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Phylogenetic Analysis and Bioactivity of Soil-Derived Nocardiopsis Species: Antibacterial and Anticancer Potentials against MCF7 and HCT16 Cell

Majdah Mohamed Al-Tuwaijri

Department of Biology Faculty of Applied Science Umm Al-Qura University Makkah Al-Mukarramah Saudi Arabia

Email: mmtuwaijri@uqu.edu.sa

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Abstract

Soil microorganisms, especially actinomycetes from the Actinobacteria species, are a valuable source of therapeutic compounds. They are known for producing abundant bioactive metabolites. Given the rise of drug-resistant pathogens, exploring new sources for novel antibacterial agents is crucial. In this study, a bacterial strain, *Nocardiopsis dassonvillei* DSM 43111, was isolated from sandy soil in Giza, Egypt. This study represents the first investigation into the biological effects of this strain, which was tested against five bacterial species to assess its bactericidal properties. The study explored the cytotoxic effects on the normal BHK cell line and anticancer activity against MCF7 and HCT16 cell lines. *Nocardiopsis dassonvillei* DSM 43111 demonstrated noteworthy anticancer activity against MCF7 and HCT16 cell lines, suggesting its promise for cancer therapy. However, slight cytotoxicity was observed against normal BHK cells, indicating the need for further investigation into the bioactive compounds from isolated actinomycete metabolites. Additionally, disc diffusion tests confirmed the strain's strong bactericidal potential toward the studied infectious bacterial pathogens. In light of these results, soil microbes have tremendous promise as sources of new medicinal molecules. Moreover, the current study introduces *Nocardiopsis* species as a promising candidate, warranting further exploration in cancer and antibacterial drug discovery endeavors.

Keywords: *Nocardiopsis dassonvillei*, Soil microorganisms, Actinobacteria, Anticancer, Antibacterial.

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1. Introduction:

The richness of our natural environment, particularly the soil ecosystem, is an abundant source of diverse microorganisms capable of producing myriad bioactive compounds. Historically, such soil microbes have paved the way for discovering a wealth of therapeutically relevant molecules, including antibiotics and anticancer agents (1,2). In the current era of growing antibiotic resistance and limited effective cancer therapies, exploiting these microbes for developing novel drugs remains pivotal. Further, these microbial-originated drugs hold the potential to exhibit minimal side effects and be biocompatible (3,4).

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Soil actinomycetes, recognized for producing therapeutic secondary metabolites, are a potent source of potential antibiotics and pharmaceutical biocompounds (5–7). Due to the limitations of traditional collection methods, researchers are now targeting actinomycetes like Nocardia, a notable source of antibiotics, to address antibiotic-resistant pathogens (8). In this context, an actinomycete strain, *Nocardiopsis dassonvillei* DSM 43111, was isolated from Egypt's under-studied Giza province for comprehensive research due to its potential advantages and Nocardiopsis genus affiliation.

Nocardiopsis, a lesser-studied actinomycete genus known for creating biologically significant compounds, along with other high G+C content bacteria, are crucial for the generation of antibiotics, with over 70% of current antibiotics being of microbial origin (9). Despite the decline in new antibiotic discoveries since the 'golden age' of antibiotic research and the recurring discovery of known antibiotics, further exploration of Nocardiopsis' medicinal applications is vital, given how environmental conditions can affect metabolite production (10).

Natural products are instrumental in developing treatments for infectious diseases and malignancies (11). However, the fast growth of multidrug-resistant (MDR) organisms has intensified the challenge of bacterial infections, given the current antibiotics' diminished effectiveness (12). The rise of MDR infections is chiefly attributed to a lack of potent medicines capable of curbing their spread (13). Developing new antibiotics is deemed the most efficient strategy to battle the spread of antibiotic-resistant microbes. The spread of MDR bacteria, a significant global health threat complicating infection treatments, results from antimicrobial resistance (AMR) when harmful bacteria, influenced by mobile genetic elements like transposons, become immune to current antibiotics (14). As a looming global health menace, AMR is forecasted to escalate alarmingly to 10 million cases by 2050 (O'Neil, 2014).

The research focuses on *Nocardiopsis dassonvillei* DSM 43111, a soil bacterium from Egypt's Giza province, to explore its potential in combating antibiotic resistance and finding innovative anticancer solutions. The study aims to identify the bacterium's phylogenetic characteristics and assess its antibacterial and anticancer effects, particularly against drug-resistant pathogens and specific cell lines. The research hopes to uncover new therapeutic avenues and contribute to cancer therapy and antibacterial drug development by delving into this underexplored area of soil-derived microorganisms.

2. Materials and methods

2.1. Soil samples collection

A soil sample was obtained from the grounds of Cairo University in Giza Province, Egypt, at coordinates 30° 1' 40.7202" N and 31° 12' 23.7823 "E in January 2023. A desert climate characterizes this location near the River Nile, with annual temperature fluctuations of approximately 15.8°C and an average yearly rainfall of 18 cm. The humidity is notably low in May, recording 36.44 %, and the precipitation pattern shows January as the wettest month and June as the driest one. The soil at this site had a sandy texture and a dark color, with a pH of 7.6 and a temperature of 24 °C. After soil collection, the samples (n=3) were placed in sterile containers and sent to the Microbiology laboratory at the Division of Botany, Cairo University. The samples were pre-heated at 60 °C for two hours, followed by serial dilution and purification to prevent any other microbial growth and enhance the growth of actinomycetes, as reported in previous studies (Sapkota et al., 2020).

2.2. Isolation and cultivation of actinomyces

For a start, 1 g of the soil sample was grabbed and diluted ten times with sterile distilled water. Starch

casein nitrate (SCN) agar plates were prepared as described previously (18), and 100 µl aliquots from each dilution of the soil sample were plated individually onto these SCN agar plates. These plates were incubated at 30°C ± 2°C for 7 d. After incubation, the developing actinomycetes colonies were picked, purified, and subcultured on the SCN. The cultures' morphological features, such as pigment formation and aerial and substrate mycelia hues, had been detected.

2.3. Sequencing and molecular identification of the isolated actinomycetes

DNA was extracted from the isolated strain grown in SCN broth for seven days, as reported earlier (19). The actinomycetes 16S rRNA gene amplification and sequencing processes were performed by Macrogen (Macrogen Inc. Geumchen-gu, South Korea) using the primers panel in Table 1.

Table 1: Primers used for 16S rRNA sequencing by Polymerase chain reaction

Primer name	Sequence	Use
27F	5' (AGA GTT TGA TCM TGG CTC AG) 3	PCR amplification
1492R	5' (TAC GGY TAC CTT GTT ACG ACT T) 3'	
785F	5' (GGA TTA GAT ACC CTG GTA) 3	PCR sequencing
907R	5' (CCG TCA ATT CMT TTR AGT TT) 3'	

The 16S rRNA gene sequences were aligned against existing sequences in the GenBank anchored by the National Center for Biotechnology Information (NCBI). Phylogenetic investigations were conducted using MEGA X software, and the sequences were analyzed using BioEdit software.

2.4. Ethyl acetate extract of Nocardiopsis culture

Pure *Nocardiopsis dassonvillei* DSM 43111 culture was cultivated further in sub-cultures on a modified Glucose Malt agar medium at 28 ºC and 180 revolutions per minute for 8 d (20). The crude extract was obtained using ethyl acetate solvent extraction of the culture broth at a 1:1 ratio. For antimicrobial bioassay, 1 mg of *Nocardiopsis dassonvillei* DSM 43111 ethyl acetate extract (EA-DSM43111) was dissolved in 100 microliters of DMSO. The ethyl acetate extract was concentrated using a rotary evaporator (Buchi, Switzerland).

2.5. Antibacterial efficacy

The crude extract (EA-DSM43111) was then used to test antibacterial activity against five pathogenic bacteria, including *Escherichia coli* ATCC 8739, *Salmonella enterica* ATCC 10708, *Staphylococcus aureus* ATCC 6538, *Listeria monocytogenes* ATCC-7644, and *Bacillus cereus* ATCC 11778 using the disc diffusion assay method according to guidelines indicated by CLSI 2020 (21). The size of the inhibition zones was calculated by measuring the inhibition zone's diameter around each disc to reveal the antimicrobial effectiveness of the ethyl acetate extract of the isolated actinobacteria.

2.6. Cell lines culture

Two cancer cell lines (MCF7, HCT16) and one normal cell line (BHK) were purchased from the National Cancer Institute at Cairo University, and the MTT assay was used to test EA-DSM43111 for its cytotoxic and anticancer activity. Cell lines were maintained as monolayer cultures in RPMI medium supplemented with 10% FBS and 2% Pen/Strep. Cells were incubated in a water-jacketed incubator at 37ºC in 5% CO2 in a high-humidity atmosphere (Thermo Fisher Scientific USA).

The lines were repeatedly sub-cultured to be kept in the exponential growth phase. Sterile conditions were achieved by working under an equipped laminar flow (Microflow Laminar flow cabinet, MDH limited, Hamsphire SP105AA, U.K.). Cells were grouped into normal BHK and cancerous MCF7 and HCT16 cells, all treated with different concentrations of the crude extract (EA-DSM43111) (12.5, 25, 50, and 100 µg/mL).

2.7. Cytotoxic Potency

After 24 h, ten µl of the MTT reagent was added to each well at a 0.5 mg/ml concentration. The microplate was incubated for 4 h. Then, 100 µl of the Solubilization solution was added to each well. After the purple formazan crystals' solubilization, a microplate (ELISA) reader was used to measure the samples' absorbance. The wavelength to measure the absorbance of the formazan product is 570 nm.

The cell viability percentage was calculated using the following equation:

$$\text{The cell viability (\%)} = [\text{ODS}/\ \text{ODC}] \times 100.$$

ODS represents the sample's mean optical density, while ODC is the control's

2.8. Statistical analysis

The trials were repeated three times to enhance accuracy, and the results were represented as the mean ± standard error of the mean (SEM). The dose-response curve for the cytotoxicity test was generated by plotting a graph of the percentage of cell viability versus the concentrations of the tested materials using GraphPad Prism 8.0.2 software.

Data for the antibacterial test were analyzed using IBM-SPSS, version 23. The Kolmogorov-Smirnov test was applied to assess normality, confirming that the data were normally distributed. Thus, all parameters were presented as the mean ± SEM. A one-way analysis of variance (ANOVA) was conducted to study the effect of the treatment on the studied parameters, followed by Duncan's test, which was used to elucidate the homogeneity among the experimental groups. A p-value of <0.05 was considered to indicate a statistically significant difference.

3. Results:

3.1. Isolation of soil sample:

The distinct environmental characteristics of the coastal Giza district's Nile Valley habitat were explored for unique Actinomyces strains. The collected sandy, black, and brown soil had a temperature of about 24°C and a pH of 7.6 (Figure 1). Soil samples were treated with 0.2% CaCO3 and pre-heated at 60°C for 2 hours to lessen microbial contamination. Numerous unique Actinomyces colonies were cultured after dilution and pour plating on 7.2-pH SCN medium. The morphological diversity of these colonies was noticed once they were cultivated further.

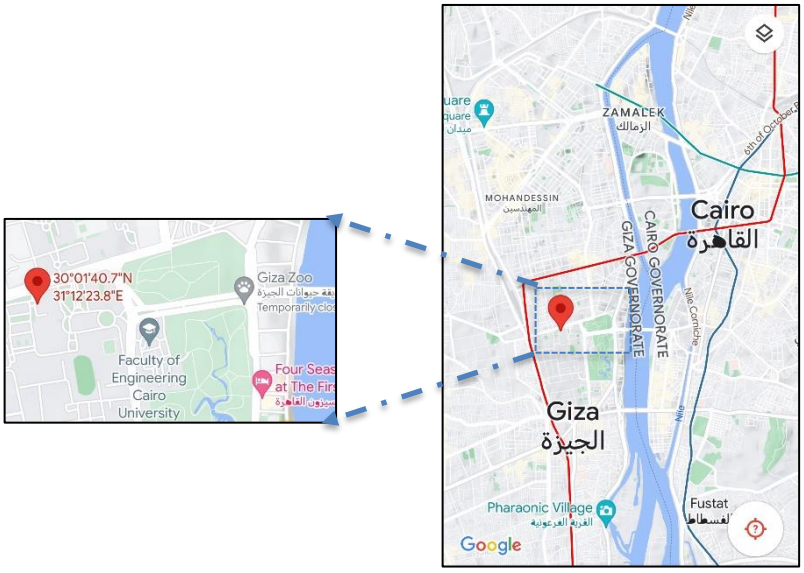

Figure 1: Geographical representation of Nile Valley, where soil sample was collected in the Giza district.

3.2. Phylogenetic Analysis:

The isolate's 16S rRNA sequence was closely related to members of the Nocardiopsis genus. The strain showed the greatest similarity (99.93%) to *Nocardiopsis dassonvillei* strain DSM 43111 16S ribosomal RNA, partial sequence (NR_074635.1). The phylogenetic tree indicated the isolated strain's relationship with known Nocardiopsis species (Figure 2).

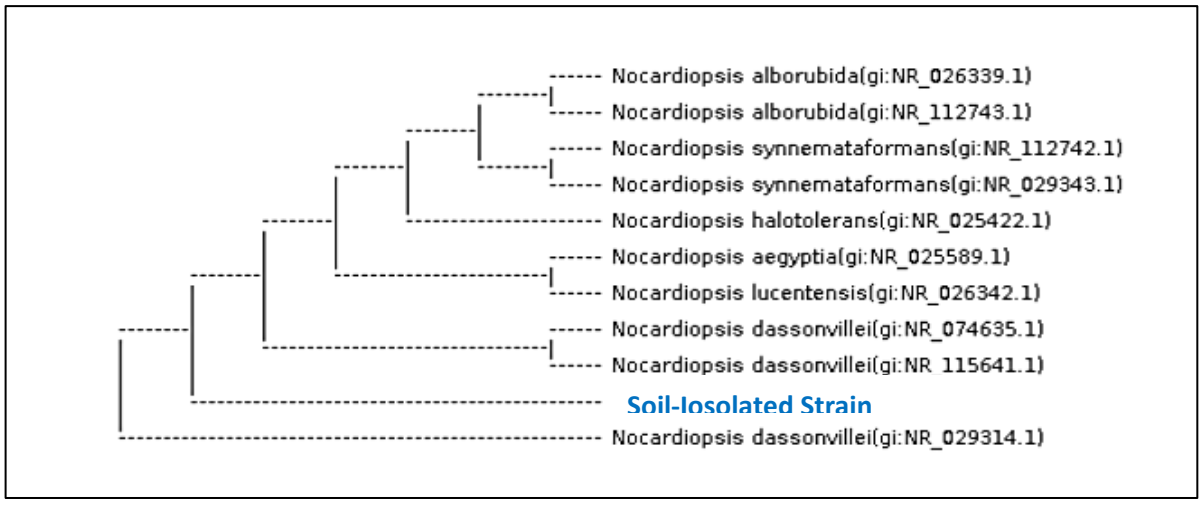

Figure 2: Phylogenetic tree constructed using the neighbor-joining method showing the position of the isolated strain within the Nocardiopsis genus. Reference sequences are indicated with accession numbers.

3.3. Antibacterial Activity:

The crude extract of the isolated strain demonstrated broad-spectrum bactericidal properties, as shown by the presence of various inhibition zones against the tested pathogenic bacteria. Antibacterial activity against bacterial pathogens was found during the screening of EA-DSM43111 for the capacity to produce antimicrobial secondary metabolites by spot inoculation on GLM agar. For *Salmonella enterica* ATCC 10708, the inhibition zone was measured at 28.83 0.01 mm (SEM), whereas *Staphylococcus aureus* ATCC 6538 was estimated at 15.16 0.14 mm (SEM). The values of a zone of inhibition against all five bacterial species are listed in Tables 2 and 3.

Table 2. One-way ANOVA to study the differences in inhibition zones among different microorganisms.

	Sum of Squares	df	Mean Square	$F_{calculated}$	Sig.
Between Groups	319.394	4	79.849	1194.028	0.000
Within Groups	0.669	10	0.067		
Total	320.063	14			

P<0.000: represents significant differences.

Table 3. The differences in inhibition zones among different microorganisms. Data is displayed as mean ± standard error.

Test organism	Zone (mm)
Ethyl acetate (vehicle)	0.00 ± 0.00
Escherichia coli ATCC 8739	20.14 ± 0.04^{b}
Salmonella enterica ATCC 10708	28.83 ± 0.01^{e}
Staphylococcus aureus ATCC 6538	15.16 ± 0.14^{a}
Listeria monocytogenes ATCC-7644	25.16 ± 0.11^{d}
Bacillus cereus ATCC 11778	22.43 ± 0.28^{c}

Means marked with the same superscript letter are insignificantly different (p>0.05), but those marked with different ones are significantly different (p<0.05).

3.4. Anticancer Activity:

EA-DSM43111 exhibited significant anticancer activity results obtained by MTT assay in MCF7 and HCT16 cells in a concentration-dependent decrease in viability. However, there was notable cytotoxicity against the BHK cell line at higher concentrations which necessitates further screening of active purified secondary metabolites for their cytotoxicity both *in vitro* and *in vivo*.

Evidence shows that EA-DSM43111 may inhibit the development of cancer cells *in vitro*, with an IC_{50} of 10.0 and 97.2 g/mL towards MCF7 and HCT16 cells, respectively, and a value of 105 g/mL against normal BHK cells.

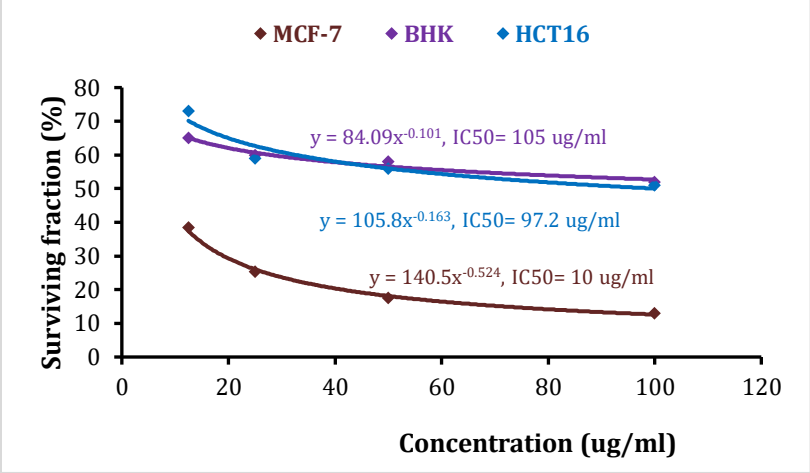

Figure 3: Dose-response curves for MCF7, HCT16, and BHK cells treated with different concentrations of the bacterial extract. The X-axis represents concentration, and the Y-axis represents cell viability.

4. Discussion

Both sides of the Nile River are home to a wide variety of microorganisms due to the river's complex ecology, which includes factories, highways, sewers, navigation sites, bridges, and residential and agricultural regions. Exploring Nile Valley soil in the coastal Giza district provided unique insights into the actinomycetes inhabiting this region. The soil's sandy, black, and brown nature, together with the temperature and pH level of the soil (24°C and 7.6, respectively), likely facilitated the growth of Actinomyces, a genus known for its diverse morphological characteristics. The biodiversity and challenging environmental conditions of the Nile allow organisms with high resistance and rich metabolism to co-exist, making the area a promising avenue for exploring unique biological effects and bioactive metabolites. A significant portion of this microbial diversity remains unexplored, as many species and lineages have not yet been isolated into pure culture.

Actinomycetes, bacteria within the phylum Actinobacteriota, are particularly noteworthy. They are among the richest providers of secondary metabolites, enriching soils and contributing to agriculture by generating antibiotics, enzymes, and other substances that combat plant pathogens and pests (22). Actinomycetia's known ability to produce bioactive secondary metabolites also offers great potential in

making biologically important compounds to combat drug-resistant pathogens. Previous reports by (23), (24), and (25) have emphasized this potential, though the agricultural area at Cairo University near the Nile River remains largely unexplored.

In this study, a Streptomyces strain, *Nocardiopsis dassonvillei* DSM 43111, was isolated from the sandy blackish soil near the Nile River in the Giza district. The role of their crude extract in antibacterial and anticancer activity was investigated, reaffirming the belief that the immense microbial diversity in soil ecosystems serves as a potential goldmine for novel bioactive compounds. This finding is consistent with (26), where Amycolatopsis keratiniphila DPA04, isolated from the Agricultural Experiment and Research Station of Cairo University, demonstrated antimicrobial properties.

These results underscore the capacity of these freshwater microorganisms to produce diverse bioactive metabolites that could play a key role in addressing humanity-threatening challenges such as antibiotic resistance (Selim *et al*., 2021). They may serve as innovative antibacterial agents against a broad range of pathogenic organisms resistant to current medications (26). Thus, the isolation of Actinomycetia strains from the referred site in this study further explores the potential of this unexplored ecosystem, accentuating the need for intensified examination of such ecological niches.

The taxonomical assignment of microorganisms is a critical step in understanding their functionality and potential applications. In our study, this was achieved through 16S rRNA gene sequencing, a well-established method that successfully linked the isolated strain to the Nocardiopsis genus, specifically *Nocardiopsis dassonvillei* DSM 43111. With a similarity of 99.93%, the phylogenetic analysis confirms the close relationship with known members of this genus, aligning with earlier literature and emphasizing the method's importance in microbial taxonomy (28).

The use of the neighbor-joining method in constructing the phylogenetic tree provides robust support for this taxonomic classification and underscores the isolate's place within the existing microbial framework. This particular actinomycete, *Nocardiopsis dassonvillei*, has previously been defined as a highly bioactive isolate from the marine ecosystem in the Red Sea and was noted for producing various bioactive compounds that act as effective antimicrobial and anticancer agents (29).

Our study significantly adds to the understanding of the Nocardiopsis genus by isolating a strain from sandy soil near the freshwater drainage of the Nile River and elucidating its complex capacity for both anticancer and antibacterial effects. This discovery is particularly salient given the rarity of species within this genus demonstrating simultaneous therapeutic potentials. While several species within the Nocardiopsis genus have been previously explored for their metabolic capacities, our findings introduce a new dimension in comprehending these microorganisms' multifaceted roles, accentuating their potential application in medicine and biotechnology.

Antibacterial resistance is a critical global health issue that demands immediate exploration of novel antibacterial agents (30). Our study initiated this endeavor by isolating *Nocardiopsis dassonvillei* strain DSM 43111 and screening the extracted crude substance for antibacterial secondary metabolites. Utilizing the spot inoculation method on agar plates, we observed remarkable activity against all tested pathogens. The inhibition zone results suggest multiple bioactive compounds or a single compound with broad-spectrum effects, revealing the highest activity against gram-positive bacteria such as *S. aureus*, *L. monocytogenes*, and *B. cereus*. Furthermore, EA-

DSM43111 also showed activity against significant gram-negative bacteria like *E. coli* and *S. enterica*, known as major drug-resistant, life-threatening pathogens. Specifically, the highest activity was against *Salmonella enterica* ATCC 10708, a finding in line with prior research (Konwar *et al*., 2023), where a similar extract demonstrated strong activity against clinically important drug-resistant pathogens such as MRSA. These results may indicate that the isolated strain harbors secondary metabolites with diverse mechanisms of action against various bacterial targets. The variations in the inhibition zones underscore the necessity for further examination of the specific compounds responsible for these effects and their potential applications in antibacterial therapies, possibly paving the way for a new class of antibiotics. Here, the extracted EA-DSM43111 demonstrated significant anticancer potential against MCF7 (IC_{50} = 10.0 µg/mL) and HCT16 (IC_{50} = 97.2 µg/mL) cell lines, evidenced by a dose-dependent decrease in cell viability. This discovery shows promising potential in the search for anticancer drugs and is consistent with a previous study that demonstrated anticancer activity in Streptomyces sp. RMS518F against HCT-116 and hepatocellular carcinoma cell line (HepG2) with IC_{50} values of 73.4 and 62.8 µg/mL, respectively (31). The dual activity against both breast and colorectal cancer cells further opens avenues for investigating its mechanism of action. Bioactive metabolites in the extract have been hypothesized to be responsible for the cytotoxic impact by interacting with the elevated G-C content areas of DNA, blocking topoisomerase-II action, and causing single-stranded genetic material to break (Selim *et al*., 2019)

Streptomyces sp. KF-15 ethyl acetate extract included many compounds with possible anticancer activity against the HeLa cell line, as shown by a separate investigation (33). However, cytotoxicity against normal BHK cells at greater doses suggests non-specific toxicity, raising important issues regarding the compound's selectivity and possible out-of-bound consequences. This characteristic poses one of the significant problems of membrane-active antimicrobial compounds, such as antimicrobial peptides and essential oils, as their high cytotoxicity toward host cells limits their potential as pharmaceutical drugs (34–36). These findings emphasize the need for further purification, identifying the active metabolites, and both *in vitro* and *in vivo* evaluation to enhance specificity and reduce potential side effects on normal tissues. The exploration of structure-activity relationships and specific anticancer mechanisms will be vital in leveraging this potential therapeutic candidate.

The distinct ecological characteristics of the Nile Valley in the Giza district have provided a unique source of Actinomyces strains specifically linked to *Nocardiopsis dassonvillei*. Interestingly, the simultaneous manifestation of both anticancer and antibacterial activities, although rare, implies the presence of a rich repertoire of secondary metabolites in the Nocardiopsis species. The subsequent discovery of broad-spectrum antibacterial activity and concentration-dependent anticancer effects lays the foundation for future research. Further work involving metabolomic profiling and purification will be pivotal in delineating these bioactive compounds and elucidating their modes of action. However, caution must be exercised regarding potential cytotoxicity. In conclusion, this research underscores the vast potential of soil microorganisms, particularly less explored genera like Nocardiopsis, in bioprospecting endeavors and has unearthed a promising avenue in the ongoing pursuit of novel antibacterial and anticancer agents from soil-derived microorganisms.

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