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Bioactivities of Free and Encapsulated *Streptomyces gougerotii* NSH Extract Isolated from Itay Al Baroud, Egypt: Antifungal, Antitumor, and Water Bioremediation Potential in El-Mex Fish Farms

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ABSTRACT

Discovering microbial capabilities in the environment has become significant to discover novel metabolites for biotechnological aspects. The present study was designated to study the diversity of actinomycetes in different soil samples (E1-E21) collected from Itay Al Baroud soil, El-Beherra, Egypt. The highest counts of actinomycetes were observed at the sampling site E15 with 267 CFU g^{-1}. Twenty-one selected isolates nominated as N1-N21 were characterized based on physiological, biochemical tests and antimicrobial activity. The isolate N7 exhibited broad spectrum of antimicrobial potential against *Staphylococcus aureus* ATCC25923, *Escherichia coli* ATCC8739, *Pseudomonas aeruginosa* ATCC9027, *Bacillus subtilis* ATCC 6633, *Enterococcus fecalis* ATCC29212 and *Klebsiella pneumoniae* ATCC13883 with mean of inhibition zone diameters as 14-16mm. N7 was identified on the basis of the morphological, cultural, biochemical characteristics, 16S rRNA sequence analysis as *Streptomyces gougerotii* NSH MW023664. The extracted bioactive metabolites exhibited antifungal activity with mean of inhibition zone diameter at 8-22mm. The antitumor activity against MCF- 7, PC 3 and HCT-116 cell lines was tested. The highest inhibition rates (90.6% and 92.5%) were observed against PC-3 and HCT-116 cell lines at a concentration of 10,000µg/ mL, with IC_{50} values of 3,460.45 and 4,113.75µg/ mL, respectively. The effectiveness of both free and encapsulated extracts in reducing fish pathogens in the rearing water of the El-Mex fish farm was investigated. The encapsulated extract showed greater efficacy, resulting in reductions of 90.2%, 83.3%, and 85.5% in *Vibrio*, *Aeromonas*, and *Pseudomonas* counts, respectively, compared to the free extract. These findings support the potential use of *S. gougerotii* NSH MW023664 as a probiotic in aquaculture.

INTRODUCTION

The overuse of antibiotics in food of animals and human has led to the global propagation of multidrug resistance **(Xu *et al.*, 2022)**. Despite a growing need, very limited novel therapies and bioactive agents have been readily accessible for clinical use in the previous decades **(Wiman *et al.*, 2023)**. Every year, thousands of people are

missing as a result of antimicrobial resistance **(O'Neill, 2014)**. This crisis prompted scientists to discover new and more active antimicrobial means for fighting multidrug resistance **(Sugden *et al.*, 2016)**. As a result of the increasing cost and complications in producing novel antibiotics, the attention is shifting to exploring alternate antibiotics with a limited risk of emerging antimicrobial resistance. Natural compounds are talented over the synthetic compounds with efficient antimicrobial actions **(Arasu *et al.*, 2013)**. Numerous researches have been directed to discover novel antimicrobial compounds from microorganisms **(Kumar *et al.*, 2020; Assou *et al.*, 2023; Diag & Nonzom, 2023; Dhaini *et al.*, 2025; Helmi, 2025)**.

Nearly two-thirds of the antibiotics are produced by actinomycetes and more than half of the natural antibiotics used in pharmaceutical industry are derived from *Streptomyces* **(Dezfully & Ramanayaka, 2015)**. After streptomycin discovery from *Streptomyces griseus* in 1944, various immense antitumor and antimicrobial metabolites produced by *Streptomyces* have gained worldwide scientific concern **(Assou *et al.*, 2023)**. Mostly, 80% of the discovered antibiotics are gained from the genus *Streptomyces* **(Lo Grasso *et al.*, 2016)**. In point of fact, Streptomyces genome can keep a panel of biosynthetic gene clusters (BGCs) accountable for the creation of innumerable secondary metabolites with antimicrobial potentiality **(Wen *et al.*, 2022)**.

The population and type of actinomycetes in soil are affected by environmental factors. They can survive both at mesophilic (25-30℃) and thermophilic (40 ℃) habitats **(Haseena *et al.*, 2016)**. The pH is also a major factor determining the activity and spreading of actinomycetes. **Vasavada *et al.* (2006)** reported that nitrogen and carbon sources, type of media and salinity affects the antibiotic production and growth of actinomycetes. The current study was designated for isolation of actinomycetes from soil samples collected from Itay Al Baroud (El-Beheira Governorate), Egypt, purification and taxonomic characterization of the isolates. Screening for antimicrobial potentiality and other biological applications of the locally isolated actinomycetes were also conducted.

MATERIALS AND METHODS

Sampling sites

Twenty-two soil samples, labeled E1 to E22, were collected from various sites in Itay Al Baroud (El-Beheira Governorate) during the winter of 2019. Fig. (1) shows the map of the study area.

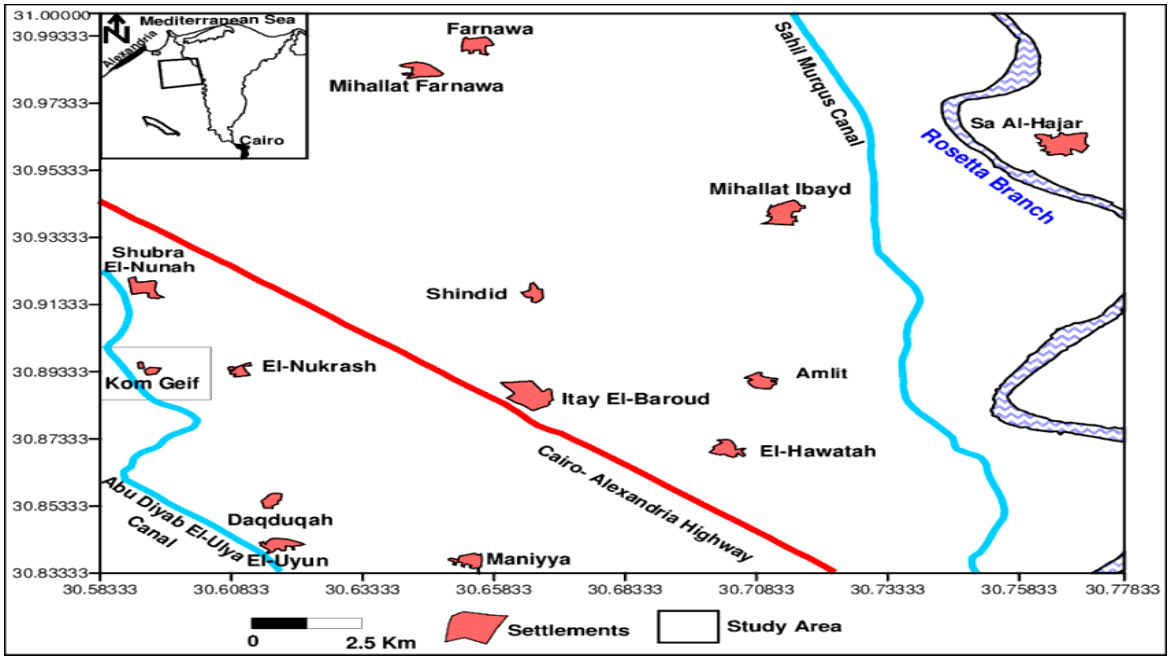

Fig. 1. Map of the study area **(Shaaban *et al*., 2016)**

Indicator pathogenic microorganisms

The used pathogenic bacterial indicators were *B. subtilis* ATCC 6633, *S. aureus* ATCC25923, *P. aeruginosa* ATCC9027, *E. coli* ATCC19404, *E. faecalis* ATCC29212 and *K. pneumoniae* ATCC13883. Fungal pathogens used were *C. albicans* (ATCC 10231), *C. glabrata*, *A. fumigatus* and *A. flavus*. These pathogens were kindly provided by the staff members of The National Institute of Oceanography and Fisheries (NIOF), Alexandria branch. Alexandria, Egypt.

Media

All culture media employed in this study were obtained from LAB M (UK). Preparation of the media involved the use of distilled water, and the pH was carefully adjusted to 7.0 ± 0.2 prior to sterilization. Sterilization was carried out by autoclaving at 121°C for 15 minutes. The media types listed below (in g/L) were consistently utilized throughout the experimental procedures.

Culture media employed for the isolation and quantitative assessment of actinomycetes

Two selective media were utilized for the isolation and enumeration of actinomycetes: Starch Nitrate Agar, as formulated by **Waksman (1959)**, and Glycerol Asparagine Agar, according to **Shirling and Gottlieb (1966)**. The composition of Starch Nitrate Agar (per liter) included: soluble starch (20.0 g), potassium nitrate (1.0 g), sodium

chloride (0.5g), dipotassium hydrogen phosphate (1.0g), magnesium sulfate heptahydrate (0.5g), calcium carbonate (3.0g), and agar (20.0g). Trace amounts of ferrous sulfate heptahydrate were optionally added to enhance sporulation. Glycerol Asparagine Agar was prepared using the following constituents per liter: L-asparagine (0.1g), glycerol (10.0mL) as the primary carbon source, dipotassium hydrogen phosphate (0.5g), magnesium sulfate heptahydrate (0.1g), sodium chloride (0.5g), and agar (15.0–20.0g). Additionally, 1.0mL of a trace salt solution typically containing minute quantities of $FeSO_4 \cdot 7H_2O$, $ZnSO_4$, and $MnCl_2$ was incorporated to support optimal growth and pigmentation.

Media for growth characterization

For the growth characterization of actinomycete isolates, five different media were prepared following established formulations. Glucose Yeast Extract Malt Extract Agar (GYM agar), as described by **Tresner *et al.* (1968)**, was composed of glucose (4.0g), yeast extract (4.0g), malt extract (10.0g), and agar (20.0g) per liter of distilled water. Oatmeal Agar, prepared according to **Küster (1959)**, contained oatmeal (20.0g) and agar (15.0–20.0g) per liter; the oatmeal was first boiled in distilled water, then filtered, and the resulting filtrate was used in the medium. Inorganic Salts Starch Agar (ISSA), based on the formulation by **Shirling and Gottlieb (1966)**, included soluble starch (10.0g), dipotassium hydrogen phosphate (1.0 g), magnesium sulfate heptahydrate (0.5g), sodium chloride (0.5g), ammonium sulfate (2.0g), calcium carbonate (0.2g), ferrous sulfate (0.01g), and agar (15.0–20.0g) per liter. Glycerol Asparagine Agar, also formulated by **Shirling and Gottlieb (1966)**, consisted of L-asparagine (0.1g), glycerol (10.0mL), dipotassium hydrogen phosphate (0.5g), magnesium sulfate heptahydrate (0.1g), sodium chloride (0.5g), trace salt solution (1.0mL), and agar (15.0–20.0 g) per liter. The trace salt solution typically contained small amounts of $FeSO_4 \cdot 7H_2O$, $ZnSO_4$, and $MnCl_2$. Peptone Yeast Extract Iron Agar, formulated by **Abd-Elnaby *et al.* (2016)**, was made with peptone (15.0g), yeast extract (1.0g), ferric ammonium citrate (0.5g), and agar (20.0g) per liter and was specifically used to detect melanin or diffusible pigment production.

Media for physiological and biochemical characterization (Hamed *et al.*, 2021)

For the physiological and biochemical characterization of actinomycete isolates, a range of selective and diagnostic media were utilized based on previously established formulations. Basal Mineral Salts Agar Medium, as described by **Hamed *et al.* (2021)**, was composed of glucose (10.0g), $(NH_4)_2SO_4$ (2.0g), K_2HPO_4 (1.0g), $MgSO_4 \cdot 7H_2O$ (0.5g), NaCl (0.5g), $CaCO_3$ (0.2g), and agar (15.0–20.0g) per liter of distilled water. Nitrate Reduction Medium, according to **Williams *et al.* (1983)**, contained peptone (5.0g), beef extract (3.0g), potassium nitrate (1.0g), and agar (15.0g) per liter. This medium was used to detect the ability of isolates to reduce nitrate to nitrite or nitrogen

gas. Urea Broth Basal Medium, formulated by **Weyland (1969)**, included urea (20.0g), KH_2PO_4 (9.1g), Na_2HPO_4 (9.5g), and phenol red (0.01g) per liter. This medium was employed to assess urease activity, as indicated by a change in color due to ammonia production. Proteolytic Activity Medium, developed by **Hankin and Anagnostakis (1975)**, consisted of casein (5.0g), yeast extract (0.5g), glucose (1.0g), K_2HPO_4 (0.5g), and agar (15.0–20.0g) per liter. The clear zones around colonies indicated protease production. Hydrogen Sulfide Production Medium (Peptone Iron Agar), as proposed by **Küster and Williams (1964)**, contained peptone (10.0g), ferric ammonium citrate (0.5g), sodium thiosulfate (0.5g), and agar (15.0g) per liter. This medium allowed detection of H_2S production through black precipitate formation. Cellulolytic Activity Medium, based on the work of **Ariffin *et al.* (2006)**, included carboxymethyl cellulose (CMC) (10.0g), $NaNO_3$ (2.0g), K_2HPO_4 (1.0g), $MgSO_4 \cdot 7H_2O$ (0.5g), NaCl (0.5g), yeast extract (0.5g), and agar (15.0–20.0g) per liter. Cellulase activity was detected via Congo red staining and clear halo formation. Modified Gelatin Medium, described by **Ventosa *et al.* (1982)**, contained gelatin (30.0g), peptone (5.0g), beef extract (3.0g), and NaCl (5.0g) per liter. This medium was used to assess gelatinase activity. Finally, Lipolytic and Chitinolytic Activity Media, both formulated by **Hankin and Anagnostakis (1975)**, were used to detect lipase and chitinase production, respectively. The lipolytic medium included Tween 80 (10mL), peptone (5.0g), NaCl (5.0g), $CaCl_2$ (0.1g), and agar (15.0g) per liter. The chitinolytic medium was prepared using colloidal chitin (5.0g), K_2HPO_4 (0.7g), $MgSO_4 \cdot 7H_2O$ (0.5g), KH_2PO_4 (0.5g), and agar (15.0–20.0g) per liter.

Media for growth of bacterial indicators

For the cultivation of bacterial indicator strains, Nutrient Agar and Nutrient Broth were employed, following the standard formulations described by **Atlas (1997)**. Nutrient agar was prepared by dissolving peptone (5.0g), beef extract (3.0g), sodium chloride (5.0g), and agar (15.0g) in one liter of distilled water. For Nutrient broth, the same composition was used but without the addition of agar. These media provided the essential nutrients necessary for the growth and maintenance of a broad range of non-fastidious bacterial species.

Chemicals

All chemicals utilized throughout this study were of analytical grade. Ethyl acetate was procured from Sigma-Aldrich (USA), while chloroform and n-hexane were obtained from Loba-Chemie (India).

Samples collection, preservation and processing

Soil samples were collected using the International Organization for Standardization's defined protocols (ISO 5667-1: 2006). Samples were collected from

Itay Al Baroud at a depth of around 15cm using sterile procedures and were immediately placed in sterile plastic bags. To preserve microbiological integrity, the samples were transferred to the laboratory in a sterile ice-cooled container and were analyzed within 24 hours of collection, following the technique outlined by **Abd-Elnaby *et al.* (2016)**. Ten grams of each soil sample were combined with thirty milliliters of distilled water that had been sterilized to create soil sample suspensions. After that, the mixes underwent thermal treatment, which involved heating them in a water bath at 50°C for an hour in order to inhibit the growth of unicellular bacteria and encourage the growth of actinomycetes. The Starch Nitrate Agar medium was mixed with 1mL of Mycostatin to lessen fungal contamination before being transferred into sterile Petri dishes. A triple set of plates was prepared and 1mL of the soil suspension was added to each dish for inoculation. In accordance with the **Jensen *et al.* (1991)** protocol, the plates were incubated at 37°C for 7–14 days.

Actinomycetes enumeration, isolation and purification

Characteristics such as branched vegetative mycelia, aerial mycelia, spore production, and thick, leathery colonies were used to identify actinomycetes. According to **Abu-Elela**'s **(1999)** instructions, actinomycetes were counted on Starch Nitrate and Glycerol Asparagine Agar plates following a 7–14-day incubation period at 37°C. The results were expressed as colony-forming units (CFU g^{-1}). To obtain pure cultures, actinomycetes colonies were purified using the streak plate method, which was repeated until pure isolates were obtained. Sub-culturing of the pure isolates was performed, and the strains were stored at 4°C. Prior to being frozen with 10% glycerol as a cryoprotectant, all isolates were cultivated at 30-37°C in 10mL of Starch Nitrate liquid medium and examined under a microscope for indications of mycelial fragmentation.

Characterization of isolated actinomycetes: Morphological, physiological, and biochemical analysis

Morphological, physiological, and biochemical characterization of the isolated actinomycetes were conducted on twenty-one isolates chosen based on their color variability and pigmentation on Starch Nitrate Agar medium following 7-14 days of incubation at 32°C. The colony morphology of the isolates was examined with respect to both substrate and aerial mycelial color, as well as their branching patterns. Cultural characteristics such as growth patterns, coloration of aerial and substrate mycelium, and the formation of soluble pigments were evaluated across five different media: Yeast Extract Malt Extract Agar, Inorganic Salts Agar, Peptone Yeast Extract Iron Agar, Glycerol Asparagine Agar, and Oatmeal Agar **(Hamed *et al.*, 2021)**. For physiological characterization, the optimal pH range (5-9), temperature range (30-60°C), and NaCl concentration (0-10%) were assessed. Additionally, the ability to utilize various carbon sources, including glucose, fructose, dextrose, maltose, sucrose, and mannitol, was

examined. Biochemical tests involved the hydrolysis of carboxymethyl cellulose, chitin, tween, and gelatin, as well as the production of urease, protease, catalase, H_2S precipitation, and nitrate reductase activity, in accordance with the methods described by **Hamed *et al.* (2021)**.

Preparation of cell free microbial supernatant and antimicrobial activity

After preparing the starch nitrate broth medium and sterilizing it in an autoclave at 121°C for 15 minutes, it was shaken at 37°C for 4–14 days and inoculated with an actinomycetes culture. The cultures' supernatants were employed for additional research after they were centrifuged at 12,000rpm **(Selvakumar *et al*., 2012)**. In this investigation, the isolates' capacity to impede the growth of indicator pathogenic bacteria was assessed using the well-cut diffusion technique. After adding 0.1mL of the indicator bacteria (OD = 1) to 30mL of Nutrient Agar medium, the mixture was transferred into sterile plates. A sterile 4mm cork borer was used to cut wells once the agar had set, making sure that there was a well in the middle and others around the periphery. 50µL of the filtered bacterial culture liquid (filtered through a 0.22µm sterile nitrocellulose syringe filter) were added to each well after the agar plugs were carefully removed. After that, the plates were incubated for 18 to 24 hours at 37°C. Following incubation, a linear measurement in millimeters was made of the diameter of the clear inhibition zone surrounding each well. According to the procedure described by **Hassan (2016)**, each experiment was carried out in triplicate, and the average outcome was determined.

Numerical analysis

Coding of data

Computational approaches were used for clustering the isolates based on the results of their tests via creating a binary matrix. The resultant patterns were analyzed using Multi Experiment Viewer v4.9 (MeV software; available at (http:// www .tm 4.o rg /m ev .html). Hierarchical clustering (HCl) was performed and dendrograms representing results obtained from the tests were generated based on the Euclidian distance matrix. MEGA version 4.0.2 was then used to display the resulting Newick trees **(Saeed *et al*., 2003)**. Almost every character received a score of either plus (1) or minus (0) and was divided into two mutually exclusive states. Qualitative multistate qualities, like physical traits and pigmentation, were rated with a score of minuses (0) for all alternative states and plus (1) for the observed state. The resultant binary pattern was analyzed using Multi Experiment Viewer v4.9 (MeV), with the data organized in an Excel spreadsheet. The first step in using MeV involves importing the data from a tab-delimited text file, which can be generated and exported from any standard spreadsheet program, such as Microsoft Excel. Clustering was subsequently performed to group similar strains based on the binary data.

Molecular identification of the most potent actinomycetes isolate

The DNA of the potent actinomycete isolate was extracted using the Gene Jet Genomic DNA Purification Kit (Fermentas). PCR amplification of the 16S rRNA gene was performed using the following primers: 27F (5' AGAGTTTGAT CCTGG C TCTCAG–3') and 1492R (5'GGTTACC TTGTTA CGACTT–3'). 200ng of DNA template, 0.4µM primers, and 25µL of Maxima Hot Start PCR Master Mix (2X) were included in the 50µL PCR reaction. Thirty cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 60 seconds, extension at 72°C for 4 minutes, and final extension at 72°C for 10 minutes comprised the amplification conditions. The initial denaturation lasted five minutes at 94°C. To verify the amplification results, 1% agarose gel electrophoresis was used. The National Center for Biotechnology Information (NCBI) website's Basic Local Alignment Search Tool (BLAST) was used to do sequence analysis and identify DNA similarities. The sequence was sent to GenBank in order to obtain an accession number. The Prodist-Neighbor-joining method (version 3.6a2.1) was used to conduct a phylogenetic analysis of the produced sequences **(Hall, 1999).**

Electron microscopy examination

Spores of the promising strain were added to Starch Nitrate agar medium, which was then cultured for seven days at 37°C for electron microscopy analysis. After removing the culture plug, it was fixed in 2.5% glutaraldehyde (v/v), rinsed with water, then post-fixed for an hour in 1% osmium tetroxide (w/v). Following two water washes, the sample was dehydrated using increasing ethanol concentrations (30, 50, 70, 90, and 100%) before being covered with gold. At Alexandria University's Electron Microscopy Unit, the material was analyzed at 15-20 kV using a JEO-JSM 5300 scanning electron microscope (SEM) (Japan).

Extraction of the antibacterial agent(s)

Solvent's efficiency

The effectiveness of *n*-hexane, ethyl acetate, and chloroform in extracting the desired active metabolite(s) was evaluated. These solvents were chosen for their varying polarities, with the expectation that the polarity of at least one would match that of the target compounds, thereby facilitating their dissolution **(Balachandran *et al.*, 2015)**.

Preparation of the extracted antibacterial agent(s)

The experimental strain's seed culture was cultivated for 4–14 days at 37°C in Starch Nitrate Broth while being shaken at 120 rpm. After that, the seed culture was moved to three liters of growth medium and cultured for four to fourteen days under typical test settings. Following incubation, the biomass was separated from the culture by centrifuging it for 20 minutes at 4°C at 8000 rpm. Using an equivalent volume of the

most effective organic solvent, the supernatant was extracted three times. Using a rotary evaporator (Stuart RE300), the organic layer was dried over anhydrous sodium sulfate and evaporated until it was completely dry **(Balachandran *et al.*, 2015)**. After being dissolved in DMSO, the resultant extract was kept for later examination.

Applications of the crude extract

Medical application

Antifungal activity

Antifungal activity of the extracted active metabolites was determined against *C. albicans*, *C. glabrata*, *A. fumigatus* and *A. falvus* by using agar well diffusion technique. Determination of inhibition zone diameter against each fungus was carried out after 7 days of incubation at 30^{o}C **(Wefky & Ghobrial, 2008)**.

Antitumor activity

The MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was used to test the cytotoxic activity of the antibacterial agent(s) generated by the chosen isolate against three tumor cell lines: the human colonic carcinoma cell line (HCT-116), the prostate cancer cell line (PC-3), and the breast cancer cell line (MCF-7). To make cell suspensions in Dulbecco's Modified Eagle Medium (DMEM), confluent cells of the tumor cell lines that had been cultured for 24 hours on 75cm² tissue culture flasks were removed. Using blank wells that contained only the medium as a control, aliquots of 100µl of the bioactive compounds were applied to each well of a 96-well tissue culture plate. The cells were kept in a humidified environment with 5% CO_2 for twenty-four hours at 37°C. The medium was aspirated and replaced with DMEM containing 2% fetal bovine serum (FBS) after a full monolayer had developed in each well. 50µl of the bioactive substance that *S. gougerotii* NSH produces was then added to each well. In place of the bioactive material, 50µl of DMEM containing 2% FBS was added to a different set of wells as a negative control. After applying a plate sealer, the treated and untreated cells were cultured for an additional twenty-four hours at 37°C in a humidified environment with 5% CO_2. Using an inverted microscope (CKX41; Olympus, Japan), the plate was inspected for morphological changes in the cells at the conclusion of the incubation period and contrasted with the control cells **(Saintigny *et al.*, 2011)**. At Science Way for Scientific Research and Consultations, the bioactive agent or agents generated by *S. gougerotii* NSH were tested for anticancer activity.

Environmental application

Reduction of some bacterial pathogens load in El-Mex fish rearing water using free and encapsulated extract

After mixing 200µl of ethyl acetate extract with 3% w/v sodium alginate solution, the mixture was dripped from a hydrodermic syringe into 100ml of 2% calcium chloride solution while being continuously stirred, and it was allowed to solidify for one hour. The beads were then stored for further use after being cleaned with sterile distilled water. 50 milliliters of fish growing water that was gathered from the El-Mex farm were used to adjust the encapsulated extract. Additionally, 50ml of fish rearing water was mixed with 200µl of free ethyl acetate crude extract, and the mixture was incubated for 24 hours at 30°C. One flask was kept as a control (the extract was not added). Afterward, count estimation of the most common fish pathogens (*Vibrio* spp., *Aeromonas* spp. and *Pseudomonas* spp.) was carried out using thiosulphate citrate bile salt (TCBS) agar, *Aeromonas* isolation medium base and *Pseudomonas* isolation medium base, respectively and compared with the control **(Hassan & Shobier, 2018)**.

RESULTS

Actinomycetes' dispersion in the soil samples that were collected

Twenty-two soil samples were collected from Itay Al Baroud during Winter, 2019. As a comparable study, the total viable counts (TVC) were estimated in the collected samples using Starch Nitrate Agar medium and Glycerol Asparagine Agar medium. Results illustrated in Fig. (2) indicate that the highest counts were observed at the sampling site E15 recording 267 CFU g^{-1} followed by E20 recording 266 CFU g^{-1}, the lowest count was recorded at E11, E 14 and E21 upon growing on Starch Nitrate Agar medium. On the other hand, generally lower counts were observed upon growing on Glycerol Asparagine Agar medium.

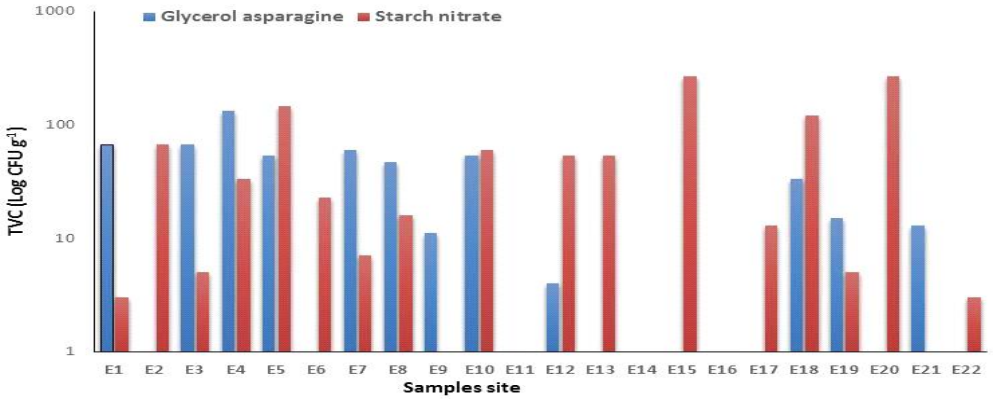

Fig. 2. Counts of actinomycetes in the collected soil samples using Glycerol Asparagine Agar medium and Starch Nitrate Agar medium

Characterization of the isolated actinomycetes

Morphological, biochemical, physiological characterization and clustering of actinomycetes strains using the SsM coefficient with the Mev4.7 depending on numerical taxonomic data

Based on their pigmentation, color variations, and leathery or powdery development, 21 actinobacterial isolates in all were chosen and assigned the N1–N21 designation. The characteristics of all isolates are summarized in Table (1). Analysis of the selected actinomycete strains using numerical techniques and the simple matching coefficient in MeV4.7 clustering produced the dendrogram shown in Fig. (3). The data revealed that at a 75% similarity level, the majority of isolates were grouped into seven main clusters: Cluster A (2 isolates), Cluster B (3 isolates), Cluster C (3 isolates), Cluster D (2 isolates), Cluster E (2 isolates), Cluster F (3 isolates), and Cluster G (3 isolates), along with 3 single clusters, each containing one isolate. Based on the overall phenotypic analysis, it can be concluded that all the soil-isolated actinomycetes grew well on all the tested media, except for N9, which did not grow on Glycerol Asparagine Agar and Glucose Yeast Extract Malt Extract media. Similarly, isolates N14, N15, N11, N8, and N10 were unable to grow on Glucose Yeast Extract Malt extract medium. The Inorganic Salts Starch Agar medium supported the growth of all isolates except for N18.

Most of the isolates had creamy substrate mycelia. The substrate mycelia of two isolates, N5 and N9, were dark brown; all members of cluster B (N17, N14&N12), 2 isolates of cluster E (N11, N15) and only one isolate (N10) from cluster G had red substrate mycelia. Most of the isolates had white aerial mycelium and some had grey, red and creamy. The majority of isolates produced red pigment. All isolates of cluster A produced dark brown pigments, and only isolate (N5) in cluster D and N19 in cluster G in addition to N20 and N21 produced black pigment. On the other hand, only N11 from cluster E, N10 from cluster G, in addition to all members in cluster B produced red pigment.

Physiological and biochemical studies indicated that all isolates grew at 30-40°C, while N12 and N21 were able to grow at higher temperature (50 and 60°C), similarly N3 and N9 tolerated high temperature up to 50°C. The majority grew at pH 7-9. All isolates grew at 0-6% NaCl and the majority was able to grow at 8% NaCl. Tolerance to higher NaCl concentration as 10% was detectable in case of N6, N10 and N21. The majority of isolates utilized maltose, dextrose and sucrose. Most isolates were able to decrease nitrate and produce urease and catalase. H_2S was not generated by any isolate.

Table 1. Phenotypic characteristics of the isolated actinomycetes

Character	Cluster A (2 isolates)		Cluster B (3 isolates)			Cluster C (3 isolates)			Cluster D (2 isolates)		Cluster E (2 isolates)		Cluster F (3 isolates)			Cluster G (3 isolates)			Cluster (single isolates)		
	N7	N9	N12	N14	N17	N1	N2	N4	N3	N5	N15	N11	N8	N13	N16	N10	N6	N19	N18	N20	N21
Growth on																					
Glucose Yeast Extract Malt Extract Agar	+	-	+	-	+	+	+	+	+	+	-	-	-	+	+	-	+	+	+	+	+
Oat Meal Agar	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Inorganic Salts Starch Agar	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
Glycerol Asparagine Agar	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peptone Yeast Extract Iron Agar	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Substrate mycelium color																					
Off white	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Grey	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pink	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
White	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Beige	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dark red	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dark brown	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Reddish dark brown	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Brown	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pale yellow	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Red	-	-	+	+	+	-	-	-	-	-	+	+	-	-	-	+	-	-	-	-	-
Creamy	-	-	-	-	-	+	+	+	+	-	-	-	+	+	+	-	+	+	+	+	+
Aerial mycelium color																					
Off white	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
Pink	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
White	-	-	-	-	-	+	+	-	+	+	-	+	+	+	+	-	-	-	+	+	+
Beige	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Grey	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
Dark brown	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pale yellow	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Red	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Brown	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dark red	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Reddish dark brown	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Creamy	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-

Cont.

Character	Cluster A (2 isolates)		Cluster B (3 isolates)			Cluster C (3 isolates)			Cluster D (2 isolates)		Cluster E (2 isolates)		Cluster F (3 isolates)			Cluster G (3 isolates)			Cluster (single isolates)		
	N7	N9	N12	N14	N17	N1	N2	N4	N3	N5	N15	N11	N8	N13	N16	N10	N6	N19	N18	N20	N21
Diffusible pigment																					
Dark brown	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Red	-	-	+	+	+	-	-	-	-	-	-	+	-	-	-	+	-	-	-	+	+
Grey	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Black	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-
Growth at ºC																					
30	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
40	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
50	-	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+
60	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Growth at pH																					
5	+	-	+	+	+	+	+	+	-	-	-	-	+	+	-	+	+	-	-	-	-
6	+	-	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	-	-	-
7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	+	+	+	+	+	+	+	-	-	-	+	-	+	+	-	+	+	+	+	+	+
9	+	+	-	+	+	+	+	-	-	-	+	-	+	+	-	+	+	+	+	+	-
Growth in NaCl (‰)																					
0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	+	-	+	+	-	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+
Utilization of																					
Glucose	+	+	-	-	+	+	-	-	-	-	-	+	+	+	+	+	+	+	-	+	+
Maltose	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fructose	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	+
Dextrose	+	+	-	-	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-	+	+
Sucrose	+	+	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mannitol	+	+	-	+	+	-	-	-	-	-	+	+	+	+	+	+	+	+	-	+	+

Cont.

Character	Cluster A (2 isolates)		Cluster B (3 isolates)			Cluster C (3 isolates)			Cluster D (2 isolates)		Cluster E (2 isolates)		Cluster F (3 isolates)			Cluster G (3 isolates)			Cluster (single isolates)		
	N7	N9	N12	N14	N17	N1	N2	N4	N3	N5	N15	N11	N8	N13	N16	N10	N6	N19	N18	N20	N21
Biochemical tests																					
Urease production	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	-	+	+
Protease production	+	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-	+	-	-	-	-
Catalase production	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
Nitrate reduction	+	+	+	+	+	-	+	-	-	+	+	-	-	+	-	+	+	+	-	-	+
H_2S production	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Degradation of																					
CMC	+	-	+	-	-	-	-	+	+	+	+	+	-	+	-	+	+	+	+	+	+
Gelatin	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tween 20	+	+	+	+	-	+	+	+	+	+	-	-	-	-	-	-	+	-	+	+	+
Tween 80	+	-	+	+	-	-	-	-	+	+	+	+	+	+	+	-	+	-	+	-	-
Chitin	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	+
Microbial inhibition																					
Staphylococcus aureus	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Escherichia coli	+	+	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bacillus subtilis	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Enterococcus faecalis	+	+	-	-	-	-	+	-	+	-	-	-	-	-	-	+	+	-	-	-	-
Klebsiella pneumoniae	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-

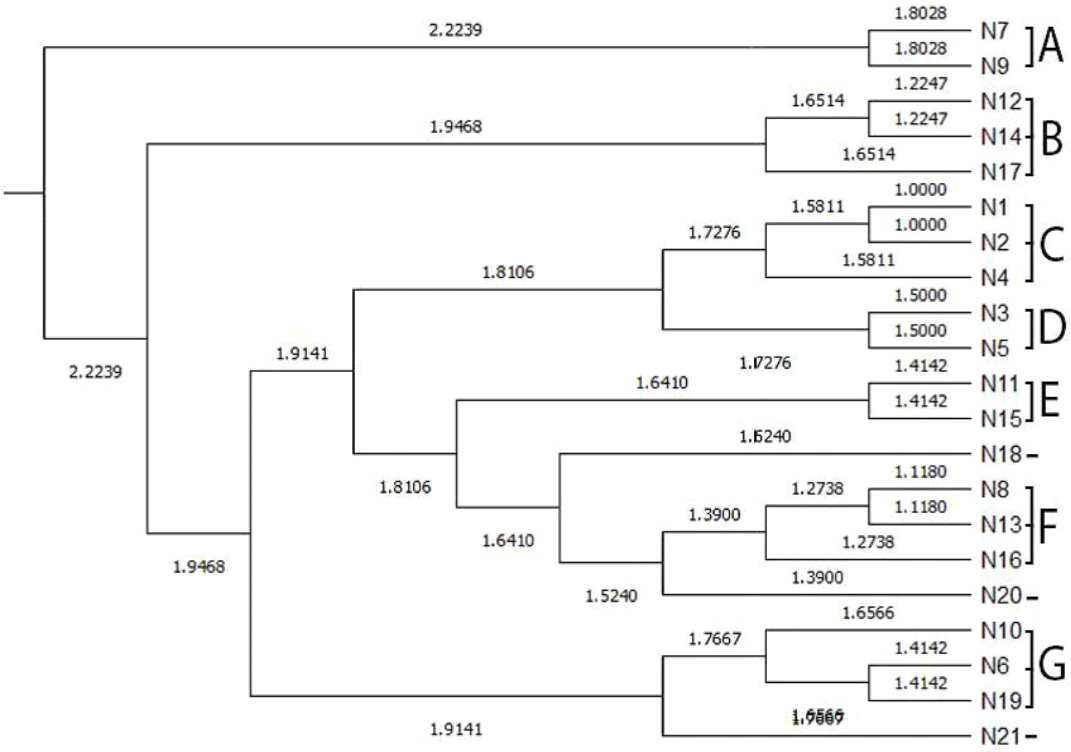

Fig. 3. Simplified dendogram showing the relationships among clusters based on the Mev4.7 analysis

Antimicrobial activities

Using the agar well diffusion technique, the isolated actinomycetes' antimicrobial ability against several Gram-positive and Gram-negative pathogenic bacteria was evaluated. Only 10 isolates, or roughly 47.6% of all isolates tested, demonstrated antagonistic activity (measured as inhibition zone diameter (mm)) against the tested pathogenic bacteria, according to the results shown in Fig. (4). It is obvious that isolates N7 and N9 showed broad spectrum of antimicrobial activity against the tested pathogens; however, N7 was selected as the most tentative antimicrobial producer showing the highest inhibition zone diameters of 14, 15, 15, 15, 16 and 16mm against *K. pneumoniae* ATCC 3883, *E. coli* ATCC 19404, *P. aeruginosa* ATCC 9027, *S. aureus* ATCC 25923, *B. subtilis* ATCC 6633 and *E. faecalis* ATCC 29212, respectively (Fig. 5A, B and C), while N1, N2, N4, N5, N6, N8 and N10 were bioactive only against one pathogen and N3 exhibited antimicrobial activity against 3 pathogens only, whereas the other isolates had no antibacterial action against the microorganisms that were examined. Thus, the isolate N7 was chosen as the most potent candidate with respect to its antagonistic potential, and *S. aureus* was selected as the most sensitive pathogenic bacteria with more clear inhibition zone.

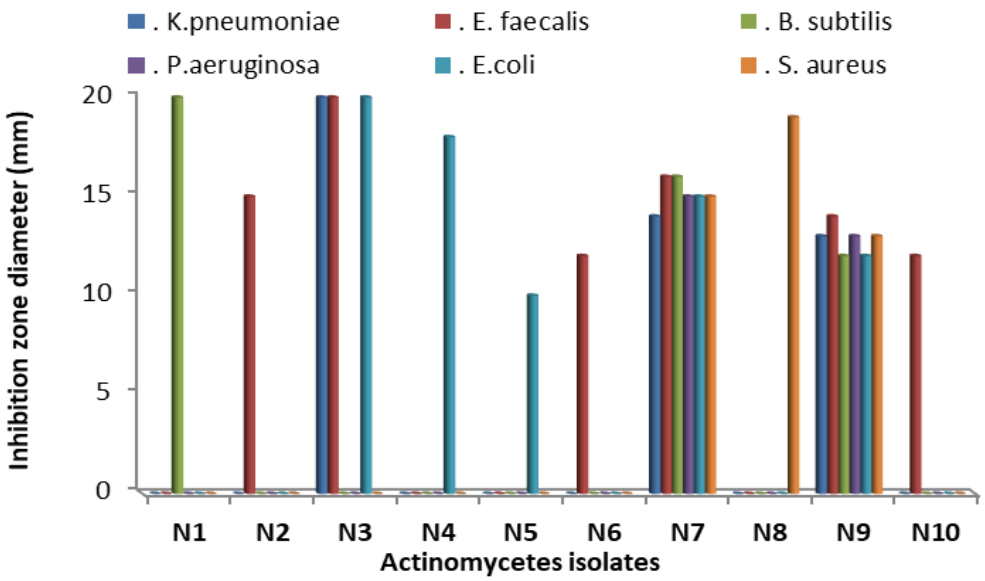

Fig. 4. Antibacterial activity of actinomycetes isolates expressed as inhibition zone diameter (mm) against some reference bacterial pathogens

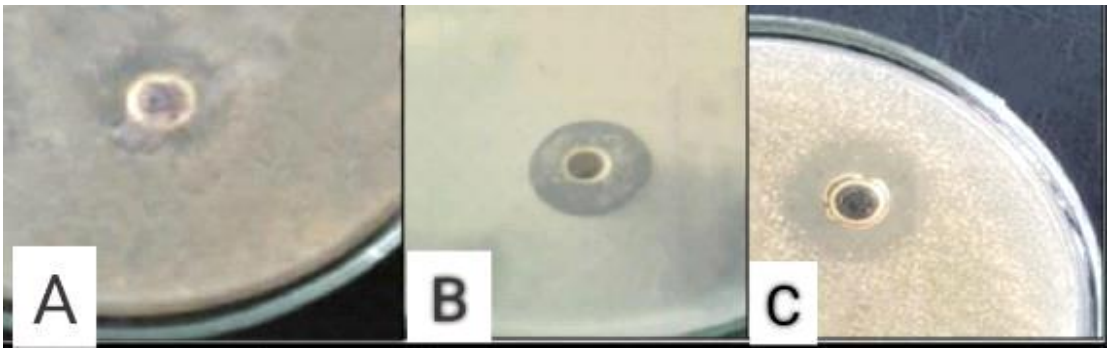

Fig. 5. Antagonistic effects of isolate N7 against **(A)** *E. faecalis*, **(B)** *S. aureus* and **(C)** *B.subtilis*

The most powerful isolate's molecular phylogeny

After amplifying the homologous 16S rRNA regions using the designed primers, the resulting amplicons were visualized through agarose gel electrophoresis (Fig. 6). The sequencing of these amplicons, performed using the ABI 3730xl sequencer, yielded a sequence of 1350 base pairs. The sequencing results indicated that the isolate under study exhibited 100% similarity to the species *Streptomyces* strain DSM 40324. The accession number MW023664 was assigned to the nucleotide sequence when it was entered into the GenBank sequence database. Fig. (7) shows N7's evolutionary location among related bacterial species. Based on these findings, N7 was identified as *Streptomyces gougerotii* NSH. A scanning electron micrograph of *S. gougerotii* NSH is shown in Fig. (8).

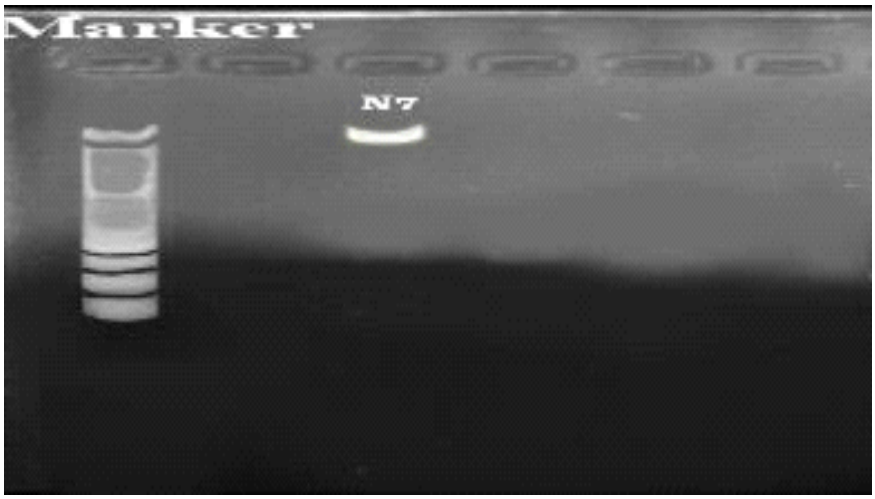

Fig. 6. Agarose gel electrophoresis of the amplified 16S rRNA gene of the isolate N7

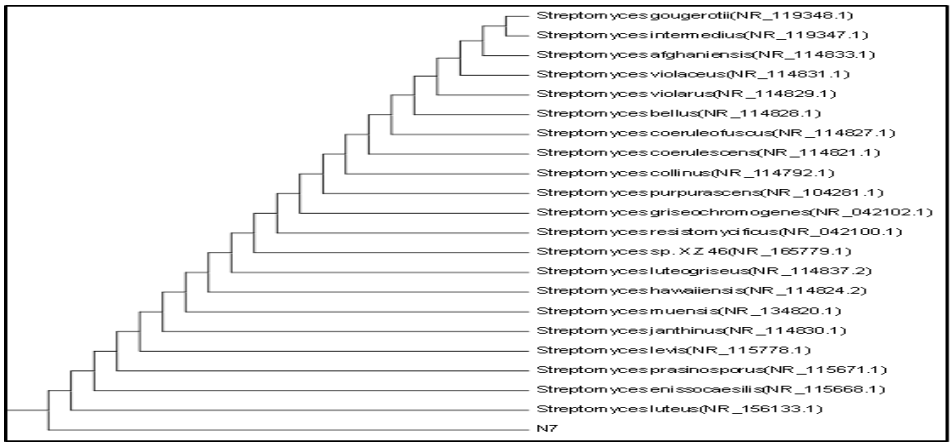

Fig. 7. Phylogenetic tree showing the position of isolate N7 with the other members of genus *Streptomyces*

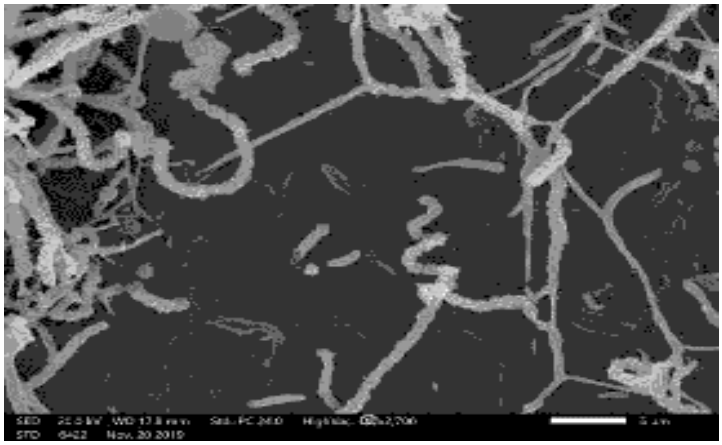

Fig. 8. Scanning electron micrograph showing spore formation of *S. gougerotii* NSH grown on starch nitrate agar medium for 14 days

Extraction of the antibacterial agents

Solvent efficiency

The effectiveness of many solvents in removing the targeted antibacterial agents was evaluated, including n-hexane, chloroform, and ethyl acetate. Ethyl acetate was the most effective solvent, producing the highest antibacterial activity (25mm), according to the results, which are shown in Table (2). In contrast, chloroform and n-hexane showed no antibacterial activity during extraction. Based on these findings, ethyl acetate was selected for further studies.

Table 2. Antibacterial activity of different solvent extracts against *S. aureus*

Solvent	Inhibition zone diameter (mm)
Ethyl acetate	**25.0**
n-Hexane	**0.0**
Chloroform	**0.0**

Applications of the extracted active metabolites

Antifungal activity

Results in Fig. (9) show that the bioactive metabolites produced by *S. gougerotii* NSH had a reasonable antifungal activity against the tested pathogenic fungi recording inhibition zone diameters as 22mm against *C. glabrata*, 15mm against *C. albicans*, 10mm against *A. fumigatus* and 8mm against *A. falvus*. As a control, amphotericin (5mg/ ml) demonstrated inhibitory zone sizes ranging from 15 to 25mm.

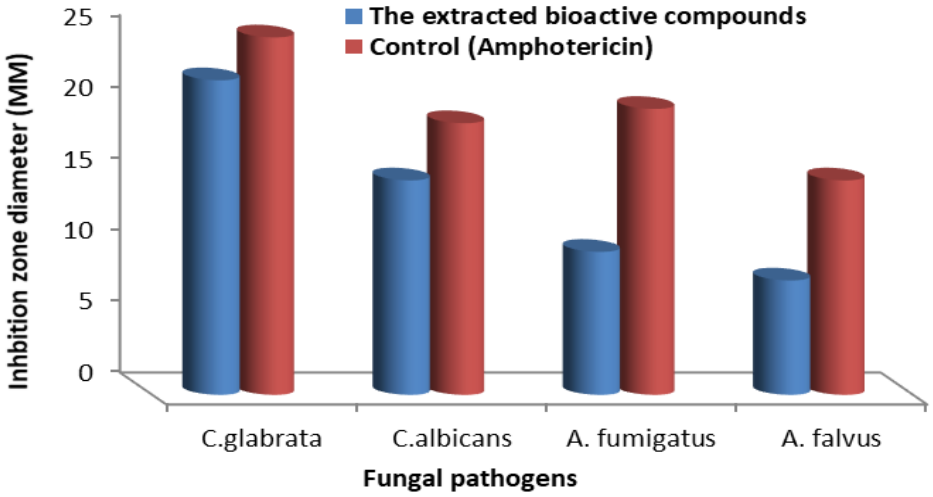

Fig. 9. Antifungal activity of the extracted bioactive compound (s) from *S. gougerotii* NSH

Anticancer activity

The growth inhibitory effect of different concentrations (125-10000µg/ ml) of the extracted compounds was evaluated against breast cancer cell line (MCF -7), human colonic carcinoma cell line (HCT-116) and prostate cancer cell line (PC3) using MTT assay. Our results highlighted the different effects of the tested extract concentrations on viability and growth of each cell line (Figs. 10 & 11) causing almost 69.4 % inhibition of MCF- 7 using the concentration 10000µg/ ml with IC50 of 7989.13µg/ ml. More inhibition was observed towards PC 3 and HCT-116 using the same concentration of the extract realizing about 90.6% and 92.5% inhibition with IC50 of 3460.45 and 4113.75µg/ ml, respectively.

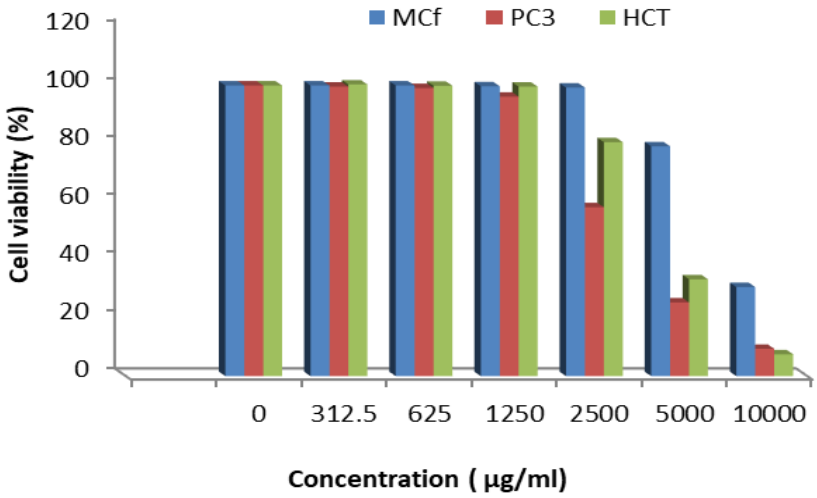

Fig. 10. Effect of different crude extract concentrations on the viability of Mcf-7, PC3 and HCT-116 cell lines

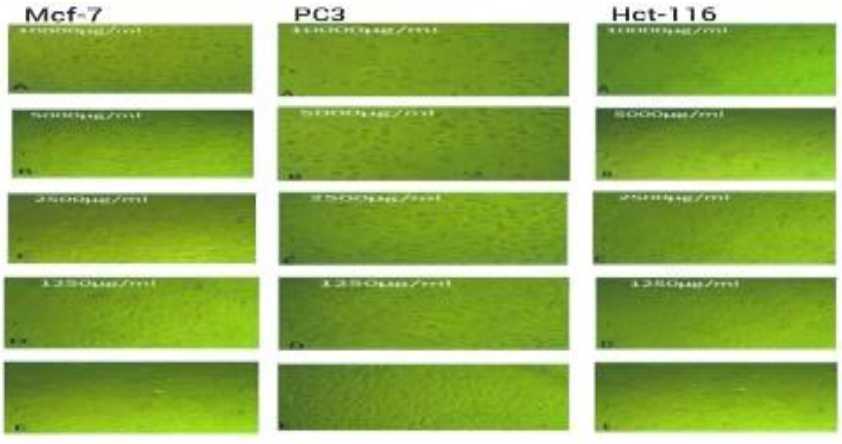

Fig. 11. Photographs showing the difference between the effect of the different concentrations (125-10000µg/ ml) (A-D) of the crude extract on growth inhibition of Mcf -7, PC3 and HCT-116 cell lines compared to control (E)

Environmental application

Fish-raising water collected from El-Mex fish farm was used in the current experiment as a test to remove the load of the main fish diseases. Counts of the most common fish pathogens (*Vibrio*, *Aeromonas* and *Pseudomonas*) were estimated in the treated rearing water using free and encapsulated crude extracts and compared to the control (untreated with crude extract). It was detected that efficiency of the encapsulated extract in reduction of fish pathogens was higher than free extract, where it caused reduction of the counts of *Vibrio*, *Aeromonas* and *Pseudomonas* by about 90.2, 83.3, 85.5%, respectively, compared to 90, 80 and 75% caused by free extract, as shown in Fig. (12).

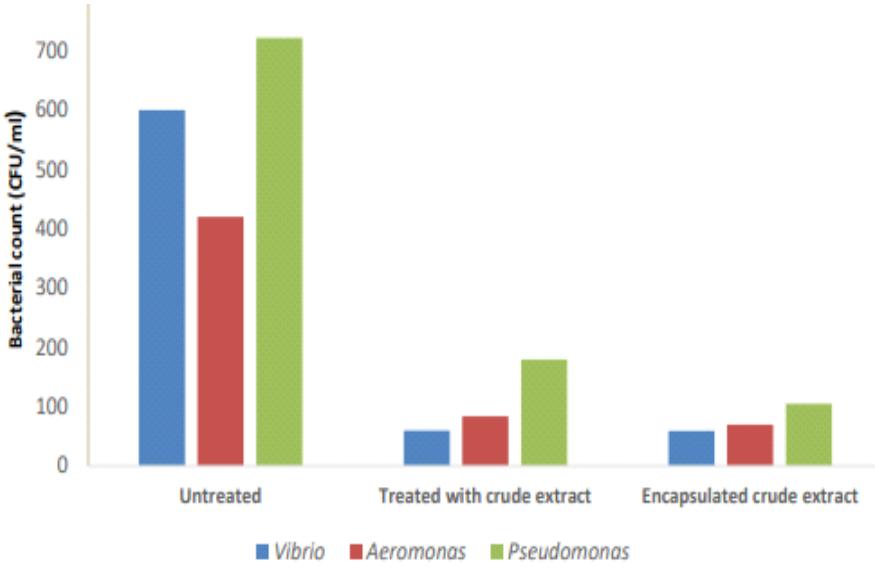

Fig. 12. Total counts of *Vibrio* spp., *Aeromonas* spp. and *Pseudomonas* spp. before and after treatment with free and encapsulated crude extract

DISCUSSION

The emergence of multidrug-resistant microorganisms as a serious danger to world health emphasizes how urgently new and improved medications must be developed **(Assou *et al.*, 2023)**. Human survival depends on actinomycetes, one of the most beneficial prokaryotes in terms of both economics and biotechnology. Actinomycetes account for around 45% of all known bioactive microbial metabolites, including over 10,000 bioactive secondary metabolites that microorganisms have been known to produce. Among actinomycetes, *Streptomyces* species produce about 1,600 different compounds **(Usha *et al.*, 2012)**. Few researchers have examined the distribution of actinomycetes in specific terrestrial environments, especially those with harsh conditions. This topic is still relevant for investigating various actinomycetes genera that can endure and adapt to these environments **(Mansour, 2003)**.

The primary objective of the current study is to investigate the diversity and taxonomic characterization of certain actinomycetes that were isolated from soil samples that were gathered from Itay Al Baroud, El-Beherra, Egypt. The study also clarifies the most powerful isolate's bioactivity. Using Glycerol Asparagine Agar and Starch Nitrate Agar media, the diversity of actinomycetes in the soil samples that were collected was examined. The highest TVC was detected upon using Starch Nitrate medium with the highest counts (267 and 266 CFU g-1) at stations E15 and E20. Starch Nitrate medium was recommended as the best medium for isolation of actinomycetes and thus was chosen to complete the study. In accordance with our results, **Ghanem *et al.* (2000)** suggested the same finding. Environmental factors like pH, temperature, moisture, nutrition, climate, and geographic location can all affect the variety of actinomycetes and their numbers **(Hong *et al*., 2009; Arifuzzaman *et al.*, 2010)**.

In order to classify organisms into clusters according to their overall similarities, numerical taxonomy applies a number of mathematical techniques to data that are numerically coded and expressed as individual features. Therefore, a useful foundation for bacterial taxonomy, including species identification, is provided by numerical taxonomy. Twenty two isolates of actinomycetes were chosen as an orientation step, and they were described using morphological, physiological, and biochemical techniques. Definitely, characterization studies aid to explain physiological, biochemical, ecological and biological characteristics, which is a significant analysis of the presence of these microbes and understanding the main physiology of soil actinomycetes isolates.

The ability of isolated actinomycetes to produce an antibacterial agent against ten chosen reference pathogenic strains was evaluated. The screening assay revealed that only 10 of the 22 isolates, or 47.6% of all isolates, exhibited antagonistic activity against the tested pathogenic bacteria. In a previous study by **Nair and Simidu (1987)**, the bacterial antagonism percentage was lower (5 to 8%) or higher (35-53.5%), as reported by **Long and Azam (2001)**. In a comparable study by **Sapkota *et al.* (2020)**, it was mentioned that when fourty-one actinomycetes isolates were exposed to primary screening for their antibacterial capabilities, 46.34% were active against the pathogenic bacteria and all active isolates had antagonistic activity against *Staphylococcus aureus* (ATCC 25923), while only 5 of them had activity against *Klebsiella pneumoniae* (ATCC 700603) and *Escherichia coli* (ATCC 25922). None had antagonistic activity against *Pseudomonas aeruginosa* (ATCC 27853). Another study done by **Elbandary *et al.* (2018)** showed that actinomycetes isolated from 100 samples collected from agricultural farming soil were screened for the antagonistic activity and only 12% actinomycetes isolates produced antimicrobial activity against *B. cereus; K. pneumoniae, S. aureus, E. coli, P. aeruginosa, S. typhimurium, Aspergillus flavus, A. niger* and *Candida albicans.*

The diverse antibacterial activity of the isolated actinomycetes may be connected to the properties of the soil due to its complexity and diversity in terms of life, nutrition, and physical attributes. As a result, they can perform a wide range of metabolic functions

and generate a large number of advantageous secondary metabolites **(Sajid *et al.*, 2011)**. The differences in sensitivity between Gram-positive and Gram-negative bacteria may also be attributed to their physical differences. Gram-positive bacteria only have an exterior layer of peptidoglycan, which is ineffective as a permeability barrier, whereas Gram-negative bacteria have an outer polysaccharide membrane that makes their cell walls impermeable to lipophilic solutes **(Sapkota *et al.*, 2020)**.

Using phylogenetic analysis and comparative 16S rRNA sequencing, isolate N7 (cluster A) was found to be 100% identical to *S. gougerotii*. Gram-positive bacteria with a high GC content (more than 70%) in their DNA have been identified in the genus *Streptomyces*. It is one of the most significant for both industrial use and human health care since it appears to produce a greater range of novel antibiotics than any other genus **(Sajid *et al.*, 2011)**.

In comparison to n-hexane and chloroform, ethyl acetate was determined to be the most effective solvent for extracting the necessary active components. The bioactive compounds generated by *Streptomyces* sp. strain FR7 **(Weslati *et al.*, 2023)** and *Streptomyces* sp. CRB46 **(Ambarwati *et al.*, 2020)** were effectively extracted using the same solvent.

Our results highlighted that the extracted bioactive metabolites from *S. gougerotii* NSH have a reasonable antifungal activity against the tested fungal pathogens. **Sharma and Parihar (2010)** conducted a parallel investigation that documented the antifungal properties of various extract concentrations made by soil actinomycetes against *A. niger*, whose inhibition zone diameter varied from 14 to 23mm, and *A. flavus* whose inhibition zone diameter ranged from 16 to 20mm. The antifungal activity of *Streptomyces* sp. ActiF450 was also proven in a recent study by **Benhadj *et al*. (2020)** which revealed a broad-spectrum activity against different pathogenic fungi including *A. fumigatus, A. niger, Candida albicans, C. krusei, C. glabarta, Arthroderma vanbreuseghemii, Rhodotorula mucilaginous, Fusarium oxysporum, F. solani* and *Microsporum canis*. The CACIS-2.15CA *Streptomyces* isolate revealed a high inhibitory action against *Aspergillus* sp., *Fusarium oxysporum*, *Sclerotinia* sp., *Botrytis cinerea*, *Phytophthora capsici*, *Colletotrichum musae* and *Fusarium* sp., with inhibition percentage from 7.3 to 61.2%, as indicated by **Rios-Muñiz and Evangelista-Martinez (2022)**.

The extracted active metabolites prepared in the present investigation exhibited reasonable degree of anticancer activity toward the tested cell lines causing almost 69.4, 90.6 and 92.5% inhibition toward breast cancer cell line MCF -7, cancer cell line prostate PC3 and carcinoma cell line HCT-116, respectively, using 10000µg/ ml of the tested compounds. Similar findings were reported by **Davies-Bolorunduro *et al.* (2019)**, who demonstrated that *Micromonospora* spp. and *Streptomyces* strains isolated from various sites in the Lagos Lagoon exhibited anticancer activity against several human cancer cell lines, including K562 (acute myelocytic leukemia), HeLa (cervical carcinoma), AGS (gastric cancer), MCF-7 (breast adenocarcinoma), and HL-60 (acute promyelocytic

leukemia). Recently, a novel *Streptomyces macrolidinus* sp. nov. isolated from a peat swamp forest soil revealed cytotoxicity against KB, Vero, NCI-H187 and MCF-7 with IC50 values of 6.15, 0.0347, 0.0352 and 3.36µg ml-1, respectively, as proven by **Kanchanasin *et al*. (2023)**.

The current experiment was designed to identify an economical and environmentally friendly method for eliminating the most harmful fish pathogens in fish rearing water collected from El-Mex fish farm using free and encapsulated crude extracts obtained from *S. gougerotii* NSH. This was done as an orientation step for applying the environmental impact of the extracted bioactive metabolites. When compared to free metabolites, the encapsulated bioactive metabolites were found to be more effective at eliminating *Vibrio* spp., *Aeromonas* spp., and *Pseudomonas* sp. To evaluate *S. gougerotii* NSH's function as a probiotic bacterium in aquaculture, more research is needed. According to earlier research, *S. carpaticus*-MK-01, which was isolated from Jeju Island's seawater, possesses bioactive chemicals that could be used as antibiotics to treat some fish diseases **(Subathradevi *et al*., 2014)**. Recent report published by Y**ang *et al*. (2022)** indicated that *Streptomyces lateritius* Z1-26 isolated from soil samples displayed wide-ranging-spectrum antagonistic action against a wide range of fish pathogens, where improved rate of fish survival and growth rate was observed after diet supplementation with *Streptomyces lateritius* Z1-26. The efficiency of *Streptomyces* as a prospective probiotic source in aquaculture was also proven in other previous studies **(Das *et al*., 2006; Tan *et al*., 2016)**. Further studies concerning the use of *S. gougerotii* NSH as a prospective probiotic in the aquaculture field will be conducted in the future study.

CONCLUSION

The present study successfully isolated and characterized diverse actinomycetes from soil samples collected at Itay Al Baroud, El-Beheira Governorate, Egypt. Among the twenty-one isolates studied, *Streptomyces gougerotii* NSH MW023664 demonstrated exceptional bioactivity, exhibiting broad-spectrum antimicrobial, antifungal, and anticancer properties. The best solvent for extracting its bioactive metabolites was found to be ethyl acetate. Furthermore, by significantly lowering the load of harmful bacteria in aquaculture settings, the encapsulated crude extract demonstrated tremendous environmental applicability. These results support *S. gougerotii* NSH MW023664's remarkable biotechnological potential as a possible probiotic option for aquaculture as well as a source of novel antibacterial and anticancer compounds. Future studies focusing on the optimization of metabolite production, structural elucidation of active compounds, and in vivo evaluations are strongly recommended to fully harness its therapeutic and environmental applications.

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