**Chlamydoconidia Production Test:** Corn meal agar used for chlamydoconidia production test. The samples were seeded with three parallel rows of agar and then placed between two slides, then incubated at 30°C for 72 hours. Observed of chlamydoconidia using an optical microscope.

**Growth on Chromo Agar Medium:** The isolates were incubated on SDA at 37°C for 48 hours before being transferred to Chromo agar medium. The medium will be then incubated for 48–72 hours at 37°C. Chromo agar is a selective medium for identifying *Candida* isolates based on the shape and color of the colonies produced by the isolate.[17]

### Isolation and Purification of Actinomycetes

Five soil samples were collected from Jizan, Saudi Arabia and transported in sterile plastic bags to the laboratory. Then, 0.1 ml of soil solution was spread on starch nitrate agar medium (SNA) using spread plate technique and incubated at 30°C for seven days. The colonies which showed morphological difference were selected and purified. For long period, strains were kept in 40% glycerol and stored at –80°C until used.

### Antifungal Activity of Actinomycetes Isolates

Screening of antifungal activity against *Candida* isolate followed two methods:

#### Primary Screening Method

The purified actinomycetes were tested for antifungal activity using agar diffusion assay as primary screening on the Muller-Hinton agar medium (MHA) and leaving them for 15 minutes to dry. Then, actinomycetes discs (7 mm) were transferred to the surface of the agar, and all plates were incubated at 37°C for 48 hours. The inhibition zone diameters around the actinomycete discs were observed.

#### Secondary Screening Method

Similarly, the most active actinomycete isolates were pre-cultured in 50 ml of starch nitrate broth (SNB) medium in 250 Erlenmeyer flasks and shaked at 120 rpm and 30°C for three days. About 2 ml (4 x $10^6$ CFU/ml) were used to inoculate 48 ml of production medium. The flasks were placed on shaker at 120 rpm for 7–8 days. After incubation, the cells free supernatant was separated by centrifugation at 10000 rpm for 10 minutes.[18] Using agar well diffusion method, the actinomycete filtrates were tested for the antimicrobial activity against *Candida* species. The suspensions of *Candida* isolates were spread over the MHA. After the agar surface dried, wells (7 mm) were fill with about 100 µl of the actinomycete filtrate. After 48 hours, the inhibition zones were measured and the test was repeated twice, and the mean of the inhibition zone diameter were calculated.

### Screening for Antifungal Non-polyenic Metabolites

The isolate NM3 was the most active isolate against Candida species, thus it was selected to grow medium medium containing ergosterol and the antifungal activity was measured. The fungal cell membrane containing ergosterol, thus its presence in medium reverse the Candida inhibition by the test actinomycete stain. SDA plates with ergosterol 50 mg/ml were prepared along with a control without ergosterol. The plates were seeded with the test organism. Actinomycetes discs (7 mm) were transferred to the surface of the agar using forceps and ergosterol inhibition was tested by disc diffusion method. Sterile filter paper discs (7 mm in diameter) were impregnated with 30 µl ergosterol suspension, dried and placed onto plates previously seeded with test microorganisms. Then, the plates were kept at 4°C for 24 hours and then incubated at 37°C for 48 hours. The zone of inhibition was were then measured.[19]

### Preparation of Ethyl Acetate Extract of the Selected Actinomycete Isolate

The selected actinomycete isolate NM3 was grown in liquid medium and described before and the culture filtrate was collected, extracted twice with the same volume of ethyl acetate (1:1, v/v), dried and finally dissolved in methanol. The absorption spectrum of active extracts in methanol was recorded in the UV region (210–400 nm) using a UV-visible spectrophotometer, and compared with those of known polyenic antifungal antibiotics.[20]

### Characterization of Selected Isolate NM3

The growth, and morphology of aerial mycelium and substrate mycelium in addition to pigment production of the selected isolate of actinobacteria was described on different agar media, following the directions given by the International Streptomyces Project (ISP). Also, he selected isolate was examined after staining using light microscopy to detect the morphology of mycelia and spore shape. The production of melanoid pigments was carried out on ISP7 agar. The ability of the isolates to utilize different carbon and nitrogen sources was determined on plates containing ISP basal medium 9 to which carbon and nitrogen sources were added separately to a final concentration of 1%.[21] The plates were incubated at 28°C, and the growth was evaluated visually for little or no observable growth.

### Molecular Identification of the most Active Isolate NM3 and Candida Species

For actinomycetes identification, the selected isolate NM3 was cultivated for 5 days at 30°C in 50 ml of (SNB) medium under orbital shaking while *Candida* species cultivated for 2 days at 37°C in 50 ml of (NB) medium. Biomass was harvested by centrifugation at 8000 rpm for 10 min and washed twice with sterile distilled water. The genomic DNA of the pellet was

extracted and the purity of isolate DNA was checked and quantified according to standard procedures. The 16S DNA gene was amplified by polymerase chain reaction (PCR). Amplification of selected actinomycetes isolate NM3 was performed using 2.5 µl of the selected primers, forward 27F (5'AGAGTTTGATCCTGGCTCAG-3') and reverse 511R (5'GCGGCTGCTGGCACRKAGT-3') with 40 µl of PCR SuperMix (Invitrogen Cat. No. 10572 - 014) and 5 µl of DNA extract in 0.2 ml PCR tubes and loaded into the thermal cycler.[22]

The primer's sequences used for *Candida* isolates were ITS86 as forward primer [5'- GTGCATCATCGAATCTTTGAAC-3'] and ITS4 as a reversed primer [5'-TCCTCCGCTTATTAGGAC-3'].[23] PCR amplification was carried out using thermocycler programmed as follows: a hot start of 95°C for 5 min, followed by 30 cycles of amplification at 95°C for 1 min, annealing at 54–55°C for 1 min, extension at 72°C for 2 min, and final extension at 72°C for 10 min.

Finally, the tubes were held at 4°C for direct use, or stored at –20°C until needed. The sequences obtained were compared for similarity with those contained in genomic database banks, using NCBI BLAST.

### *Fermentation and Extraction of the Antifungal Compounds*

Crude antifungal extract was prepared from the culture filtrate of each active isolate by solvent extraction using ethyl acetate. Ethyl acetate was added to the filtrate with 1:1 (v/v) ratio, and shaken vigorously for 20 min. The organic phases were collected, and the organic solvent was removed using a vacuum evaporator at 40°C to obtain a crude extract. The absorption spectrum of active extracts in methanol as recorded in the UV region (210–400 nm) using a UV-visible spectrophotometer, and compared with those of known polyenic antifungal antibiotics.[20]

## Determination of the Antifungal Activity

Antifungal activity of purified extracellular crude extracts was determined by agar well diffusion method. Cell Concentration of all test *Candida* was adjusted at 0.5 McFarland turbidity standards and inoculated on MHA plates by using sterilized cotton swabs. Wells were bored by sterilized a cork borer (7 mm), and 100 µl of each crude extract was poured into wells. Plates were incubated at 37°C for 24 h.[24]

## Results

Out of the 24 isolates from vaginal swabs, urine, genital vaginal bronchial and trachea samples, *C. albicans* had the highest occurrence with 29%, followed by *C. auris* (25%), *C. tropicalis* (25%), both *C. glabrata* and *C. ciferrii* (9%), and *C. parapsilosis* were the least isolated species (5%) on the basis of Vitek 2, as shown in Table 1. All isolates were resistant to different antifungal agents (Table 1).

The highest percentage of infection (Table 2) was recorded for urine (41%), followed by blood (29%), genital vagina (25%). Of the twenty-five samples obtained from patients infected with *Cantida*, the results showed that 54% were females and 46% were males (Figure 1).

The shape of *Candida* species under light microscope after staining with crystal vilote were oval shape. *Candida* species on SDA medium showed white yellowish creamy colonies. It is well known that CHROM agar is a rapid method to spectate the various *Candida* species. It facilitates the detection and identification of *Candida* species from mixed culture and provides results in 24–48 hours. All *C. albicans* isolates formed green colonies; whereas *C. auris* produced cream coloured colonies. Other colonies observed were pink (*C. glabrata*), blue-green (*C. tropicalis*), purple (*C. ciferrii*), and cream to yellow (*C. parapsilosis*) as shown in Figure 2. For germ tube formation, *C. albicans* had a positive result as shown in Figure 3-A by forming short lateral extension from mother cell. Figure (3-B), shows the presence of filaments and chlamydospores of *C. albicans*. After stained with lactophenol blue the cells appeared spherical with a thick wall, and where this character dispersed the *C. albicans* from other species, where they are negative of the chlamydospores. The isolates of *Candida* were molecular identified and the Phylogenetic tree of the *Candida* isolates and the most related genera were shown in Figure 4.

*Actinomycetes* have been intensively studied in several underexplored environments; niche and extreme habitats in various parts of the world in the last few years. In this study, five soil samples collected from different places in Jazan (16°54'34.3"N 42°43'26.8"E-), Saudi Arabia. A total 39 *Actinomycetes* isolates were obtained. All the isolates were found to be positive for Gram staining and had different morphological structures. The description of soil samples with the number of isolates is presented in Table 3.

The isolates were screened for their inhibitory activity against seven *Candida* species. Both the primary and secondary screening methods were used to screen the actinomycetes for antifungal activity. The first screening was used to determine the range of actinomycetes that had antifungal activity against *Candida* isolates. The secondary screening method was crucial to select the isolates for further studies. The results of antifungal activity depend on the measuring of inhibition diameter around actinomycetes discs. All the experimental measurements were carried out in triplicates. Out of 39 actinomycetes, the isolate NM3 exhibited high activity against tested *Candida* species in the primary screening. In the well diffusion method, the filtrate of actinobacterial strain NM3 showed good activity against all tested *Candida* (Table 4). This promising isolate was identified as *Streptomyces plicatus* NM3 and has strong antifungal activity in both primary and secondary screening methods (Figure 5).

The production of non-polyenic antifungal substances by the active isolates showing antifungal activities after assessing their activity against the tested *Candida* in the presence of ergosterol which decreased the inhibition activity of the tested isolate. Ergosterol present in fungal cell membrane has a very high affinity towards polyene antibiotics. Polyene drugs form complexes with ergosterol, forming channels in the fungal membrane that cause leakage of critical intracellular constituents and subsequent cell death. This behavior is exploited in a detection method developed to identify the presence of polyene class antifungals. Hence, variations in the inhibition diameters in the presence and absence of exogenous ergosterol in the culture medium indicates the implication of sterols as target of these active substances. Table 4 shows that the isolate NM3 had a reduced inhibition zone in the presence of ergosterol in the medium. Evaluating the UV- visible spectra of active extracts confirm the non polyenic structure (Figure 6) of the antifungal active extract of the isolate NM3.

Table 1. **Identification results obtained with the Vitek 2 and molecular method for Candida species and the resistance to different antifungal agents**

| NO. | Vitec identification | Molecular identification | *AmB | *CIS | *FLU | *5-FC | *VORI | *MICI |
|---|---|---|---|---|---|---|---|---|
| C1 | *Candida auris* | *Candida auris* | R | R | R | R | R | R |
| C2 | *Candida sp.* | *Candida auris* | R | R | R | R | R | R |
| C3 | *C.tropicalis* | *C.tropicalis* | R | R | R | R | R | R |
| C4 | *C.ciferrii* | *C.ciferrii* | R | R | R | R | R | R |
| C5 | *C.albicans* | *C.albicans* | R | R | R | R | R | R |
| C6 | *Candida auris* | *Candida auris* | R | R | R | R | R | R |
| C7 | *C.albicans* | *C.albicans* | S | S | S | S | S | S |
| C8 | *C.albicans* | *C.albicans* | S | S | S | S | S | S |
| C9 | *Candida auris* | *Candida auris* | R | R | R | R | R | R |
| C10 | *C.tropicalis* | *C.tropicalis* | S | S | S | S | S | S |
| C11 | *C.albicans* | *C.albicans* | S | S | S | S | S | S |
| C12 | *C.ciferrii* | *C.ciferrii* | R | R | R | R | R | R |
| C13 | *C.tropicalis* | *C.tropicalis* | R | R | R | R | R | R |
| C14 | *C.auris* | *C.auris* | R | R | R | R | R | R |
| C15 | *C.albicans* | *C.albicans* | R | R | R | R | R | R |
| C16 | *Candida sp.* | *C. tropicalis* | R | R | R | R | R | R |
| C17 | *Candida sp.* | *C. glabrata* | R | R | R | R | R | R |
| C18 | *C.tropicalis* | *C.tropicalis* | S | S | S | S | S | S |
| C19 | *C.albicans* | *C.albicans* | S | S | S | S | S | S |
| C20 | *C.auris* | *C.auris* | R | R | R | R | R | R |
| C21 | *C.parapsilosis* | *C.parapsilosis* | S | S | S | S | S | S |
| C22 | *C.glabrata* | *C.glabrata* | S | S | S | S | S | S |
| C23 | *C.tropicalis* | *C.tropicalis* | S | S | S | S | S | S |
| C24 | *C.albicans* | *C.albicans* | S | s | S | S | S | S |

*Amphotericin B (AmB), Caspofungin (CISA), Fluconazol (FLU), Flucytosine (5-FC), Voriconazole (VORI), Micafungin (MICI), Resistant (R), Sensitive (S).

Table 2. **Distribution of *Candida* Species According to their origin**

| Species (n) | Urine | Blood | Genital vaginal | Bron-chial | Trachea | Total |
|---|---|---|---|---|---|---|
| *C.albicans* | 2 | - | 4 | - | 1 | 7 |
| *C.glabrata* | - | 1 | 1 | - | - | 2 |
| *C.parapsilosis* | - | 1 | - | - | - | 1 |
| *Candida auris* | 4 | 2 | - | - | - | 6 |
| *C.tropicalis* | 3 | 1 | 1 | 1 | - | 6 |
| *C.ciferrii* | - | 2 | - | - | - | 2 |
| Total (24) | 10 | 7 | 6 | 1 | 1 | 24 |

-: No isolate was detected.

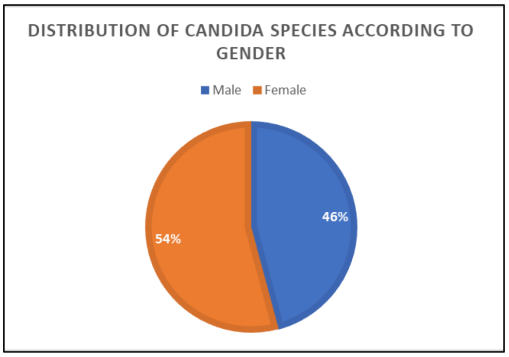


Fig. 1 **Distribution of Candida species based on gender.**

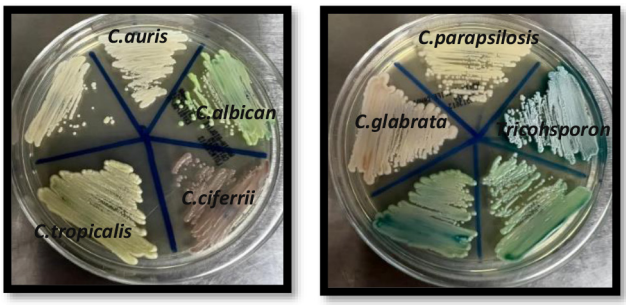


Fig. 2 **Growth of different Candida spp. on CHROM agar showing chromogenic color change.**

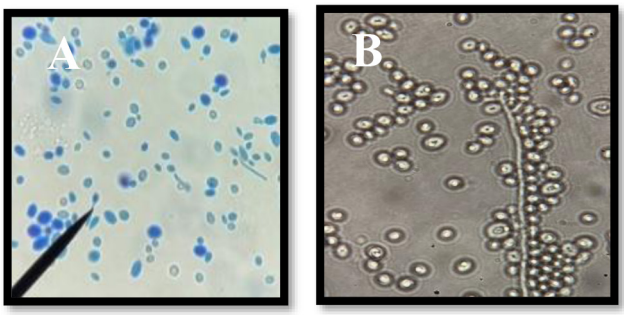


Fig. 3 **(A) Germ Tube stain with Lactophenol (B) Chlamydoconidia production of C. albican (1000X).**

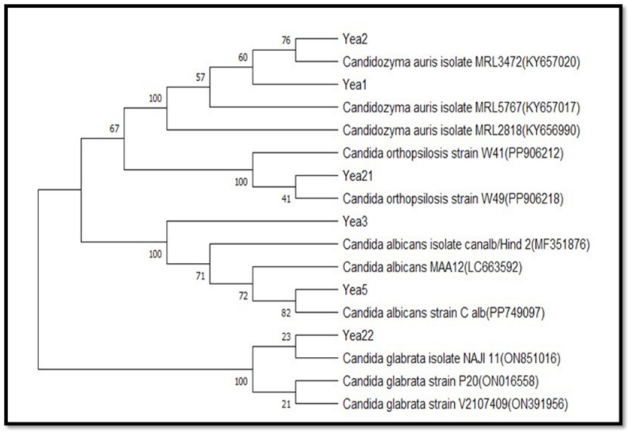


Fig. 4 **The phylogenetic tree of Candida isolates and the most related isolates.**

Table 3. **Description of soil samples and the total number of isolates and active isolates**

| NO. | Source of sample | Name of isolates | Number of isolates |
|---|---|---|---|
| 1 | Soil around Hot Spring 5 cm | NM27, NM2, NM3*, NM33 NM23, NM24, NM32 | 7 |
| 2 | Agriculture 1 (5 cm) | NM29, NM31, NM5, NM1, NM28, NM6 | 6 |
| 3 | Agriculture 2 (5 cm) | NM36, NM38 NM35, NM39, NM34 NM37, NM7, NM4 | 8 |
| | | NM9, NM10 | 2 |
| 4 | Agriculture 3 (5 cm) | NM16, NM8, NM13, NM14, NM15 | 5 |
| | | NM11, NM12, NM26 | 3 |
| | | NM30, NM17, NM18, NM21 | 4 |
| 5 | Agriculture 4 (5 cm) | NM19, NM25, NM22 | 3 |
| | | NM20 | 1 |

Total of Isolates: 39 Isolates, *: the most active isolate.

Table 4. **Antifungal activities of the active actinomycetes isolate NM3 against seven *Candida* species using the disc diffusion, well diffusion and ethyl acetate extract methods.**

| *Candida spp.* | Zone of inhibition (mm) | | | |
|---|---|---|---|---|
| | Disc diffusion method | Well diffusion method (F) | Ethyl Acetate Extract (E)* | Ergosterol effect |
| *C. auris* | 20.3 | 21.4 | 23.5 | 10 |
| *C. cefferii* | 10.4 | 19.6 | 18.0 | ND |
| *C. albican* | 11.6 | 17.5 | 18.8 | ND |
| *C. parapsilosis* | 21.6 | 21.5 | 15.5 | ND |
| *C. glabrata* | 15.7 | 14.5 | 15.4 | ND |
| *C. tropicalis* | 19.7 | 16.5 | 15.5 | ND |

Ethyl acetate extract (30 mg/ml); ND: no inhibition.

The selected actinomycete strain NM3, isolated from soil attracted our attention based on the strong non-polyenic antifungal activity against all tested *Candida*. Therefore, it was identified using the morphological, physiological, and molecular characterization methods. The morphology of strain NM3 in different ISP media showed filamentous bacterium with extensively branched aerial mycelia and grew well on starch nitrate agar medium (Figure 7). The growth ranged from heavy and moderate to poor for all isolates. The aerial mycelium of strain appeared white on starch nitrate agar and ISP2, ISP7, and ISP9 media. It was creamy on ISP2, white and gray on ISP4 medium and the substrate mycelium was beige, creamy, light purple, and light brown (Table 5). Many carbon and nitrogen sources were investigated for their effect on the growth of the isolate NM3, it can grow well with the six kinds of carbon sources and seven kinds of nitrogen sources. The isolate NM3 had high growth on starch, sucrose, dextrose, maltose, and lactose as carbon sources and on peptone, yeast extract, and nitrogen sources (Table 6). According to different morphological, physiological, and molecular identification, Isolate NM3 belonged to the genus Streptomyces and was identified as *Streptomyces plicatus* NM3 (Figure 8).

## Discussion

*Candida* species is an important cause of systemic mycosis in hospitalized patients, and morbidity and mortality worldwide, especially in critically ill patients.[25] Among *Candida* species, *C. albicans, C. glabrata, C. tropicalis, C. parapsilosis*, and *C. krusei* were the most common species encountered in routine clinical laboratory samples.[26] In our study, we have found *C. albicans* (29%) as the predominant species followed by *C. auris* and *C. tropicalis* (25%), *C. glabrata* and *C. ciferrii* (9%) and *C. parapsilosis* (2.9%), and, which is consistent with a previous study of Jha and others in 2006 in which the majority of *Candida* species were *C. albicans* (70%) followed by *C. tropicalis* (13.33%), *C. krusei* (10%), *C. parapsilosis* (3.33%), and *C. stellatoidea* (3.33%).[27] A study from South India by Kumari et al. in 2014 reported with overall predominance of NAC spp. and the predominant species identified was *C. albicans*.[28]

In this study, we compared the fully automated Vitek2 ID system with molecular methods for identification of *Candida* species. Although, our results show that Vitek 2 was able to correctly identify (88%) of tested samples of *Candida* isolates with a high level of confidence and there were no false-positive results of *Candida* isolates that were reported by Vitek 2 among the *Candida* strains, three isolates (12%) were misidentified. Massonet and others in 2004 in their prospective study reported that Vitek2 identified 41 (67.21%) *Candida* isolates correctly, 10 (16.39%) were not identified, 3 (4.91%) were misidentified, and 7 (11.47%) isolates were identified with low discrimination (Massonet et al.,2004)[29] whereas other studies reported that Vitek2 system to correctly identify 98.5%[30] and 100%[31] of clinical isolates. In this study, the strains were simultaneously tested by the molecular techniques. Molecular techniques are excellent tools for identification and methods are highly reproducible, more discriminatory, high throughput, easy-to-use, these techniques have been used in a number of studies with *Candida* species.[32]

The development of new antimicrobial drugs remains a major challenge to overcome the spread of drug resistance of clinically-relevant microbes, as strains are increasingly

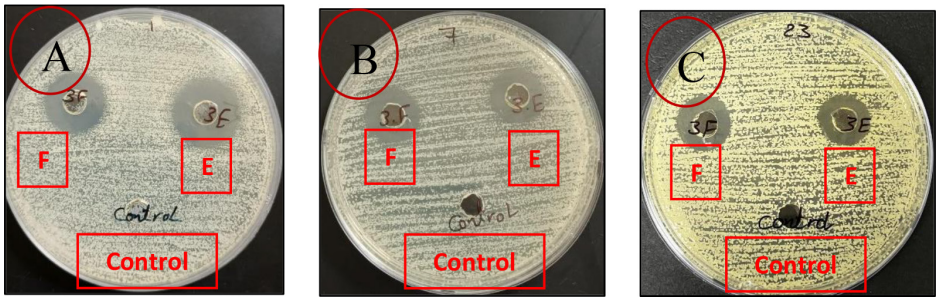


Fig. 5 **The inhibitory effect of the culture filtrate (F) and ethyl acetate extract (E) of the selected isolate NM3 on three different Candida isolates, A: Candida auris; B: Candida albican: C: Candida tropicalis.**

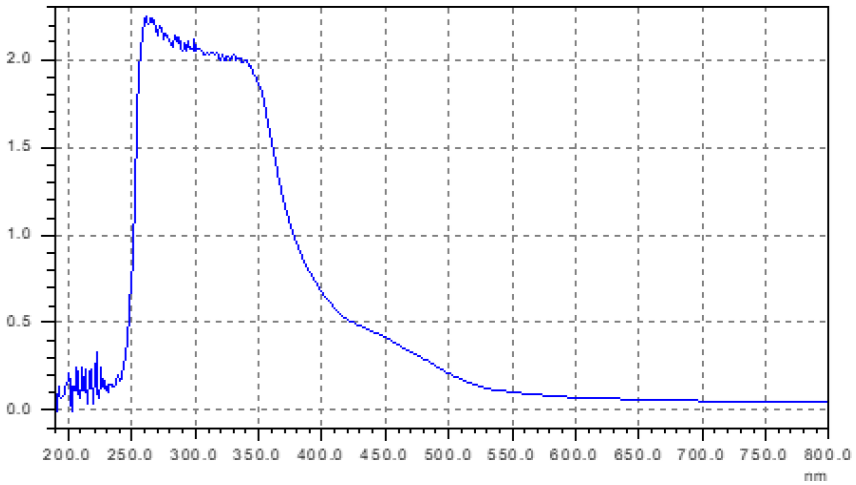


Fig. 6 **UV-visible spectra of the ethyl acetate extract of the most active isolate NM3.**

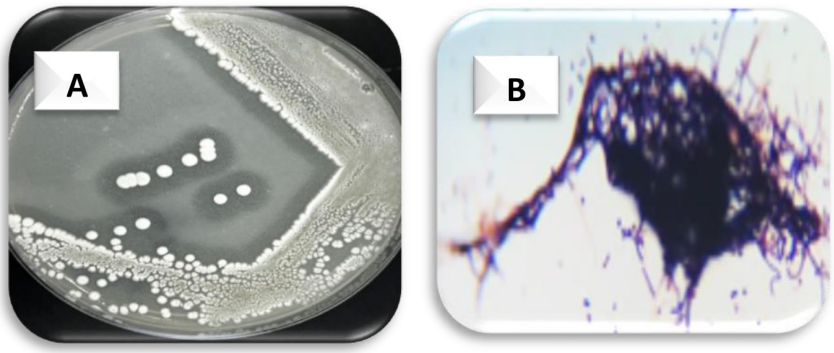


Fig. 7 **(A) The selected isolate NM3 on starch Nitrate agar; (B) the isolate NM3 under light microscope (X1000).**

Table 5. **Cultural characteristics of the bacterial isolate NM3**

| Media | Growth | Color of aerial mycelium | Color of substrate mycelium | Color of soluble pigment |
|---|---|---|---|---|
| **Starch-nitrate agar** | Heavy | White | beige | No pigment |
| **ISP-2 (yeast malt extract agar)** | Scanty | Creamy | Creamy | No pigment |
| **ISP-4 (inorganic salt starch agar)** | Heavy | White and gray | Light purple | No pigment |
| **ISP-5 (glycerol asparagine agar)** | Heavy | White | Light brown | No pigment |
| **ISP-7 (Tyrosine agar)** | Heavy | White | Light purple | No pigment |
| **E- Medium ISP-9** | Moderate | White | White | No pigment |

Table 6. **Effect of different carbon and nitrogen sources in ISP-9 medium on growth of the selected isolates NM3**

| Carbon Source | Isolates NM3 | Nitrogen Source Isolates | Isolates NM3 |
|---|---|---|---|
| Negative control | ++ | Ammonium chloride | ++ |
| Glucose | ++ | Potassium nitrate | ++ |
| Starch | +++ | Sodium nitrate | ++ |
| Sucrose | +++ | Peptone | +++ |
| Dextrose | + | Yeast extract | +++ |
| Lactose | +++ | Glycine | ++ |
| Maltose | ++ | Asparagine | +++ |

+++: high growth, ++: moderate growth and +: weak growth.

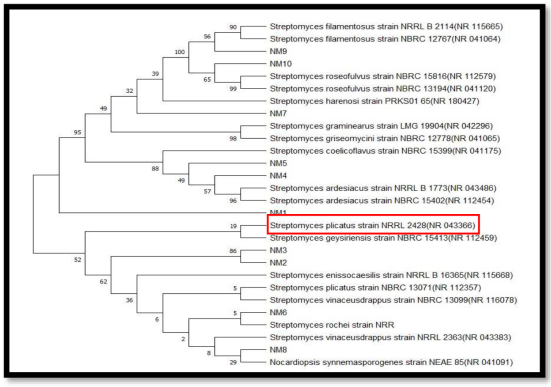


Fig. 8 **The Phylogenetic tree of the isolate NM3 and the most related Isolates.**

becoming less sensitive to conventional antibiotics. Actinomycetes is the most biotechnologically valuable prokaryotes responsible for the production of about half of the discovered bioactive secondary metabolites including antibiotics.[33] They are the main source of clinically important antibiotics, most of which are too complex to be synthesized by combinatorial chemistry. Thus, microbial natural products still appear as the most promising sources for developing future antibiotics.[34] Isolation of an antibiotic from culture filtrate largely determined by its chemical nature. Solvent extraction is usually employed for the extraction of antibiotics from the culture filtrates. Organic solvents with different polarities have been used by many researchers for the extraction of antimicrobial compounds from actinomycetes.[24] This result clearly indicated that the antimicrobial activity of the potential strain is due to the production of extracellular bioactive compounds. The published literature stated that most of the antibiotics from actinomycetes are extracellular in nature.[35] From a natural products perspective, marine bacteria remain a relatively unexplored resource for novel secondary metabolites. However, data suggest that actinomycetes, in particular genus *Streptomyces* are widely distributed in marine environments.[36] In our screening program for bioactive compounds, an antifungal activity of as *Streptomyces plicatus* NM3 isolated from soil around hot spring Jazan region highlights its importance as candidate for further investigation in biological control of pathogen *Candida* species.

## Conclusion

The significance of actinomycetes extends beyond their antimicrobial properties. They are integral to the discovery and isolation of novel antifungal metabolites which not only lead to new therapeutic agents but can also provide insight into the evolutionary dynamics of host-pathogen interactions. Moreover, harnessing the potential of actinomycetes for antifungal development has several advantages, they can be cultured under laboratory conditions, manipulated genetically to enhance yields, and their metabolites can often be modified for improved efficacy and safety. Research into actinomycetes and their antifungal properties is therefore vital in the global effort to combat fungal infections, especially in immunocompromised populations where the risk of morbidity and mortality is significantly heightened. In summary, actinomycetes represent a promising avenue for the development of new antifungal agents, with the potential to address the growing challenge of antifungal resistance and to enhance treatment options for infected patients.

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## Conflict of Interest

None.

## References

1. Brown, G. D., Denning, D. W., Gow, N. A., Levitz, S. M., Netea, M. G., & White, T. C. (2012). Hidden killers: human fungal infections. Science Translational Medicine, 4(165), 165rv13–165rv13.
2. Rana, J. S., Khan, S. S., Lloyd-Jones, D. M., & Sidney, S. (2021). Changes in mortality in top 10 causes of death from 2011 to 2018. Journal of General Internal Medicine, 36, 2517–2518.
3. Nami, S., Mohammadi, R., Vakili, M., Khezripour, K., Mirzaei, H., & Morovati, H. (2019). Fungal vaccines, mechanism of actions and immunology: A comprehensive review. Biomedicine & Pharmacotherapy, 109, 333–344.
4. Sakagami, T., Kawano, T., Yamashita, K., Yamada, E., Fujino, N., Kaeriyama, M., ... & Mikamo, H. (2019). Antifungal susceptibility trend and analysis of resistance mechanism for Candida species isolated from bloodstream at a Japanese university hospital. Journal of Infection and Chemotherapy, 25(1), 34–40.
5. Marak, M. B., & Dhanashree, B. (2018). Antifungal susceptibility and biofilm production of Candida spp. isolated from clinical samples. International Journal of Microbiology, 2018(1), 7495218.
6. Kainz, K., Bauer, M. A., Madeo, F., & Carmona-Gutierrez, D. (2020). Fungal infections in humans: the silent crisis. Microbial Cell, 7(6), 143.
7. Veríssimo, C. (2016). Fungal infections. In Environmental Mycology in Public Health, 27–34.
8. Dadar, M., Tiwari, R., Karthik, K., Chakraborty, S., Shahali, Y., & Dhama, K. (2018). *Candida albicans*-Biology, molecular characterization, pathogenicity,

and advances in diagnosis and control–An update. Microbial Pathogenesis, 117, 128–138.
9. Talapko, J., Juzbašić, M., Matijević, T., Pustijanac, E., Bekić, S., Kotris, I., & Škrlec, I. (2021). *Candida albicans*-the virulence factors and clinical manifestations of infection. Journal of Fungi, 7(2), 79.
10. Develoux, M., & Bretagne, S. (2005). Candidoses et levuroses diverses. EMC-Maladies infectieuses, 2(3), 119–139.
11. Tamo, S. B. (2020). Candida infections: Clinical features, diagnosis and treatment. Infect Dis Clin Microbiol, 2(2), 91–102.
12. Silva, S., Negri, M., Henriques, M., Oliveira, R., Williams, D. W., & Azeredo, J. (2012). *Candida glabrata*, *Candida parapsilosis* and *Candida tropicalis*: Biology, epidemiology, pathogenicity and antifungal resistance. FEMS Microbiology Reviews, 36(2), 288–305.
13. Costa-de-Oliveira S, Rodrigues AG. *Candida albicans* Antifungal Resistance and Tolerance in Bloodstream Infections: The Triad Yeast-Host-Antifungal. *Microorganisms*, 2020; 8(2):154. https://doi.org/10.3390/microorganisms8020154.
14. Houšť, J., Spížek, J., & Havlíček, V. (2020). Antifungal drugs. In Metabolites (Vol. 10, Issue 3). MDPI AG. https://doi.org/10.3390/metabo10030106
15. Genilloud, O. (2017). Actinomycetes: Still a source of novel antibiotics. Natural Product Reports, 34(10), 1203–1232.
16. Vanreppelen, G., Wuyts, J., Van Dijck, P., & Vandecruys, P. (2023). Sources of antifungal drugs. Journal of Fungi, 9(2), 171.
17. Hospenthal, D. R., Beckius, M. L., Floyd, K. L., Horvath, L. L., & Murray, C. K. (2006). Presumptive identification of *Candida* species other than *C. albicans*, *C. krusei*, and *C. tropicalis* with the chromogenic medium CHROMagar Candida. Annals of Clinical Microbiology and Antimicrobials, 5, 1–5.
18. Radhakrishnan, M., Balaji, S., & Balagurunathan, R. (2007). Thermotolerant actinomycetes from the Himalayan mountain-antagonistic potential, characterization and identification of selected strains. Malaysian Applied Biology, 36(1), 59.
19. Lemriss, S., Laurent, F., Couble, A., Casoli, E., Lancelin, J. M., Saintpierre-Bonaccio, D., ... & Boiron, P. (2003). Screening of nonpolyenic antifungal metabolites produced by clinical isolates of actinomycetes. Canadian Journal of Microbiology, 49(11), 669–674.
20. Boussaber, E. A., & Brahim, S. (2014). Idrissi SEl. Extraction and preliminary characterization of bioactive molecules produced by a new Streptomyces strain. J Environ Treat Tech, 2, 50–5.
21. Ayari, A., Morakchi, H., & Djamila, K. G. (2012). Identification and antifungal activity of Streptomyces sp. S72 isolated from Lake Oubeira sediments in North-East of Algeria. African Journal of Biotechnology, 11(2), 305–311.
22. Myer, P. R., Kim, M., Freetly, H. C., & Smith, T. P. (2016). Evaluation of 16S rRNA amplicon sequencing using two next-generation sequencing technologies for phylogenetic analysis of the rumen bacterial community in steers. Journal of Microbiological Methods, 127, 132–140.
23. Gutzmer, R., Mommert, S., Küttler, U., Werfel, T., & Kapp, A. (2004). Rapid identification and differentiation of fungal DNA in dermatological specimens by LightCycler PCR. Journal of Medical Microbiology, 53(12), 1207–1214.
24. Selvameenal, L., Radhakrishnan, M., & Balagurunathan, R. (2009). Antibiotic pigment from desert soil actinomycetes; biological activity, purification and chemical screening. Indian Journal of Pharmaceutical Sciences, 71(5), 499.
25. Sardi, J. C. O., Scorzoni, L., Bernardi, T., Fusco-Almeida, A. M., & Mendes Giannini, M. J. S. (2013). Candida species: Current epidemiology, pathogenicity, biofilm formation, natural antifungal products and new therapeutic options. Journal of Medical Microbiology, 62(1), 10–24.
26. Graf, B., Adam, T., Zill, E., & Göbel, U. B. (2000). Evaluation of the VITEK 2 system for rapid identification of yeasts and yeast-like organisms. Journal of Clinical Microbiology, 38(5), 1782–1785.
27. Jha, B. J., Dey, S., Tamang, M. D., Joshy, M. E., Shivananda, P. G., & Brahmadatan, K. N. (2006). Characterization of Candida species isolated from cases of lower respiratory tract infection. Kathmandu University Medical Journal (KUMJ), 4(3), 290–294.
28. Kumari, K. S., Raghunath, P., Harshavardhan, B., & Chaudhury, A. (2014). Distribution of *Candida albicans* and the non-albicans *Candida* species in different clinical specimens from South India. Int J Microbiol Res, 5, 1–5.
29. Massonet, C., Van Eldere, J., Vaneechoutte, M., De Baere, T., Verhaegen, J., & Lagrou, K. (2004). Comparison of VITEK 2 with ITS2-fragment length polymorphism analysis for identification of yeast species. Journal of Clinical Microbiology, 42(5), 2209–2211.
30. Hata, D. J., Hall, L., Fothergill, A. W., Larone, D. H., & Wengenack, N. L. (2007). Multicenter evaluation of the new VITEK 2 advanced colorimetric yeast identification card. Journal of Clinical Microbiology, 45(4), 1087–1092.
31. Melhem, M. S. C., Bertoletti, A., Lucca, H. R. L., Silva, R. B. O., Meneghin, F. A., & Szeszs, M. W. (2013). Use of the VITEK 2 system to identify and test the antifungal susceptibility of clinically relevant yeast species. Brazilian Journal of Microbiology, 44, 1257–1266.
32. Soll, D. R. (2000). The ins and outs of DNA fingerprinting the infectious fungi. Clinical Microbiology Reviews, 13(2), 332–370.
33. Yuan, J., Zhao, M., Li, R., Huang, Q., Rensing, C., Raza, W., & Shen, Q. (2016). Antibacterial compounds-macrolactin alters the soil bacterial community and abundance of the gene encoding PKS. Frontiers in Microbiology, 7, 1904.
34. Narasimhan, B., Sharma, D., & Kumar, P. (2011). Biological importance of imidazole nucleus in the new millennium. Medicinal Chemistry Research, 20, 1119–1140.
35. Arasu, M. V., Duraipandiyan, V., Agastian, P., & Ignacimuthu, S. (2008). Antimicrobial activity of Streptomyces spp. ERI-26 recovered from Western Ghats of Tamil Nadu. Journal de Mycologie Médicale, 18(3), 147–153.
36. Sergey BZ (2012). Marine actinomycetes as an emerging resource for the drug development pipelines. J. Biotechnol. 158:168–17.