sciendo

ADVANCED RESEARCH IN LIFE SCIENCES
7, 2023, 73 - 82

www.degruyter.com/view/j/arls

DOI: 10.2478/arls-2023-0009
Research Article

New Alkali-tolerant and Halo-tolerant *Nocardiopsis* Strain B20 from Algerian Saharan Soil: Isolation, Taxonomy, and Antifungal Activity

Khaoula Bouznada[1], Dalila Boubetra[1], Samira Tata[1], Noureddine Bouras[1,2], Mahfoud Bakli[3,*] and Atika Meklat[1]

[1]Laboratoire de Biologie des Systèmes Microbiens (LBSM), Ecole Normale Supérieure de Kouba, Alger, Algeria.
[2]Laboratoire de Valorisation et Conservation des Écosystèmes Arides (LVCEA), Faculté des Sciences de la Nature et de la Vie et Sciences de la Terre, Université de Ghardaïa, Ghardaïa, Algeria.
[3]Département de Biologie, Faculté des Sciences et Technologie, Université de Aïn Temouchent, Aïn Temouchent, Algeria.

Received May, 2023; Revised August, 2023; Accepted August, 2023

Abstract
A newly characterized actinobacterial strain, designated B20, displayed remarkable tolerance to extreme pH and NaCl conditions, while showcasing a diversity in carbon source utilization. Phylogenetic analysis confirmed its taxonomic assignment within the genus *Nocardiopsis*. However, several physiological features were found to differentiate B20 from its most closely related *Nocardiopsis* species. Notably, *Nocardiopsis* sp. B20 demonstrated potent and selective antifungal activity against various micro-fungi, suggesting its potential as a valuable source for targeted antifungal compounds. Ethyl acetate extract contains a bioactive compound, B20-1, displaying antifungal properties. Chemical revelations indicated the presence of sugars and polycyclic aromatic hydrocarbons (PAHs) in B20-1 compound. The discovery of antifungal PAHs derived from natural sources presents promising opportunity for addressing fungal infections while mitigating environmental concerns and resistance risks associated with synthetic fungicides. However, further research is warranted to evaluate efficacy, assess ecological impacts, and explore potential applications across diverse industries.

Keywords: *Nocardiopsis*, alkali-tolerance, halo-tolerance, Saharan soil, taxonomy, antifungal activity, polycyclic aromatic hydrocarbons.

Introduction

Actinobacteria, a heterogeneous bacterial group, plays an indispensable role in the decomposition of organic compounds in soil and can be found thriving in diverse extreme habitats [1,2]. *Nocardiopsis*, a genus within the *Actinobacteria* phylum, is renowned for its diverse bioactive compound production ability, making it a valuable subject of study. These bioactive compounds, which include antibacterial, antifungal, antiviral, and anticancer agents, have significant potential for application in the medical and agricultural sectors [3,4]. In the soil ecosystem, *Nocardiopsis* species contribute to the breakdown of complex organic matter, enhancing nutrient cycling, and suppressing soil-borne diseases through the production of these bioactive compounds [5].

Of special interest are basophilic *Actinobacteria* that grow in alkaline environments due to their exceptional metabolic proficiency [6]. Nevertheless, despite the extensive research on *Actinobacteria*, a significant portion of microbial diversity remains undiscovered, and there exists vast potential to uncover novel bioactive compounds from these unexplored sources [7,8]. The Algerian Sahara, a distinctive ecosystem,

* Corresponding author: **M. Bakli, Email:** mahfoud.bakli@gmail.com

© 2023 M. Bakli et al., published by De Gruyter Open.
This work was licensed under the Creative Commons Attribution-NonCommercial-NoDerivs 3.0 License

holds untapped potential in terms of its actinobacterial diversity, as only a limited number of studies have been conducted on *Actinobacteria* from this region [9]. This unique and largely unexplored ecosystem presents a promising niche for the exploration of novel actinobacterial species and bioactive compounds.

Considering the value of *Nocardiopsis* and the prospective discovery of novel bioactive compounds from uncharted sources, this study aims to selectively isolate, identify, and characterize a new *Nocardiopsis* strain from an Algerian Saharan soil sample. Additionally, the study will examine the antifungal activity of this new strain against several fungal strains and partially characterize the antifungal compounds.

Materials and Methods

Sample collection and processing

The soil samples from the Algerian Sahara were taken from Mzab region in the southern Algerian province of Ghardaïa (32°28'N 3°40'E). The distant location and minimal anthropogenic effect of the sampling site made it highly likely to find novel microbial strains. At a depth of up to 20 cm, the samples were taken with a sterile soil auger, and put in sterile plastic bags. After being air dried at ambient temperature for 48 hours, the samples were sieved using a 2-mm mesh to remove large debris and stones.

Strain isolation

Nocardiopsis sp. B20 was isolated via the dilution plate method on a selective Complex Medium Agar (CMA) [10]. The CMA was prepared by combining Difco casamino acids (7.5 g), yeast extract (10 g), $MgSO_4.7H_2O$ (10 g), sodium citrate (3 g), KCl (1 g), $FeSO_4.7H_2O$ (at 4.98%, 1 mL), agar (18 g), and distilled water (1000 mL). The isolation medium was supplemented by cycloheximide at a concentration of 50 mg/L to prevent fungal contamination. A buffer consisting of 0.1 M $NaHCO_3$/0.1 M Na_2CO_3 was used to adjust the pH to 10.0. After incubation at 30°C for 2 weeks, the strain was purified and stored on the same medium at 4°C and in 20% (v/v) glycerol suspensions at -20°C.

Phenotypic characterization

To characterize the morphology of *Nocardiopsis* sp. B20, we utilized standard techniques. The strain was cultured on two types of International *Streptomyces* Project (ISP) media (ISP2 and ISP4) [11], and on CM agar. The cultures were incubated for three weeks at 30 °C and pH 10.0. The observations were carried out using light microscopy (Motic, B1 Series).

To obtain the biomass for chemotaxonomic analysis, liquid cultures were centrifuged after six days of growth in CM broth (pH 7.5) on a rotary shaker (250 rpm) at 30°C. The biomass was obtained by centrifuging the cultures at 4000 *g*. Subsequently, it was thoroughly subjected to multiple washes (3-4 times) with distilled water to ensure the total elimination of the remaining culture medium. A brief rinse with 70 % ethanol was then performed to expedite the subsequent drying process which was carried out at a temperature of 40 °C overnight. The identification of the isomeric form of diaminopimelic acid (DAP), and the determination of glycine presence in the cell wall was conducted according to Becker et al. [12]. Additionally, the whole cell sugar composition was assessed [13] and the analysis of phospholipids was performed [14].

To determine the growth conditions and biochemical characteristics of the *Nocardiopsis* sp. B20, we conducted several physiological tests. Each of these tests was performed in triplicate to ensure consistency and reliability of the results. We assessed the utilization of twenty carbon sources and the degradation of nine organic acids using the methods established by Gordon et al. [15]. The carbone sources were sterilized with ether overnight before being added separately and aseptically at a final concentration of 10 g/L. In parallel, the organic acids, utilized in the form of sodium salts, were incorporated into the medium at a final concentration of 2 g/L prior to the sterilization. Additionally, we examined the degradation of starch, cellulose, milk casein, gelatin, tween 80, adenine, guanine, xanthine, hypoxanthine, testosterone, L-tyrosine, and according to the protocols outlined by Goodfellow [16] and Marchal et al. [17]. Furthermore, we evaluated the strain's ability to grow under 0, 7, and 10 % (w/v) of NaCl and at different pH levels, with all tests performed at pH 7 (except those for pH resistance). The following buffers: pH 6.0, 7.0, and 8.0, 0.1 M KH_2PO_4/0.1 M NaOH; pH 9.0 and 10.0, 0.1 M $NaHCO_3$/0.1 M Na_2CO_3; pH 11.0, 0.05 M Na_2HPO_4/0.1 M NaOH; and pH 12.0, 0.2 M KCl/0.2 M NaOH were used to determine the pH range of growth on nutrient broth.

16S rRNA gene sequencing and phylogenetic analysis

The genomic DNA of *Nocardiopsis* sp. B20 was extracted using the method developed by Liu et al. [18], and the 16S rRNA gene was amplified by PCR using the forward FC27 (5'-AGAGTTTGATCCTGGCTCAG-3') and reverse RC1492 (5'-GGTTACCTTGTTACGACTT-3') primer pair, as outlined by Rainey et al. [19]. The resulting PCR products were analyzed by agarose gel electrophoresis, purified, and sequenced by

the Genewiz Company (United Kingdom). The 16S rRNA gene sequence of the *Nocardiopsis* sp. B20 was compared to the corresponding sequences of type strains available on the EzBioCloud server (https://eztaxon-e.ezbiocloud.net) [20]. MEGA version X software was used to conduct the phylogenetic analysis [21]. The CLUSTAL W algorithm [22] with default parameters was used to align the 16S rRNA gene sequence against the 16S rRNA gene sequences of the neighbouring *Nocardiopsis* type species. Maximum-Likelihood method [23] was employed with the Tamura and Nei model [24] to calculate evolutionary distances [25] and construct the phylogenetic tree. Bootstrap resampling with 100 replicates was performed to assess the tree structure's reliability, as proposed by Felsenstein [26].

Antimicrobial assay

The antimicrobial activity of *Nocardiopsis* sp. B20 was assessed against several microbial strains, including bacteria (*Bacillus subtilis* ATCC 6633, *Micrococcus luteus* ATCC 9314, *Staphylococcus aureus* CIP 7625 and *Klebsiella pneumoniae* CIP 8291), fungi (*Umbelopsis ramanniana* NRRL 1829, *Penicillium expansum* B831P, *P. glabrum*, and *Aspergillus carbonarius* M333), and yeast (*Saccharomyces cerevisiae* ATCC 4226). The experimental setup, conducted in three repetitions, involved streaking the actinobacterial inoculum in a straight line across the surface of 90 mm-diameter media plates, followed by incubation at 30°C for 10 days. After the growth of the actinobacterial strain, the target microorganisms were streaked in a cross line over the actinobacterial culture. The extent of antimicrobial activity was determined by measuring the length of the zone of inhibition between the target microorganisms and the actinobacterial colony margins.

Production, extraction, and detection of bioactive compounds

In this study, *Nocardiopsis* sp. B20 was grown in liquid ISP2 medium at 30°C with constant agitation at 250 rpm. To extract bioactive compounds, the fermentation broth was centrifuged at 8000 rpm for 20 minutes, and the resulting cell-free supernatant was subjected to liquid-liquid extraction using, separately, *n*-hexane, dichloromethane, ethyl acetate, and *n*-butanol, in a 1:1 ratio. The organic extracts were then vacuum-dried with a Rotavapor R-205 (Buchi, Switzerland). For the biological assay, the Kirby-Bauer method was employed [27]. Paper disks (6 mm in diameter) were loaded with 60 µl of each extract and placed on semi-solid ISP2 medium (12 g/L agar) inoculated with *U. ramanniana* NRRL 1829 and *A. carbonarius* M333 as target microorganisms. After 48 hours of incubation at 30°C, the diameter of the inhibition distances was measured, with each measurement representing the mean value derived from three independent repetitions. The control consisted of a paper disk containing the same amount of methanol. Furthermore, five silica gel plates (Merck Art. 5735, Kiesselgel 60HF 254-365) were prepared and then activated at 105°C for one hour. A volume of 120 µl of the most active organic extract was spotted on the plates, which were subsequently developed with a solvent system of ethyl acetate/methanol (100:15 v/v). To evaporate all solvent, the developed silica gel thin layer chromatography (TLC) plates were air-dried overnight at 40°C. In addition to being visible to the naked eye as colored spots, the separated compounds were also visualized under ultraviolet (UV) light at 254 nm (absorbance) and 365 nm (fluorescence). To detect the active spots, we employed the bioautography method developed by Betina et al. [28]. One TLC plate, placed in a plastic bioassay dish, was overlaid with 100 ml/plate of ISP2 medium (7 g/L of agar) inoculated with *U. ramanniana* NRRL 1829. After 48 h of incubation, the clear areas observed on the TLC plate served as visual evidence of the inhibition of microorganism growth caused by the antimicrobial compounds. The retention factor value (Rf) for each active spot was recorded. The antimicrobial compounds were subsequently detected through the separate application of specific chemical revelators, including ninhydrin, naphthoresorcinol-sulfuric acid, ferrous iron chloride, and formaldehyde-sulfuric acid [29].

Results and discussion

Nocardiopsis sp. B20 was characterized by its morphology, chemotaxonomy, and physiological properties. The strain was found to produce fragmented substrate mycelium into coccoid and rod-shaped elements, with a branched growth pattern. During sporulation, the aerial hyphae initially exhibited a zig-zag shape, which will be later fragmented into branched and straight to flexuous spore chains comprising rod-shaped spores at maturity (Figure 1). The chemotaxonomic analyses revealed the presence of *meso*-diaminopimelic acid in the peptidoglycan, phosphatidylcholine as the diagnostic phospholipid in the cell membrane, and ribose and glucose (no diagnostic sugars) in the whole-cells. The morphological and chemotaxonomic properties of this strain confirm its identification as a member of the genus *Nocardiopsis* [30].

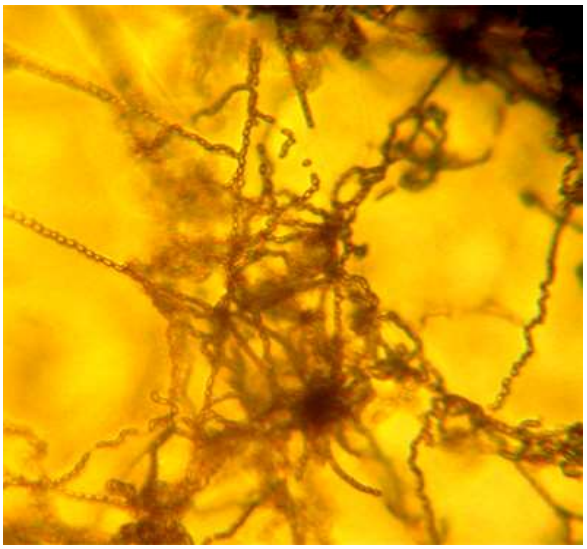


Figure 1. Morphological characteristics of *Nocardiopsis* sp. B20 on ISP2 medium after 2 weeks of incubation at 30 °C. Bar: 5 µm

Table 1. Differential phenotypic properties of *Nocardiopsis* sp. B20 compared with closely related type strains belonging to the genus *Nocardiopsis*

Characteristics	*Nocardiopsis* sp. B20	Type strains			
		1	2	3	4
pH range (optimum)	6–12 (11)	7–9 (7)	6–9 (7)	7–10 (7)	7-9(7)
NaCl concentration range (%, w/v)	0–10	0–12	0–13	0–13	0–12
Milk coagulation	+	–	+	w	w
Carbon source utilization					
D-Glucose	+	+	–	–	+
D-Lactose	–	–	+	+	–
Esculin	+	–	+	–	–
L-Arabinose	+	–	–	+	–
D-Fructose	+	+	+	–	+
α-D-Glucose and D-Galactose	+	+	+	–	–
Glycerol	+	+	–	+	+
Myo-inositol	–	–	–	+	–
D-Maltose	+	+	+	–	+
D-Mannose	+	+	+	+	–
D-Melibiose	–	–	–	–	+
Mannitol	+	Nd	+	+	v
D-Raffinose	–	–	–	–	+
L-Rhamnose	+	+	+	+	–
D-Salicin	+	–	+	+	+
D-Sorbitol	+	+	+	–	+
Sucrose	+	–	+	+	–
D-Trehalose	+	–	–	–	+
Xylose	+	Nd	–	–	v
Degradation of					
Starch	–	+	+	+	–
Cellulose	+	Nd	–	–	v
Gelatin	+	Nd	+	+	+
Na-citrate	+	–	+	+	+
Tween 80	+	Nd	Nd	Nd	Nd
Na-succinate and Na-acetate	+	Nd	Nd	Nd	Nd
Adenine, guanine, tyrosine, xanthine and hypoxanthine	+	Nd	Nd	Nd	Nd
Testosterone, Na-benzoate, Na-butyrate, Na-oxalate, Na-propionate, Na-pyruvate and Na-tartrate	–	Nd	Nd	Nd	Nd

1, Nocardiopsis deserti KCTC 49249^T [54]; 2, N. dassonvillei DSM 43111^T [55]; 3, N. alborubida NBRC 13392^T [56]; 4, N. synnematoformans DSM 44143^T [57]. +, positive; –, negative; V, variable; w, weak positive.

Nocardiopsis sp. B20 grew at pH 6.0-12.0 with the highest growth rate observed at pH 11.0 and 30°C. Its alkaliphilic capacity is comparable to other extremophilic *Nocardiopsis* such as *N. ganjiahuensis* and *N. alkaliphile* which grow optimally at pH 9 and 10, respectively [31,32]. It grew under various NaCl concentrations (up to 10 %), similar to halo-tolerant strains like *N. salina* [33]. The strain utilized sixteen carbon sources (out of twenty tested), highlighting a remarkable versatility like several *Nocardiopsis* strains isolated from Algerian Saharan Soils [34]. However, it only degraded three organic acids (out of nine tested). Furthermore, it was able to degrade cellulose, gelatin, milk casein, adenine, guanine, tyrosine, xanthine, and hypoxanthine, but not starch or testosterone (Table 1).

The ability of *Nocardiopsis* sp. B20 to grow under extreme pH and NaCl conditions and utilization of a variety of carbon sources highlight its potential for application as a promising candidate for biotechnological and agricultural applications [5,34]. Moreover, the ability to exhibit metabolic flexibility is a crucial trait for soil microorganisms, including species of *Nocardiopsis*. This flexibility facilitates their adaptation to the feast-famine dynamics that can characterize nutrition cycles in desert soils and other alkaliphilic niches [35,36]. Such metabolic versatility is important in nutrient cycling, organic matter decomposition, plant-microbe interactions, and contributes to the overall health and functioning of soil ecosystems [37].

The 16S rRNA gene sequence has been deposited in the GenBank database under accession number KJ470133, and was compared to the corresponding sequences of type strains available on the EzBioCloud server (https://eztaxon-e.ezbiocloud.net). The taxonomic assignment of *Nocardiopsis* sp. B20 was confirmed through a phylogenetic analysis based on the nearly complete 16S rRNA gene sequence. The analysis showed that *Nocardiopsis* sp. B20 exhibited the highest degree of similarity (99.5%) to *N. dassonvillei* subsp. *dassonvillei* DSM43111^T and *N. dassonvillei* subsp. *crassaminis* CECT 30033^T, followed by *N. deserti* KCTC 49249^T (99.3%), *N. alborubida* NBRC 13392^T, and *N. synnemataformans* DSM 44143^T (99.2%). These relationships are supported by the maximum likelihood phylogenetic tree (Figure 2).

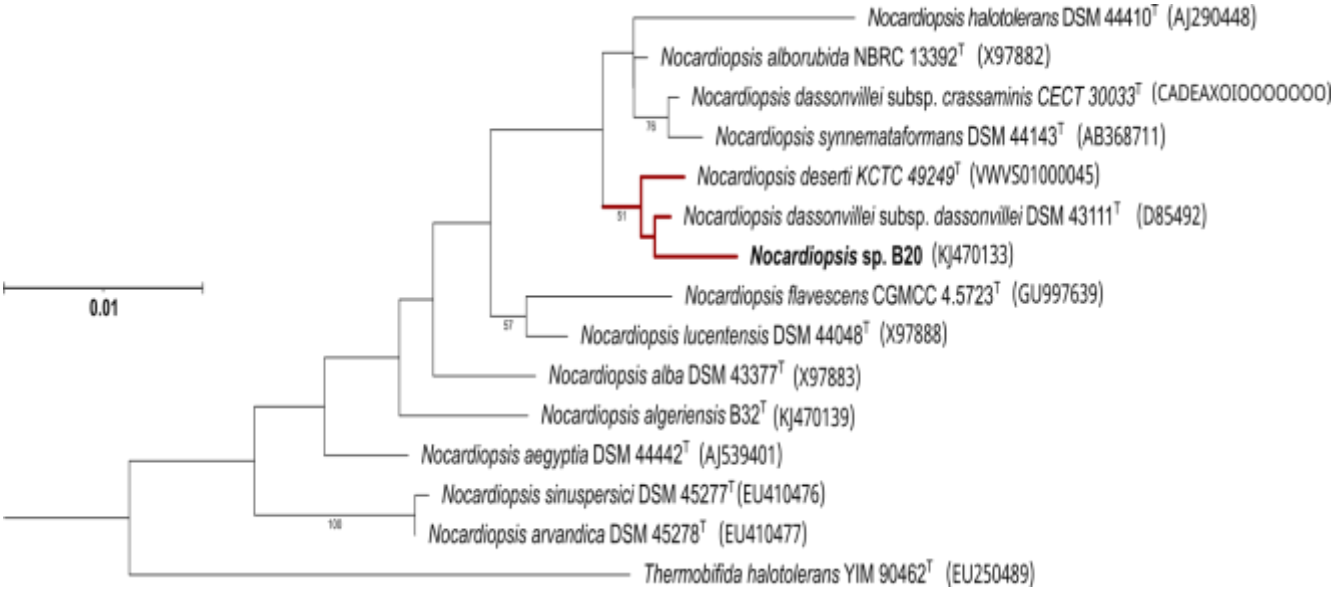


Figure 2. Phylogenetic tree inferred for the near-complete 16S rRNA gene sequences using Maximum Likelihood [24], with Tamura and Nei model [22]. This illustrates the evolutionary relationship between *Nocardiopsis* sp. B20 and its taxonomic position among the most closely related *Nocardiopsis* type strains. Numbers at the nodes are bootstrap values, expressed as a percentage of 100 resamplings (only values > 50 % are shown). *Thermobifida halotolerans* YIM 90462^T was used as an outgroup. Bar: 0.01 nucleotide substitution per site

Despite its close phylogenetic relationship with these strains, *Nocardiopsis* sp. B20 exhibited differences in its extremophilic tolerances and metabolic activities compared to the most related type strains, as can be seen from the differential physiological characters given in Table 1. Key distinguishing features are its broad alkaliphilic range, cellulolytic ability, and expanded purine base degradation. Furthermore, its taxonomic position at the species level can only be

determined after DNA-DNA hybridization, as very high 16S rRNA gene sequence similarities have been found between several type strains of validly published *Nocardiopsis* species [38].

The evaluation of the antimicrobial activity of *Nocardiopsis* sp. B20 against various microorganisms, including bacteria, fungi, and yeast, exhibited potent antifungal activity against all the tested fungi, with *Umbelopsis ramanniana* NRRL 1829 being the most susceptible (29 mm as distance of inhibition) (Table 2).

Table 2. Antimicrobial activity of *Nocardiopsis* sp. B20 by the streak assay method on ISP2 agar

Target microorganisms	Inhibition distance (mm)
Bacillus subtilis ATCC 6633	13 ± 0.58
Micrococcus luteus ATCC 9314	0
Staphylococcus aureus CIP 7625	0
Klebsiella pneumoniae CIP 8291	0
Saccharomyces cerevisiae ATCC 4226	15 ± 0.58
Umbelopsis ramanniana NRRL 1829	29 ± 1
Penicillium expansum B831P	21 ± 0.58
P. glabrum	16 ± 0.58
Aspergillus carbonarius M333	23 ± 1

The other tested fungi, *Aspergillus carbonarius* M333, *Penicillium expansum* B831P, and *P. glabrum*, also showed significant sensitivity to the antifungal compounds of *Nocardiopsis* sp. B20, with distances of inhibition of 23 mm, 21 mm, and 16 mm, respectively. The genus *Nocardiopsis* is known for its ability to produce a wide variety of secondary metabolites, which account for its diverse biological activities. These secondary metabolites have shown promise mainly as cytotoxic compounds, anticancer, antimicrobial agents, tumor promoters, kinase, and P-glycoprotein inhibitors, immunoregulators, plant growth promoters, and natural products with other multiple bioactivities [4,34,39–41]. *Nocardiopsis* species have also been identified as potential sources of diverse and novel extracellular enzymes [42]. Furthermore, recent studies have reported correlations between extremophilic traits like alkaliphily and halo-tolerance with increased production of bioactive compounds in *Actinobacteria*, suggesting that adaptation to extreme conditions requires synthesis of compatible solutes, antioxidants, and other stress response molecules with potential bioactivities [43,44]. It is noteworthy that the compatible solutes play a crucial role as stabilizers for biomolecules or stress-protective agents in extremophilic *Actinobacteria*, including *Nocardiopsis*, and can be used in various biotechnological applications, including industrial, pharmaceutical, agricultural, and environmental aspects [5,45,46].

Interestingly, the antimicrobial activity of *Nocardiopsis* sp. B20 was selective, as it did not show any significant activity against the tested bacteria (except against *B. subtilis* ATCC 6633). This selectivity of *Nocardiopsis* sp. B20 towards fungi suggests that the secondary metabolites produced by this strain have a higher affinity for fungal targets [3]. This selectivity could be advantageous for developing targeted antifungal treatments that minimize the impact on beneficial bacterial populations [47].

The ethyl acetate extract showed higher antimicrobial activity against *U. ramanniana* NRRL 1829 (Table 3, Figure 3).

Table 3. Antifungal activity of four extracts from *Nocardiopsis* sp. B20 against *U. ramanniana* NRRL 1829 and *A. carbonarius* M333

Target microorganisms	Inhibition distance (mm)			
	***n*-Hexane**	**Dichloromethane**	**Ethyl acetate**	***n*-Butanol**
Umbelopsis ramanniana NRRL 1829	0	14 ± 0.76	17 ± 0.76	16 ± 0.50
Aspergillus carbonarius M333	0	12 ± 0.58	12 ± 0.29	11 ± 0.58

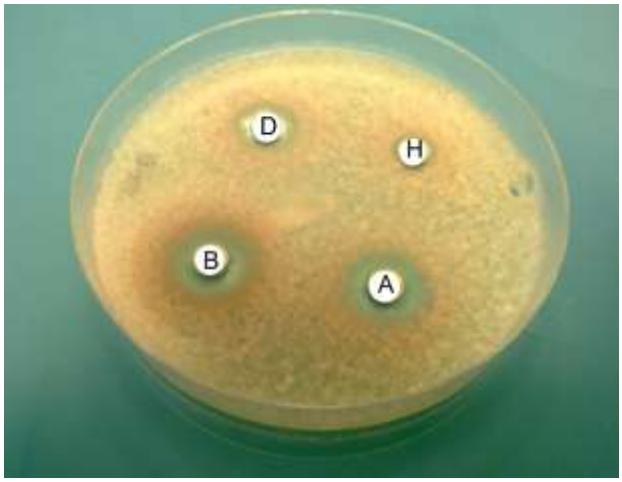


Figure 3. Antimicrobial activities of *n*-butanol (B), ethyl acetate (A), dichloromethane (D) and *n*-hexane (H) extracts against *U. ramanniana* NRRL 1829

The TLC of the ethyl acetate extract revealed multiple spots, with only one spot (Rf = 0.6), identified as B20-1, exhibiting antifungal activity after bioautography. Spot B20-1 was characterized by a brown color under 254 nm and a purple color under 365 nm. The chemical revelators used in this study provide valuable insights regarding the functional groups present in the bioactive compounds produced by newly isolated *Nocardiopsis* sp. B20. The absence of a positive reaction with ninhydrin and ferrous iron chloride suggests the absence or low presence of amine groups and phenolic compounds (and hydroxamic acid), respectively. In contrast, the positive reactions observed with naphtoresorcinol-sulfuric acid and formaldehyde-sulfuric acid indicate the presence of sugars and polycyclic aromatic hydrocarbons, respectively. Polycyclic aromatic hydrocarbons (PAHs) are organic compounds composed of multiple fused aromatic rings [48]. Memic *et al.* unveiled the potential of PAHs as antifungal agents against ligninolytic fungi [49]. This discovery opens new possibilities for their use in various industries, including agriculture, forestry, wood preservation, and the paper industry, where ligninolytic fungi can cause significant damage.

PAHs derived from natural sources offer a potential alternative to synthetic fungicides. However, their suitability depends on a proper assessment of toxicity and risks. While some PAHs exhibit carcinogenic and mutagenic effects in humans, toxicity varies greatly among different PAH structures and is not an inherent property of all PAHs [50]. Mildly toxic PAHs with relatively rapid environmental biodegradation may provide selective antifungal activity with minimal health or ecological impacts [51,52]. Furthermore, the PAHs produced by *Nocardiopsis* sp. B20 could potentially be modified or used as a starting point for the development of new compounds with reduced toxicity and enhanced antifungal activity. Further research is needed to understand the mechanisms underlying their antifungal activity, evaluate their efficacy, assess potential ecological impacts, and address any limitations or risks associated with their use [53].

Conclusions

The results of current research revealed the taxonomic position of strain B20 as a member of the genus *Nocardiopsis*. The ability of *Nocardiopsis* sp. B20 to thrive under extreme pH conditions and a wide range of NaCl concentrations, coupled with diverse carbon source utilization, showcases its potential for diverse biotechnological applications. The strain displayed potent and selective antifungal activity against various fungi by producing bioactive compounds containing sugars and PAHs. The use of natural PAHs as antifungal agents represents a significant advancement in addressing fungal infections while minimizing environmental concerns and resistance risks associated with synthetic fungicides. While the potential health risks associated with PAHs should not be overlooked, the antifungal activity demonstrated by the PAHs produced by *Nocardiopsis* sp. B20 presents a promising opportunity for the development of new antifungal agents. Future investigations should focus on elucidating the genomic basis of alkaliphily/halo-tolerance, characterizing the chemical structure of antifungal compounds to identify key functional groups, screening extracts against agricultural fungal pathogens, evaluating ecological impacts of the

PAHs, optimizing their therapeutic potential, and assessing their biodegradability and delivery techniques to ensure their safe use.

Acknowledgments

This publication honors the legacy of the late Prof. Sabaou Nasserdine, a leading expert in bacterial taxonomy, natural products, and open communication. He was a dedicated scientist, teacher, and mentor who worked tirelessly to advance the field of applied microbiology and biotechnology, and to improve the lives of people around the world. He represents an inspiration to many, a role model in his profession, and an exemplary humanitarian.

References

1. **Hazarika, S. N., & Thakur, D.** (2020). Chapter 21—*Actinobacteria*. In N. Amaresan, M. Senthil Kumar, K. Annapurna, K. Kumar, & A. Sankaranarayanan (Eds.), *Beneficial Microbes in Agro-Ecology* (pp. 443–476). Academic Press. https://doi.org/10.1016/B978-0-12-823414-3.00021-6

2. **Narsing Rao, M. P., & Li, W.-J.** (2022). Diversity of *Actinobacteria* in Various Habitats. In L. Karthik (Ed.), *Actinobacteria* (pp. 37–58). Springer Nature Singapore. https://doi.org/10.1007/978-981-16-5835-8_2

3. **Ibrahim, A. H., Desoukey, S. Y., Fouad, M. A., Kamel, M. S., Gulder, T. A. M., & Abdelmohsen, U. R.** (2018). Natural Product Potential of the Genus *Nocardiopsis*. *Marine Drugs*, *16*(5), 147. https://doi.org/10.3390/md16050147

4. **Shi, T., Wang, Y.-F., Wang, H., & Wang, B.** (2022). Genus *Nocardiopsis*: A Prolific Producer of Natural Products. *Marine Drugs*, *20*(6), 374. https://doi.org/10.3390/md20060374

5. **Bennur, T., Kumar, A. R., Zinjarde, S., & Javdekar, V.** (2015). *Nocardiopsis* species: Incidence, ecological roles and adaptations. *Microbiological Research*, *174*, 33–47. https://doi.org/10.1016/j.micres.2015.03.010

6. **van Bergeijk, D. A., Terlouw, B. R., Medema, M. H., & van Wezel, G. P.** (2020). Ecology and genomics of *Actinobacteria*: New concepts for natural product discovery. *Nature Reviews Microbiology*, *18*(10), 546–558. https://doi.org/10.1038/s41579-020-0379-y

7. **Selim, M. S. M., Abdelhamid, S. A., & Mohamed, S. S.** (2021). Secondary metabolites and biodiversity of actinomycetes. *Journal of Genetic Engineering and Biotechnology*, *19*(1), 72. https://doi.org/10.1186/s43141-021-00156-9

8. **Xie, F., & Pathom-aree, W.** (2021). *Actinobacteria* From Desert: Diversity and Biotechnological Applications. *Frontiers in Microbiology*, *12*. https://www.frontiersin.org/articles/10.3389/fmicb.2021.765531

9. **Gacem, M. A., Ould-El-Hadj-Khelil, A., Abd-Elsalam, K. A., & Wink, J.** (2021). *Actinobacteria* in the Algerian Sahara: Diversity, adaptation mechanism and special unexploited biotopes for the isolation of novel rare taxa. *Biologia*, *76*(12), 3787–3799. https://doi.org/10.1007/s11756-021-00928-1

10. **Chun, J., Bae, K. S., Moon, E. Y., Jung, S. O., Lee, H. K., & Kim, S. J.** (2000). *Nocardiopsis kunsanensis* sp. Nov., a moderately halophilic actinomycete isolated from a saltern. *International Journal of Systematic and Evolutionary Microbiology*, *50*(5), 1909–1913. https://doi.org/10.1099/00207713-50-5-1909

11. **Shirling, E. B., & Gottlieb, D.** (1966). Methods for characterization of *Streptomyces* species. *International Journal of Systematic Bacteriology*, *16*(3), 313–340. https://doi.org/10.1099/00207713-16-3-313

12. **Becker, B., Lechevalier, M. P., & Lechevalier, H. A.** (1965). Chemical Composition of Cell-Wall Preparations from Strains of Various Form-Genera of Aerobic Actinomycetes. *Applied Microbiology*, *13*(2), 236–243. https://doi.org/10.1128/am.13.2.236-243.1965

13. **Lechevalier, M. P., & Lechevalier, H.** (1970). Chemical composition as a criterion in the classification of aerobic actinomycetes. *International Journal of Systematic Bacteriology*, *20*(4), 435–443. https://doi.org/10.1099/00207713-20-4-435

14. **Minnikin, D. E., Patel, P. V., Alshamaony, L., & Goodfellow, M.** (1977). Polar Lipid Composition in the Classification of *Nocardia* and Related *Bacteria*. *International Journal of Systematic Bacteriology*, *27*(2), 104–117. https://doi.org/10.1099/00207713-27-2-104

15. **Gordon, R. E., Barnett, D. A., Handerhan, J. E., & Pang, C. H.-N.** (1974). *Nocardia coeliaca*, *Nocardia autotrophica*, and the Nocardin Strain. *International Journal of Systematic Bacteriology*, *24*(1), 54–63. https://doi.org/10.1099/00207713-24-1-54

16. **Goodfellow, M.** (1971). Numerical Taxonomy of Some Nocardioform *Bacteria*. *Journal of General Microbiology*, *69*(1), 33–80. https://doi.org/10.1099/00221287-69-1-33

17. **Marchal, N., Bourdon, J.-L., & Richard, C.** (1982). *Les milieux de culture pour l'isolement et l'identification biochimique des bactéries*. Doin Paris.

18. **Liu, D., Coloe, S., Baird, R., & Pedersen, J.** (2000). Rapid mini-preparation of fungal DNA for PCR. *Journal of Clinical Microbiology*, *38*(1), 471–471.

19. **Rainey, F. A., Ward-Rainey, N., Kroppenstedt, R. M., & Stackebrandt, E.** (1996). The genus *Nocardiopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage: Proposal of Nocardiopsaceae fam. Nov. *International Journal of Systematic and Evolutionary Microbiology*, *46*(4), 1088–1092.

20. **Yoon, S.-H., Ha, S.-M., Kwon, S., Lim, J., Kim, Y., Seo, H., & Chun, J.** (2017). Introducing EzBioCloud: A taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *International Journal of Systematic and Evolutionary Microbiology*, *67*(5), 1613–1617. https://doi.org/10.1099/ijsem.0.001755

21. **Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K.** (2018). MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Molecular Biology and Evolution*, *35*(6), 1547–1549. https://doi.org/10.1093/molbev/msy096

22. **Thompson, J. D., Higgins, D. G., & Gibson, T. J.** (1994). CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, *22*(22), 4673–4680. https://doi.org/10.1093/nar/22.22.4673
23. **Felsenstein, J.** (1981). Evolutionary trees from DNA sequences: A maximum likelihood approach. *Journal of Molecular Evolution*, *17*(6), 368–376. https://doi.org/10.1007/BF01734359
24. **Tamura, K., & Nei, M.** (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, *10*(3), 512–526. https://doi.org/10.1093/oxfordjournals.molbev.a040023
25. **Tamura, K., Nei, M., & Kumar, S.** (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(30), 11030–11035. https://doi.org/10.1073/pnas.0404206101
26. **Felsenstein, J.** (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution; International Journal of Organic Evolution*, *39*(4), 783–791. https://doi.org/10.1111/j.1558-5646.1985.tb00420.x
27. **BAUER, A. W., PERRY, D. M., & KIRBY, W. M. M.** (1959). Single-Disk Antibiotic-Sensitivity Testing of Staphylococci: An Analysis of Technique and Results. *A.M.A. Archives of Internal Medicine*, *104*(2), 208–216. https://doi.org/10.1001/archinte.1959.00270080034004
28. **Betina, V.** (1973). Bioautography in paper and thin-layer chromatography and its scope in the antibiotic field. *Journal of Chromatography A*, *78*(1), 41–51.
29. **Boudjella, H., Bouti, K., Zitouni, A., Mathieu, F., Lebrihi, A., & Sabaou, N.** (2006). Taxonomy and chemical characterization of antibiotics of *Streptosporangium* Sg 10 isolated from a Saharan soil. *Microbiological Research*, *161*(4), 288–298. https://doi.org/10.1016/j.micres.2005.10.004
30. **Meyer, J.** (1976). *Nocardiopsis*, a new genus of the order *Actinomycetales*. *International Journal of Systematic and Evolutionary Microbiology*, *26*(4), 487–493.
31. **Zhang, X., Zhang, L.-P., Yang, R., Shi, N., Lu, Z., Chen, W. X., Jiang, C.-L., & Xu, L.-H.** (2008). *Nocardiopsis ganjiahuensis* sp. Nov., isolated from a soil from Ganjiahu, China. *INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY*, *58*(1), 195–199. https://doi.org/10.1099/ijs.0.65145-0
32. **Hozzein, W. N., Li, W.-J., Ali, M. I. A., Hammouda, O., Mousa, A. S., Xu, L.-H., & Jiang, C.-L.** (2004). *Nocardiopsis alkaliphila* sp. Nov., a novel alkaliphilic actinomycete isolated from desert soil in Egypt. *International Journal of Systematic and Evolutionary Microbiology*, *54*(1), 247–252. https://doi.org/10.1099/ijs.0.02832-0
33. **Li, W.-J., Park, D.-J., Tang, S.-K., Wang, D., Lee, J.-C., Xu, L.-H., Kim, C.-J., & Jiang, C.-L.** (2004). *Nocardiopsis salina* sp. Nov., a novel halophilic actinomycete isolated from saline soil in China. *International Journal of Systematic and Evolutionary Microbiology*, *54*(5), 1805–1809. https://doi.org/10.1099/ijs.0.63127-0
34. **Boudjelal, F., Zitouni, A., Bouras, N., Spröer, C., Klenk, H.-P., Smaoui, S., & Mathieu, F.** (2023). Rare Halophilic *Nocardiopsis* from Algerian Saharan Soils as Tools for Biotechnological Processes in Pharmaceutical Industry. *BioMed Research International*, *2023*, 1–13. https://doi.org/10.1155/2023/1061176
35. **Jiao, S., Chen, W., Wang, J., Du, N., Li, Q., & Wei, G.** (2018). Soil microbiomes with distinct assemblies through vertical soil profiles drive the cycling of multiple nutrients in reforested ecosystems. *Microbiome*, *6*(1), 146. https://doi.org/10.1186/s40168-018-0526-0
36. **Ortiz, M., Leung, P. M., Shelley, G., Jirapanjawat, T., Nauer, P. A., Van Goethem, M. W., Bay, S. K., Islam, Z. F., Jordaan, K., Vikram, S., Chown, S. L., Hogg, I. D., Makhalanyane, T. P., Grinter, R., Cowan, D. A., & Greening, C.** (2021). Multiple energy sources and metabolic strategies sustain microbial diversity in Antarctic desert soils. *Proceedings of the National Academy of Sciences*, *118*(45), e2025322118. https://doi.org/10.1073/pnas.2025322118
37. **Yurgel, S. N., Nearing, J. T., Douglas, G. M., & Langille, M. G. I.** (2019). Metagenomic Functional Shifts to Plant Induced Environmental Changes. *Frontiers in Microbiology*, *10*, 1682. https://doi.org/10.3389/fmicb.2019.01682
38. **Li, W.-J., Kroppenstedt, R. M., Wang, D., Tang, S.-K., Lee, J.-C., Park, D.-J., Kim, C.-J., Xu, L.-H., & Jiang, C.-L.** (2006). Five novel species of the genus *Nocardiopsis* isolated from hypersaline soils and emended description of *Nocardiopsis* salina Li et al. 2004. *International Journal of Systematic and Evolutionary Microbiology*, *56*(5), 1089–1096. https://doi.org/10.1099/ijs.0.64033-0
39. **Allali, K., Goudjal, Y., Zamoum, M., Bouznada, K., Sabaou, N., & Zitouni, A.** (2019). *Nocardiopsis dassonvillei* strain MB22 from the Algerian Sahara promotes wheat seedlings growth and potentially controls the common root rot pathogen Bipolaris sorokiniana. *Journal of Plant Pathology*, *101*(4), 1115–1125. https://doi.org/10.1007/s42161-019-00347-x
40. **Muthukrishnan, P., Chithra Devi, D., Mostafa, A. A., Alsamhary, K. I., Abdel-Raouf, N., & Nageh Sholkamy, E.** (2020). Antimicrobial efficacy of *Nocardiopsis* sp. MK_MSt033 against selected multidrug resistant clinical microbial pathogens. *Journal of Infection and Public Health*, *13*(10), 1522–1532. https://doi.org/10.1016/j.jiph.2020.06.025
41. **Sabu, R., Soumya, K. R., & Radhakrishnan, E. K.** (2017). Endophytic *Nocardiopsis* sp. From Zingiber officinale with both antiphytopathogenic mechanisms and antibiofilm activity against clinical isolates. *3 Biotech*, *7*(2), 115. https://doi.org/10.1007/s13205-017-0735-4
42. **Chen, D., Mo, P., & Li, B.** (2022). Complete Genome Sequence of *Nocardiopsis exhalans* Strain JCM 11759T, Isolated from Indoor Air of a Water-Damaged Private House in Finland. *Microbiology Resource Announcements*, *11*(12), e00930-22. https://doi.org/10.1128/mra.00930-22
43. **Hui, M. L.-Y., Tan, L. T.-H., Letchumanan, V., He, Y.-W., Fang, C.-M., Chan, K.-G., Law, J. W.-F., & Lee, L.-H.** (2021). The Extremophilic *Actinobacteria*: From

Microbes to Medicine. *Antibiotics*, *10*(6), 682. https://doi.org/10.3390/antibiotics10060682
44. A**min, D. H., Abdallah, N. A., Abolmaaty, A., Tolba, S., & Wellington, E. M. H.** (2020). Microbiological and molecular insights on rare *Actinobacteria* harboring bioactive prospective. *Bulletin of the National Research Centre*, *44*(1), 5. https://doi.org/10.1186/s42269-019-0266-8
45. **Menasria, T., Monteoliva-Sánchez, M., Benhadj, M., Benammar, L., Boukoucha, M., & Aguilera, M.** (2022). Unraveling the enzymatic and antibacterial potential of rare halophilic actinomycetes from Algerian hypersaline wetland ecosystems. *Journal of Basic Microbiology*, *62*(10), 1202–1215. https://doi.org/10.1002/jobm.202200085
46. **Souagui, S., Boudries, H., Djoudi, W., Djinni, I., Laincer, F., Keramane, B., & Kecha, M.** (2023). Characterization of a Haloalkaline *Nocardiopsis* sp. Strain S10 Isolatedfrom Wastewater and Optimization of Culture Medium for ImprovingProduction of Antifungal Compounds. *Anti-Infective Agents*, *21*(3), e240223214038. https://doi.org/10.2174/2211352521666230224150318
47. **Lo, W.-H., Deng, F.-S., Chang, C.-J., & Lin, C.-H.** (2020). Synergistic Antifungal Activity of Chitosan with Fluconazole against Candida albicans, Candida tropicalis, and Fluconazole-Resistant Strains. *Molecules*, *25*(21), 5114. https://doi.org/10.3390/molecules25215114
48. **Gachanja, A. N.** (2019). Polycyclic Aromatic Hydrocarbons | Environmental Applications☆. In P. Worsfold, C. Poole, A. Townshend, & M. Miró (Eds.), *Encyclopedia of Analytical Science (Third Edition)* (pp. 341–349). Academic Press. https://doi.org/10.1016/B978-0-12-409547-2.14108-3
49. **Memic, M., Selovic, A., & Sulejmanovic, J.** (2011). Antifungal activity of polycyclic aromatic hydrocarbons against Ligninolytic fungi. *Hemijska Industrija*, *65*(5), 575–581. https://doi.org/10.2298/HEMIND110408039M
50. **Abdel-Shafy, H. I., & Mansour, M. S. M.** (2016). A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. *Egyptian Journal of Petroleum*, *25*(1), 107–123. https://doi.org/10.1016/j.ejpe.2015.03.011
51. **Haritash, A. K., & Kaushik, C. P.** (2009). Biodegradation aspects of Polycyclic Aromatic Hydrocarbons (PAHs): A review. *Journal of Hazardous Materials*, *169*(1–3), 1–15. https://doi.org/10.1016/j.jhazmat.2009.03.137
52. **Sivaram, A. K., Logeshwaran, P., Lockington, R., Naidu, R., & Megharaj, M.** (2019). Phytoremediation efficacy assessment of polycyclic aromatic hydrocarbons contaminated soils using garden pea (Pisum sativum) and earthworms (Eisenia fetida). *Chemosphere*, *229*, 227–235. https://doi.org/10.1016/j.chemosphere.2019.05.005
53. **Barbosa, F., Rocha, B. A., Souza, M. C. O., Bocato, M. Z., Azevedo, L. F., Adeyemi, J. A., Santana, A., & Campiglia, A. D.** (2023). Polycyclic aromatic hydrocarbons (PAHs): Updated aspects of their determination, kinetics in the human body, and toxicity. *Journal of Toxicology and Environmental Health. Part B, Critical Reviews*, *26*(1), 28–65. https://doi.org/10.1080/10937404.2022.2164390
54. **Asem, M. D., Salam, N., Idris, H., Zhang, X.-T., Bull, A. T., Li, W.-J., & Goodfellow, M.** (2020). *Nocardiopsis deserti* sp. Nov., isolated from a high altitude Atacama Desert soil. *International Journal of Systematic and Evolutionary Microbiology*, *70*(5), 3210–3218. https://doi.org/10.1099/ijsem.0.004158
55. **Miyashita, K., Mikami, Y., & Arai, T.** (1984). Alkalophilic Actinomycete, *Nocardiopsis dassonvillei* subsp. *prasina* subsp. Nov., Isolated from Soil. *International Journal of Systematic Bacteriology*, *34*(4), 405–409. https://doi.org/10.1099/00207713-34-4-405
56. **Grund, E., & Kroppenstedt, R. M.** (1990). Chemotaxonomy and Numerical Taxonomy of the Genus *Nocardiopsis* Meyer 1976. *International Journal of Systematic Bacteriology*, *40*(1), 5–11. https://doi.org/10.1099/00207713-40-1-5
57. **Yassin, A. F., Rainey, F. A., Burghardt, J., Gierth, D., Ungerechts, J., Lux, I., Seifert, P., Bal, C., & Schaal, K. P.** (1997). Description of *Nocardiopsis* synnemataformans sp. Nov., Elevation of *Nocardiopsis alba* subsp. *prasina* to *Nocardiopsis prasina* comb. Nov., and Designation of *Nocardiopsis antarctica* and *Nocardiopsis alborubida* as Later Subjective Synonyms of *Nocardiopsis dassonvillei*. *International Journal of Systematic Bacteriology*, *47*(4), 983–988. https://doi.org/10.1099/00207713-47-4-983