

Virulence Factors of Non-dermatophytes Isolated From Inmates of Owerri Correctional Center, IMO State, Southeast Nigeria

MaryRose Ogechi Echeta

maryrose.echeta@uaes.edu.ng

University of Agriculture & Environmental sciences

Ugueri Udochukwu

UNIVERSITY OF AGRICULTURE & ENVIRONMENTAL SCIENCES

Ifeanyi Mirian Oyem

University of Delta

Maximus Chibuoyim Korie

Imo State Polytechnic

Calista Odinachi Itubochi

Kingsley Ozumba Mbadiwe University

Valentine Nnachetam Unegbu

University of Agriculture & Environmental sciences

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University of Agriculture & Environmental sciences

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University of Agriculture & Environmental sciences

Vivian Chika Igiri

University of Agriculture & Environmental sciences

Ijeoma Monica Izundu

Nnamdi Azikiwe University

Udoka Laurina Onyebuchi-Ogwuegbu

University of Agriculture & Environmental sciences

Joy Nwachukwu Egbucha

University of Agriculture & Environmental sciences

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Abstract

Background

Non-dermatophytic fungi are opportunistic organisms that cause cutaneous infections, typically following skin damage induced by dermatophytes or other agents. These fungi produce virulence factors such as keratinase, protease, lipase, urease, and cellulase, which contribute to their pathogenicity. This study aimed to evaluate the ability of non-dermatophytes isolated from inmates of the Owerri Correctional Center to produce selected virulence-associated enzymes.

Methods

Non-dermatophytic fungi were isolated through standard culture techniques and screened for extracellular enzyme production using plate assay methods.

Results

A number of isolates demonstrated keratinase production. *Candida parapsilosis* and *Paecilomyces dactylethromorph* exhibited the highest activity with zones of clearance measuring 40 mm each. Other keratinase producers included *Purpureocillium lilacinum* (35 mm), *Aspergillus flavus* (30 mm), *Trichoderma harzianum* (28 mm), *Aspergillus tamarii* (20 mm), and *Trichoderma lixii* (10 mm). However, *Aspergillus sydowii*, *Candida tropicalis*, *Candida orthopsilosis*, *Candida spp.*, *Candida haemulonii*, *Fusarium solani*, and *Pseudallescheria boydii* showed no keratinase activity. Protease production was highest in *P. dactylethromorph* (50 mm), followed by *C. haemulonii* (48 mm), *P. lilacinum* (42 mm), *T. harzianum* (36 mm), *C. parapsilosis* (35 mm), *C. tropicalis* (33 mm), and *Candida spp.* (28 mm). Moderate activity was observed in *C. orthopsilosis* and *A. flavus* (25 mm each), while *T. lixii* (21 mm) and *A. sydowii* (20 mm) showed lower activity. *F. solani*, *A. tamarii*, and *P. boydii* did not produce protease. Amylase production was recorded in ten isolates, with *C. parapsilosis* showing the highest activity (55 mm). Other producers included *C. tropicalis* (43 mm), *P. dactylethromorph* (42 mm), *A. flavus* (37 mm), *T. harzianum* (35 mm), *A. sydowii* (34 mm), *P. lilacinum* (30 mm), *A. tamarii* and *Candida spp.* (22 mm each), and *F. solani* (15 mm). *C. orthopsilosis*, *C. haemulonii*, *P. boydii*, and *T. lixii* did not exhibit amylase activity. Statistical analysis revealed significant differences in enzyme production among isolates ($P < 0.05$).

Conclusion

A substantial proportion of the non-dermatophytic fungi demonstrated the ability to produce keratinase, protease, and amylase, suggesting their potential role in disease pathogenesis through extracellular enzyme activity.

Introduction

Non-dermatophytic fungi are opportunistic pathogens capable of causing superficial or cutaneous infections, particularly following skin damage caused by dermatophytes or other microorganisms. These fungi produce several virulence factors, including keratinase, protease, lipase, urease, and cellulase, which facilitate host tissue invasion and disease progression. Among these, keratinase, protease, cellulase, lipase, and amylase are key extracellular enzymes synthesized by fungi that contribute to their pathogenic mechanisms [1]. Several pathogenic fungi have been reported to produce proteinases, phospholipases, keratinase, and amylase, indicating that these enzymes play significant roles in fungal virulence. For instance, *Candida albicans* and related species are known to produce proteinases and phospholipases, which enhance their ability to colonize and infect host tissues [2][3][4][5]. Keratin, a structural protein abundant in epithelial cells of vertebrates, constitutes a major component of skin, hair, nails, and feathers. Microorganisms capable of producing keratinase can degrade these substrates, including feathers, wool, nails, and hair. Keratinophilic fungi are therefore characterized by their ability to utilize keratinized tissues as nutrient sources through enzymatic degradation. Proteases are enzymes that hydrolyze peptide bonds in proteins, resulting in the formation of amino acids and smaller peptides. These enzymes are widely distributed in plants, animals, and microorganisms, including bacteria and fungi, and play essential roles in both metabolism and pathogenicity [6].

Materials and Methods

Study Area

This study was carried out at the Owerri Correctional Center located in Owerri Municipal, Imo State, Nigeria, along the Owerri–Okigwe Road, in close proximity to the Imo State Government House. The facility, originally constructed during the colonial era to accommodate approximately 500 inmates, is currently overcrowded, housing more than 2,200 inmates [7]. Owerri lies at latitude 5.478310°N and longitude 7.025853°E and serves as the capital of Imo State. It is situated near the Otamiri River and functions as a major hub for commerce, religion, and social activities, with a predominantly Christian population. Owerri Municipal Local Government Area covers approximately 58 km² and had a population of 127,213 according to the 2006 census [8]. The city is an important commercial center for agricultural products such as palm produce, maize, yam, and cassava, with Eke Ukwu Owerri serving as the main market. The area also hosts notable institutions including the Federal University of Technology Owerri, Imo State University, and Alvan Ikoku Federal College of Education, as well as healthcare facilities such as the Federal Medical Centre (FMC) Owerri.

Sample Collection

Skin, nail, and scalp scrapings were collected from inmates following disinfection with 70% (v/v) ethanol. Sterile instruments including razor blades, scalpel blades, swab sticks, and toothbrushes were used for

sample collection. Each specimen was properly labeled with the subject's code and site of infection and stored in sterile envelopes. Each sample was divided into two portions. One portion was prepared for direct microscopic examination by adding a drop of 20% (w/v) potassium hydroxide (KOH) to the specimen mounted on a slide. After 15 minutes, the preparation was examined under low ($\times 10$) and high ($\times 40$) magnification for the presence of fungal elements [9].

Culture Procedure

All specimens were cultured regardless of the results of direct microscopy. Samples were inoculated onto Sabouraud's dextrose agar (SDA) supplemented with chloramphenicol (0.05 mg/mL), prepared according to the manufacturer's instructions. The inoculated plates were incubated aerobically in an inverted position at 30°C for 72 hours and monitored daily for fungal growth. Emerging colonies were sub-cultured onto fresh SDA plates to obtain pure isolates. Microslide culture techniques were employed to observe fungal morphology, including intact fruiting structures and spore formation. Identification of mold isolates was based on macroscopic features such as colony texture, growth rate, surface topography, and pigmentation (both obverse and reverse), as well as microscopic characteristics. For microscopic examination, coverslips from microslide cultures were transferred onto slides containing lactophenol cotton blue stain. Standard identification manuals were used for definitive classification [11].

Screening for Keratinase Production

Keratinase production was evaluated using the plate assay method [12]. Fungal isolates were inoculated onto feather meal agar plates and incubated at 28°C for 72 hours. A clear zone surrounding the fungal colony indicated keratinase activity. Feather meal agar was prepared using processed chicken feathers that were washed, oven-dried at 50°C, and ground into powder. The medium composition included feather meal, ammonium chloride, sodium chloride, dipotassium hydrogen phosphate, potassium dihydrogen phosphate, magnesium chloride hexahydrate, yeast extract, and agar at pH 7.5 [13]. The chicken feathers were obtained from Delight farms Nigeria after they have processed their chicken for sale. The farm owner was briefed on the use of the chicken feathers for research purpose only and their consent was obtained.

Screening for Protease Production

Protease activity was determined using a gelatin-based plate assay [14]. Fungal isolates were spot-inoculated onto nutrient agar supplemented with 1% gelatin and incubated at 28°C for 72 hours. Following incubation, plates were flooded with mercuric chloride reagent, which reacts with undegraded gelatin to form a white precipitate. Clear zones around colonies indicated gelatin hydrolysis and protease activity. The diameter of the clearing zone was measured to quantify enzyme production.

Screening for Amylase Production

Amylase production was assessed by inoculating isolates onto nutrient agar containing 1% soluble starch. Plates were incubated at room temperature for 7 days. After incubation, Lugol's iodine solution was added to the plates. The appearance of clear zones around colonies indicated starch hydrolysis and positive amylase activity [15].

Statistical Analysis

Statistical evaluation revealed a significant difference in extracellular enzyme production among the fungal isolates ($P < 0.05$), indicating that the observed enzyme activities are meaningful and may contribute to the pathogenic potential of the organisms.

Results

The majority of the fungal isolates demonstrated the ability to produce extracellular enzymes, as presented in Table 1 and Plates 1–6.

Keratinase Activity

Out of the 14 non-dermatophytic fungal isolates screened, 7 exhibited keratinase activity with varying zones of hydrolysis. *Candida parapsilosis* and *Paecilomyces dactylethromorph* showed the highest activity (40 mm each), followed by *Purpureocillium lilacinum* (35 mm), *Aspergillus flavus* (30 mm), *Trichoderma harzianum* (28 mm), *Aspergillus tamarii* (20 mm), and *Trichoderma lixii* (10 mm).

No keratinase activity was observed in *Aspergillus sydowii*, *Candida tropicalis*, *Candida orthopsilosis*, *Candida spp.*, *Candida haemulonii*, *Fusarium solani*, and *Pseudallescheria boydii*.

Protease Activity

Protease production was detected in 11 of the 14 isolates. *Paecilomyces dactylethromorph* exhibited the highest activity (50 mm), followed by *Candida haemulonii* (48 mm), *Purpureocillium lilacinum* (42 mm), *Trichoderma harzianum* (36 mm), *Candida parapsilosis* (35 mm), *Candida tropicalis* (33 mm), and *Candida spp.* (28 mm). Moderate activity was observed in *Candida orthopsilosis* and *Aspergillus flavus* (25 mm each), while *Trichoderma lixii* (21 mm) and *Aspergillus sydowii* (20 mm) showed relatively lower activity. *Fusarium solani*, *Aspergillus tamarii*, and *Pseudallescheria boydii* did not exhibit protease activity.

Amylase Activity

Amylase production was observed in 10 of the isolates. *Candida parapsilosis* recorded the highest activity (55 mm), followed by *Candida tropicalis* (43 mm), *Paecilomyces dactylethromorph* (42 mm), *Aspergillus flavus* (37 mm), *Trichoderma harzianum* (35 mm), *Aspergillus sydowii* (34 mm), *Purpureocillium lilacinum* (30 mm), and both *Aspergillus tamarii* and *Candida spp.* (22 mm each).

Fusarium solani showed the lowest activity (15 mm). No amylase production was observed in *Candida orthopsilosis*, *Candida haemulonii*, *Pseudallescheria boydii*, and *Trichoderma lixii*.

Table 1
Extracellular Enzyme Production by the 14 fungal Isolates. Enzymes and their Zones of Hydrolysis in millimeter (mm)

Isolates	Keratinase	Protease	Amylase
<i>Aspergillus flavus</i>	30	25	37
<i>A. sydowii</i>	-	20	34
<i>A. tamari</i>	20		22
<i>Candida tropicalis</i>	-	33	43
<i>C. parasilosis</i>	40	35	53
<i>C. orthopsilosis</i>	-	25	-
<i>C. spp</i>	-	28	22
<i>C. haemulonis</i>	-	48	
<i>F. solani</i>	-	-	15
<i>Paceolomyces dactylethromorph</i>	40	50	42
<i>P. boydii</i>	-	-	-
<i>P. liacinum</i>	35	42	30
<i>T. harzianum</i>	28	36	35
<i>T. lixii</i>	10	21	-

Key:

- - means negative.

A. flavus=*Aspergillus flavus*, *A. sydowii*= *Aspergillus sydowii*, *A. tamarri*= *Aspergillus tamari*, *C. tropicalis*=*Candida tropicalis*, *C. parasilosis*=*Candida parasilosis*, *C. orthopsilosis*=*Candida orthopsilosis*, *C. haemulonis* = *Candida haemulonis*, *F. solani* = *Fusarium solani*. *Paecilomyces* = *Paecilomyces dactylethromorp*, *P. boydii* = *Pseudallescheria boydii*, *P. lilacinum* = *Purpureocilium lilacinum*, *T. harzianum* = *Trichoderma harzianum*. *T. lixii*=*Trichoderma lixii*.

Plate 1: Hydrolysis of protease enzyme by *C. tropicalis*.

Plate 2: *Candida haemulonis* showing protease activity.

Plate 3: Amylolytic activity of *Fusarium solani* on starch agar showing clear zone around the organism.

Plate 4: Amylolytic activity of *Pseudallescheria boydii* showing hydrolysis.

Plate 5: *T. harzianum* Clear zone around the colony showing amylolytic activity.

Plate 6: Amylolytic activity of *P. dactylethromorph* showing hydrolysis of starch.

Discussion

The findings of this study indicate that the majority of fungal isolates obtained from the correctional center environment were capable of producing extracellular enzymes. Specifically, *Aspergillus flavus*, *A. tamarii*, *Candida parapsilosis*, *Paecilomyces dactylethromorph*, *Purpureocillium lilacinum*, *Trichoderma harzianum*, and *T. lixii* demonstrated keratinase activity, whereas *A. sydowii*, *C. tropicalis*, *C. orthopsilosis*, *Candida spp.*, *C. haemulonii*, *Fusarium solani*, and *Pseudallescheria boydii* did not.

The ability of these fungi to produce keratinase highlights their capacity to degrade keratin, a highly resistant structural protein, thereby supporting their role in the development of cutaneous mycoses. These findings are consistent with previous studies [16–18], which identified several genera, including *Aspergillus*, *Penicillium*, and *Cladosporium*, as effective keratin degraders. Similarly, earlier reports [19] [20] have documented the contribution of non-dermatophytic fungi, particularly *Aspergillus flavus*, to keratinase production, further supporting the present results.

Protease production was observed in a wide range of isolates, including *A. flavus*, *A. sydowii*, *Candida tropicalis*, *C. parapsilosis*, *C. orthopsilosis*, *Candida spp.*, *C. haemulonii*, *P. dactylethromorph*, *P. lilacinum*, *T. harzianum*, and *T. lixii*, whereas *A. tamarii*, *F. solani*, and *P. boydii* did not exhibit proteolytic activity. The ability of these organisms to secrete proteases aligns with earlier findings [21][22], which established filamentous fungi as prolific producers of hydrolytic enzymes, particularly proteases.

Furthermore, studies [23–26] have demonstrated that genera such as *Aspergillus*, *Penicillium*, and *Rhizopus*, as well as yeasts like *Candida*, are important sources of proteolytic enzymes. The present findings are also consistent with reports [27][28] indicating that various *Candida* species associated with cutaneous infections produce protease and phospholipase, enhancing their virulence. In addition, previous studies [29][30] have highlighted the role of *Trichoderma harzianum* in protease production, suggesting that these enzymes may function as virulence determinants and contribute to antagonistic interactions with other microorganisms. The detection of protease activity in *P. lilacinum* further corroborates earlier findings [31] on its ability to produce keratinolytic serine proteases.

Amylase production was also widely observed among the isolates. Species such as *A. flavus*, *A. sydowii*, *A. tamarii*, *C. tropicalis*, *C. parapsilosis*, *Candida spp.*, *F. solani*, *P. dactylethromorph*, *P. lilacinum*, and *T. harzianum* demonstrated amylolytic activity, while *C. orthopsilosis*, *C. haemulonii*, *P. boydii*, and *T. lixii* did not. These findings are consistent with previous studies [33][34], which identified filamentous fungi, particularly *Aspergillus* species, as efficient producers of amylase. The results also align with earlier reports [35][36], which documented amylase production by environmental fungal isolates, including

Candida species. However, the present findings differ from some studies [35] that reported the absence of amylase production in certain genera such as *Fusarium*, *Trichoderma*, and *Paecilomyces*. Overall, the ability of these fungi to produce amylase further emphasizes their metabolic versatility and potential ecological and clinical significance.

Conclusion

This study investigated the isolation and enzymatic characterization of non-dermatophytic fungi with respect to keratinase, protease, and amylase production. The findings revealed that a large proportion of the isolates were capable of synthesizing these extracellular enzymes. *Candida parapsilosis* and *Paecilomyces dactylethromorph* exhibited the highest keratinase activity, while *P. dactylethromorph* and *Candida haemulonii* showed the greatest protease activity. In addition, *C. parapsilosis* and *C. tropicalis* demonstrated the highest amylase production. The production of these enzymes suggests that non-dermatophytic fungi possess significant pathogenic potential, as these extracellular enzymes facilitate tissue invasion and disease progression. Further investigations incorporating additional virulence factors and enzyme systems are recommended to better understand their pathogenic mechanisms. Moreover, these enzymes may have promising applications in industrial and biotechnological processes.

Declarations

Ethical Approval and Consent to participate

Ethical approval was obtained from the Imo State Ministry of Health Research Ethics Committee, Nigeria who approved that we collect skin samples from the inmates. The inmates were briefed after getting the approval on the need to screen them for fungal infection and those that consented were enrolled in the study. Those that did not give their consent were excluded from the study.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

There is no conflict of interest.

Clinical Trial Number

Clinical trial is not applicable in this study.

Funding

There was no external funding for this study.

Authors' Contributions

MOE, UU, IMY and VNU conceived and designed the study. COT, COU and CTP collected the data for the study and subsequently analyzed it. COA, VCI, IMI, ULO and JNE carried out statistical analysis and interpretation. All authors were substantially involved in drafting the manuscript.

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Consent to publish declaration

Not applicable

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Plates

Plates 1 to 6 are available in the Supplementary Files section.

Supplementary Files

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