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1 **An overview on *Nocardioopsis* species originating from North African**
2 **biotopes as a promising source of bioactive compounds & *in silico* genome**
3 **mining analysis of three sequenced genomes**

4
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6
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16
17 **Abbreviations**

18 **BGC**, gene cluster; **HR- ESI- MS**, high- resolution electrospray ionization mass
19 spectrometry; **HRMS**, high resolution mass spectrometry; **ISP2**, yeast- malt extract agar;
20 **MIC**, minimum inhibitory concentrations; **MRSA**, methicillin- resistant *Staphylococcus*
21 *aureus*; **NMR**, nuclear magnetic resonance; **NRPS**, nonribosomal peptide synthetases; **PKS**,
22 polyketide synthetases.

28 Coverage: from the first study in 2004 to December 2023

29 **ABSTRACT**

30 Actinobacteria are renowned for their prolific production of diverse bioactive secondary
31 metabolites. In recent years, there has been an increasing focus on exploring “rare” genera
32 within this phylum for biodiscovery purposes, notably the *Nocardiopsis* genus, which will be
33 the subject of the present study. Recognizing the absence of articles describing the research
34 process of finding bioactive molecules from the genus *Nocardiopsis* in North African
35 environments. We, therefore, present a historical overview of the discoveries of bioactive
36 molecules of the genus *Nocardiopsis* originating from the region, highlighting their biological
37 activities and associated reported molecules, providing a snapshot of the current state of the
38 field, and offering insights into future opportunities and challenges for drug discovery.
39 Additionally, we present a genome mining analysis of three genomes deposited in public
40 databases that have been reported to be bioactive. A total of 36 biosynthetic gene clusters
41 (BGCs) were identified, including those known to encode bioactive molecules. Notably, a
42 substantial portion of the BGCs showed little to no similarity to those previously described,
43 suggesting the possibility that the analyzed strains could be potential producers of new
44 compounds. Further research on these genomes is essential to fully uncovering their
45 biotechnological potential. Moving forward, we discuss the experimental designs adopted in
46 the reported studies and new avenues to guide the exploration of the *Nocardiopsis* genus in
47 North Africa.

48 **Keywords:** Actinobacteria, bioactive potential, genome mining analysis, *Nocardiopsis* sp,
49 North African biotopes.

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57 **1 Introduction**

58 Actinobacteria (or more recently Actinomycetota [1], owing to their remarkable diversity and
59 capacity to produce new bioactive compounds, are considered one of the most valuable
60 sources of natural products with many industrial and medicinal applications. Their ability to
61 generate a wide range of unique compounds has positioned them as a focal point in
62 bioprospecting and drug discovery endeavors [2–4]. Members of this bacterial group have
63 indeed proven to be a particularly rich source of secondary metabolites exhibiting various
64 biological activities [5–8], notably as antibiotics [9, 10]. Additionally, genomic insights have
65 revealed that actinobacterial strains can produce a multitude of compounds that go well
66 beyond what has already been described [11]. Several studies indicate that their genetic
67 potential for the production of secondary metabolites is estimated to be about 10 times higher
68 than what has been identified through screening and chemical analysis [12, 13]. Additionally,
69 it is estimated that each strain of Actinomycetota has the genetic potential to produce
70 approximately 15–25 secondary metabolites [7, 14]. Recently, research has been oriented
71 toward other genera belonging to the so- called “rare” Actinobacteria [15–17], and several
72 ongoing discoveries have been reported [18, 19]. Among these genera, *Nocardiopsis* has been
73 particularly highlighted as a promising source of bioactive compounds [20]. This genus is a
74 potent producer of a variety of bioactive compounds, including antibacterial agents [21],
75 antioxidants and enzyme inhibitors [22], antibiofilm compounds [23], biosurfactants [24], and
76 anticancer agents [25]. The genus *Nocardiopsis*, initially described by Meyer in 1976 [26],
77 comprises aerobic, Gram positive, nonacid- fast, and catalase- positive actinomycetes. Their
78 colonies resemble those of the *Nocardia* and *Actinomadura* genera [27]. They also exhibit
79 variations and novelties in their teichoic acid composition [28]. Significantly, a considerable
80 number of *Nocardiopsis* species have been isolated from saline or alkaline environments, with
81 approximately two- thirds of them being halophilic or halotolerant [29–32].

82 Despite the above- mentioned interest, there are very few reviews on the genus *Nocardiopsis*.
83 Bennur and colleagues took the initiative in 2015 to discuss the ecology and distribution of
84 this genus [33]. In 2016, the same group published another review paper highlighting the
85 chemical diversity of the genus [34]. Three years later, Ibrahim et al. reported on the potential
86 of *Nocardiopsis* to produce natural products [20], covering the period from January 2015 to
87 February 2018. Subsequently, Shi et al. provided a summary of the sources, distribution,
88 bioactivities, biosynthesis, and structural characteristics of compounds isolated from
89 *Nocardiopsis*, covering the period from March 2018 to 2021 [35]. However, these reviews did

90 not encompass all reports of bioactive compounds isolated from *Nocardioopsis* strains
91 originating from North Africa, nor did they provide analyses of the methodology employed.
92 Hence, we risk overlooking the chemical diversity within the *Nocardioopsis* genus, originating
93 from North Africa. By filling this gap, we could focus on unexplored strains and potentially
94 discover new bioactive molecules, on the one hand. On the other, we could pave the way for
95 significant advances in the field of natural product chemistry in North Africa.

96 In this context, we aim to provide an updated summary of the geographical distribution and
97 biological activities of *Nocardioopsis* strains isolated from North African ecosystems, along
98 with their isolated molecules. It is important to note that the reported publications were
99 limited to those carried out in Algeria and Egypt, so each country will be presented separately.
100 The compiled data on the compounds identified from the study area are listed in Table 1,
101 including their sampling sources, producer strains, and evaluated biological activities. We
102 have analyzed the genomes of three *Nocardioopsis* strains for the presence of biosynthetic gene
103 clusters (BGCs) of secondary metabolites using AntiSMASH. Finally, we provide an
104 overview of the position of the reported studies and the progress made in the field of drug
105 discovery.

106 **2 Literature Survey**

107 **2.1 *Nocardioopsis* Strains Retrieved From Algerian Biotopes**

108 Algeria is the largest country in Africa, boasting a diverse climate that could serve as a
109 valuable source of biodiversity and natural products. However, most of these resources
110 remain underexplored. We have come across a limited number of studies that specifically
111 focus on isolating strains belonging to the *Nocardioopsis* genus from Algerian environments.
112 We compile and present all available studies on this genus, providing details about the
113 sampling sites, isolated compounds, and their biological activities. Several of these strains
114 were found to produce interesting bioactive compounds, including known and novel
115 compounds.

116 **2.1.1 | Isolated Strains and Their Biological Potential**

117 The first report on the *Nocardioopsis* genus in Algeria was conducted by Zitouni et al. [42] In
118 their study, they collected samples from Saharan soils to isolate rare actinomycetes. By
119 examining morphological and chemotaxonomic properties, they were able to distinguish and
120 identify a total of 86 isolates. Among these, 54 isolates were classified as belonging to the

121 *Nocardiopsis* genus, while 32 were categorized as members of the *Saccharothrix* genus. The
122 isolates were screened by the streak assay against a broad spectrum of pathogenic
123 microorganisms, including *Micrococcus luteus* ATCC 9314, *Escherichia coli* ATCC 10536;
124 *Pseudomonas fluorescens*, *Saccharomyces cerevisiae* ATCC 4226, and *Mucor ramannianus*
125 NRLL 1829. Findings showed that several isolates exhibited interesting antagonistic potential
126 against the targeted strains. The strain SA 103, among them, was consequently chosen for the
127 identification of its antifungal antibiotic activities [42]. A later study conducted by Meklat et
128 al. isolated 52 halophilic actinomycetes from Saharan soils and investigated their diversity
129 using a polyphasic approach, including phenotypical identification and phylogenetic
130 characterization [43]. The isolates were found to belong to several genera: *Actinopolyspora*,
131 *Nocardiopsis*, *Saccharomonospora*, and *Streptomonospora*. These strains were screened for
132 their antimicrobial activities by the cylinder plate method on yeast- malt extract agar (ISP2)
133 medium supplemented with 15% NaCl. Additionally, they were examined for the presence of
134 genes encoding polyketide synthetases (PKS) and nonribosomal peptide synthetases (NRPS).
135 Remarkably, approximately 50% of the bacterial collection exhibited activity against at least
136 one of the tested pathogenic microorganisms, and notably, among active strains, 16 belonged
137 to the *Nocardiopsis* genus. Additionally, out of the 26 strains that were tested for the presence
138 of PKS type I and NRPS coding genes, 23 strains (88.5%) were found to possess at least one
139 of these genes, indicating the potential for these strains to produce bioactive compounds.
140 However, despite their promising genetic characteristics, the chemical profile of these strains
141 has not yet been explored.

142 In another study conducted by Aouar et al. [44], 72 actinobacteria strains were isolated from
143 various rhizosphere semi- arid soils in Algeria. These strains were screened for their
144 antagonistic activity against several plant pathogenic fungi, including *Verticillium dahliae*,
145 *Fusarium culmorum*, *Drechslera teres*, *Microdochium nivale*, *Bipolaris sorokiniana*, *Botrytis*
146 *fabae*, and *Fusarium oxysporum*. Results showed that all isolates exhibit chitinolytic activity.
147 However, only six isolates (Lac1, Lac3, Vic8, Pin10, Pru1, and Pru16) were able to inhibit the
148 growth of five or more pathogenic fungi. Among these active isolates, Vic8 and Pin10 were
149 identified as belonging to the *Nocardiopsis* genus, while the remaining active isolates were
150 classified as members of the *Streptomyces* genus. Additionally, the research team also
151 evaluated the immunostimulatory effect of crude extracts obtained from *Nocardiopsis*
152 *dassonvillei* (Vic8) using a carbon clearance test. The crude extract prepared on ISP2 medium

153 demonstrated a significant increase in phagocytic activity, but the compounds responsible for
154 the stimulatory effect have not been identified [45].

155 Furthermore, the first officially named *Nocardiopsis* species from Algeria, *Nocardiopsis*
156 *algeriensis* B32T, was isolated from a soil sample collected in the Adrar province in its
157 southern region. The isolation and characterization of this strain were carried out using a
158 polyphasic taxonomic approach [31]. The *N. algeriensis* B32T was identified as an
159 alkali- tolerant actinomycete. Although the bioactivity of *N. algeriensis* B32T has not been
160 reported thus far, its genome sequence has recently become available at:
161 <https://www.ncbi.nlm.nih.gov/datasets/taxonomy/1478215/>.

162 A study that focused on the isolation of marine actinomycetes from polluted environments
163 along the Coast of Bejaia resulted in the isolation of 103 isolates. The isolates were then
164 screened for their antimicrobial activities using agar and well diffusion methods. Only 10% of
165 them were found to be active against one of the tested Gram- positive bacteria, including
166 methicillin resistant *Staphylococcus aureus* ATCC 43300, *S. aureus* ATCC 25923, and
167 *Bacillus subtilis* ATCC 6633. The 10 active isolates underwent molecular identification of the
168 16S rRNA gene, revealing that two isolates belong to the *Nocardiopsis* genus and the rest to
169 the *Streptomyces* genus [46]. More recently, a total of 114 actinobacterial isolates were
170 obtained from sediments and seawater samples collected from the Algerian Coast. These
171 isolates were subjected to 16S rRNA gene sequencing, which led to the identification of
172 several genera. Among them, three strains belonged to the *Nocardiopsis* genus. To assess
173 their potential, the isolated strains were screened for antibacterial activity against a set of
174 human pathogenic bacteria, including methicillin- resistant *S. aureus* ATCC 43300, *S. aureus*
175 ATCC 6538, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 14579, *Listeria*
176 *monocytogenes* ATCC 13932, *Micrococcus luteus* ATCC 9314, *Enterococcus faecalis* ATCC
177 10541, *Esherichia coli* ATCC 8739, *Klebsiella pneumoniae* ATCC 13883, *K. oxytoca* ATCC
178 13182, *Salmonella typhimurium* ATCC 14028, *Vibrio cholera* ATCC 14035, and
179 *Pseudomonas aeruginosa* ATCC 7320. The screening revealed that 36% of the screened
180 isolates were active against the tested Gram- positive bacteria [47].

181 In the study carried out by Laassami et al. [48], various genera of actinobacteria were isolated
182 from vineyard soils and identified based on 16 rRNA gene sequencing. These isolates were
183 then screened for their antifungal activity against grape vine trunk pathogens as well as plant
184 growth-promoting features (PGP). Among those, two *Nocardiopsis* isolates (Ms10 and Md45)

185 did not inhibit fungal growth, but they produced the PGPs indol- 3 acetic acid (IAA) and
186 siderophores [48].

187 Moreover, the diversity and antimicrobial activity of Actinobacteria isolated from Saharan
188 environments. Specifically, the collection was isolated by Messaoudi et al. from the
189 rhizosphere of date palms and saline environments [49]. Molecular identification based on
190 16S rRNA gene sequence was performed on 40 strains, revealing their affiliation to six
191 clusters corresponding to eight genera. The identified genera included 23 strains of
192 *Streptomyces*, 10 strains of *Nocardiopsis*, 2 *Micromonospora*, and 1 strain each of
193 (*Saccharopolyspora*, *Actinomadura*, *Actinocorallia*, *Couchioplanes*, and *Planomonospora*).
194 Additionally, four strains were classified as new Actinobacteria taxa. Out of the 40 tested
195 strains, 16 isolates displayed moderate to strong antimicrobial activities against fungi and
196 bacteria. Notably, three strains belonging to the *Nocardiopsis* genus, namely CG3, T1, and
197 B015, exhibited antimicrobial activity. The CG3 isolate displayed a broad spectrum of
198 antimicrobial activity, effectively inhibiting the growth of all tested microorganisms except
199 for *K. pneumoniae*. The extract of *Nocardiopsis* sp. CG3 demonstrates antimicrobial and
200 antifungal activity against various target pathogens, with minimum inhibitory concentrations
201 (MIC) ranging from 0.52 to 66.66 µg/mL [49].

202 Other recent studies have been conducted on the genus *Nocardiopsis* from Algerian biotopes,
203 mainly from the Sahara. Menasria et al. collected rare actinomycetes from the hypersaline
204 soils of Algerian inland wetland ecosystems, known as “Sebkhas- Chotts,” situated in arid
205 and hot hyperarid regions. Based on 16S rRNA gene sequencing, they identified 31 isolates
206 belonging to rare halophilic actinobacterial genera, specifically *Actinopolyspora* and
207 *Nocardiopsis*. *Actinopolyspora* accounted for 74.19% of the isolates (23 out of 31), while
208 *Nocardiopsis* represented 25.8% (8 out of 31) of the isolates. This bacterial collection was
209 screened for its enzymatic and antibacterial activities. Results showed that the majority of
210 isolates displayed significant both enzymatic and antibacterial activities, primarily against
211 Gram- positive pathogens: *B. cereus*, *B. subtilis*, *M. luteus*, and *S. aureus* [50].

212 Furthermore, a total of 23 halophilic actinobacteria were isolated from soil samples collected
213 from the Algerian Sahara. Identification by polyphasic approach allowed their assignment to
214 the genus *Nocardiopsis*. Additionally, the isolated strains were examined for their antagonistic
215 properties against pathogenic microorganisms using the agar cylinder method. Except for one
216 isolate (AH37), all *Nocardiopsis* isolates exhibited moderate to high biological activities
217 against *Pseudomonas syringae* and *Salmonella enterica*, with some isolates demonstrating

218 activity against *Agrobacterium tumefaciens*, *Serratia marcescens*, and *K. pneumoniae*.
219 However, none of the isolates displayed activity against *B. subtilis*, *Aspergillus flavus*, or *A.*
220 *niger* [51]. More recently, a haloalkaliphilic actinobacterium designated S10 was isolated
221 from a wastewater treatment plant in the Souk- El Tenine region (Bejaia, Algeria). It
222 exhibited a broad spectrum of activity against bacteria, fungi, and yeast. *Nocardiosis* sp. S10
223 was identified based on morphological and chemical analyses and sequencing of the 16S
224 rRNA gene. Additionally, the optimization of antifungal production was performed using the
225 response surface methodology [52]. Furthermore, a *Nocardiosis* strain labeled B20 was
226 isolated from the Mزاب region in the southern Algerian province of Ghardaia. It exhibits
227 remarkable tolerance to extreme pH and NaCl conditions. Phylogenetic analysis confirmed
228 the assignment of the isolated strain to the *Nocardiosis* genus. Analysis of the ethyl acetate
229 extract revealed the presence of a bioactive compound named B20- 1, which possesses
230 antifungal properties. Further chemical analysis detected the presence of sugars and
231 polycyclic aromatic hydrocarbons (PAHs) within compound B20- 1 [53]. Moreover, another
232 *Nocardiosis* strain, named GSBS4, exhibiting broad spectrum antimicrobial activity, was
233 collected from Saharan soil in the province of El- Oued in Algeria and identified as
234 *Nocardiosis dassonvillei* based on a polyphasic approach. Its crude ethyl acetate extract
235 demonstrated potent antibacterial activity against pathogenic bacteria and *Candida albicans*.
236 It inhibited the biofilm formation of both *S. aureus* and methicillin resistant *S. aureus*, with
237 estimated MICs of 0.144 and 1.15 mg/mL, respectively. A 44% reduction in the biofilm was
238 observed for *S. aureus* and a 61% reduction for *P. aeruginosa*. It has also provided significant
239 antioxidant activity, suggesting that it is a promising candidate for further exploration [54].

240 All the reported studies suggest that the Algerian desert could be a potential source of
241 bioactive molecules.

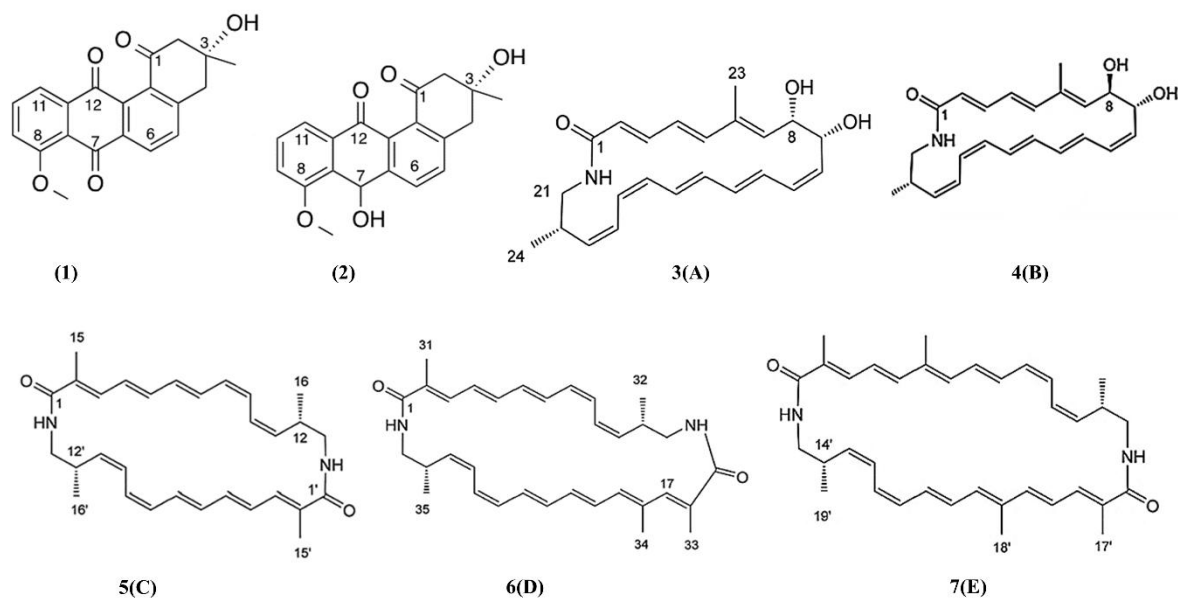
242 **2.1.2 Bioactive Strains With Characterized Molecules**

243 Extensive research has been carried out on isolating compounds from other *Nocardiosis*
244 strains originating from Algerian biotopes. In the study led by Hadj Rabia- Boukhalfa [36],
245 the *Nocardiosis* sp. HR- 4 strain was investigated. This strain was isolated from the
246 salt- lake soil in the Saharan desert region of Algeria. Through various analyses, including
247 16S rRNA gene sequencing, morphological observations, and physiological characteristics, it
248 was determined that this strain represents a novel species within the *Nocardiosis* genus. The
249 HR- 4 strain exhibited broad antimicrobial activity against various bacteria, filamentous
250 fungi, and yeasts. Metabolites obtained from HR- 4 were produced in ISP2 liquid medium

251 supplemented with 10% NaCl and incubated at 30°C for 8 days. From the culture supernatant,
252 two predominant compounds were identified, one of which was identified as a novel
253 molecule. The chemical structure was confirmed through nuclear magnetic resonance (NMR)
254 analysis, revealing that the isolated compounds belong to the angucyclinone group.
255 **Compound 1** was identified as 8 O- methyltetrangomycin [C₂₀H₁₆O₅], and **Compound 2**
256 [C₂₀H₁₈O₅] was identified as a novel natural molecule named
257 (–)-7- deoxy- 8- O- methyltetrangomycin. Both compounds 1 and 2 displayed good
258 antibacterial activity against *S. aureus* ATCC 25923, *S. aureus* ATCC 43300, *E. faecalis*
259 ATCC 29212, and *M. luteus* ATCC 4698. However, no activity was noted against
260 Gram- negative bacteria or yeast tested [36].

261 In a different study, five new polyene macrolactams, macrolactams A–E (designated
262 Compounds 3–7 in the present study), were isolated from *Nocardiosis* CG3 (DSM 106572)
263 previously described. This novel species of *Nocardiosis* was isolated from the saltpan of
264 Kenadsa in southern Algeria. The molecular formulas of the isolated compounds were
265 elucidated by high- resolution mass spectrometry (HRMS) and NMR. The following
266 molecular formulas were assigned: **Compound 3** (A) C₂₃H₂₉NO₃; **Compound 4** (B)-
267 C₂₃H₂₉NO₃ (an isomer of compound A, with UV, mass, and NMR spectra similar to
268 compound A); **Compound 5** (C)- C₃₀H₃₈N₂O₂; **Compound 6** (D)- C₃₃H₄₂N₂O₂; and
269 **Compound 7** (E)- C₃₆H₄₆N₂O₂. Macro lactams A, C, D, and E; were assessed for their
270 antimicrobial, cytotoxic, and antiviral activities. Macrolactams C, D, and E demonstrated
271 moderate activity against *S. aureus* Newman, with MIC values measured at 66.7, 16.7, and
272 66.7µg/mL, respectively. Among them, only compound C exhibited moderate antifungal
273 activity against *Mucor hiemalis* DSM2656 and *Candida albicans* DSM1665, with a MIC
274 value of 66.7µg/mL. Compound A did not exhibit any antimicrobial activity against the
275 targeted microorganisms. Additionally, macrolactams C, D, and E exhibited significant but
276 weak cytotoxicity against various human cancer cell lines. Compound E demonstrated the
277 highest potency, inhibiting HeLa cells, KB3.1, human prostate cancer PC- 3, SKOV- 3, and
278 human lung carcinoma A549 cell lines, with IC50 values ranging between 2.1 and 5.5 µM.
279 However, Compound A did not exhibit any activity against KB3.1 and L929 cell lines.
280 Compounds C, D, and E displayed weak antiviral activity against HCV, while compound A
281 showed no activity in this regard [37]. This suggests that *Nocardiosis* species could
282 potentially serve as a source of antiviral compounds. More recently, two fractions (F2 and F3)
283 from the culture of the CG3 strain in a soy medium, which exhibited pronounced antiviral

284 activity against the Luc- Jc1 strain of HCV, underwent chemical profiling using HPLC- ESI
 285 HRMS. This analysis revealed the strain's potential to produce other molecules in addition to
 286 the previously identified kenalactams. Three isoflavone metabolites were identified, along
 287 with mitomycin C and a p- terphenyl derivative [55]. The structures of all the isolated
 288 compounds mentioned above can be found in Figure 1.



289
 290 **Figure 1.** Structural formulas of **Compounds (1-7)** isolated from *Nocardiosis* strains
 291 obtained from Algerian ecosystems.

292
 293 **2.2 *Nocardiosis* Strains Collected From Egyptian Biotopes**

294 **2.2.1 Isolated Strains and Their Biological Potential**

295 In the past 20 years, several new *Nocardiosis* strains from Egyptian ecosystems have been
 296 obtained and described using a polyphasic approach. However, not all these strains have been
 297 extensively explored in terms of bioactivity or isolated compounds, even though certain
 298 strains have yielded interesting compounds with potential biological activities, such as
 299 antimicrobial and cytotoxic properties. Here, we shed light on all the studies carried out in this
 300 context.

301 A novel alkaliphilic actinomycete isolated by Hozzein et al. from desert soil in Egypt was
 302 described by polyphasic taxonomy and designated *Nocardiosis alkaliphila* YIM 80379 [56].
 303 Its complete genome was later sequenced [57]. In the same year, another novel strain of

304 *Nocardiosis*, *N. aegyptia*, designated SNG49T, was isolated from the marine sediment of
305 Abu Qir Bay, located on the Western seashore of Alexandria in Egypt [58]. The strain was
306 also subjected to an optimization study using the Plackett–Burman method for the production
307 of amylase [59]. *Nocardiosis arabia* sp. nov., a halotolerant actinomycete, was originally
308 isolated from sand- dune soil in Egypt [60]. However, it was later renamed *Strenomonospora*
309 *arabica* [61]. *N. dassonvillei* WA52, isolated from Wadi Araba in Egypt, may be considered a
310 new subspecies of *N. dassonvillei* and has been found to produce a macrolide antibiotic
311 compound, WA52- A. This compound displayed antimicrobial activity against
312 Gram- positive bacteria, including *B. subtilis* NCTC10400, *B. pumilus* NCTC 8214, *S. aureus*
313 NCTC 7447, and *M. luteus* ATCC 9341, with MIC values of 1.56, 3.12, 6.25, and 6.25
314 µg/mL, respectively. However, no activity against Gram negative bacteria was observed,
315 except for *K. pneumoniae* NCIB 9111, which showed a MIC of 50µg/mL. Additionally, it did
316 not exhibit activity against yeast or fungi. This study also revealed, for the first time, that *N.*
317 *dassonvillei* is a new producer of one of the erythromycins [62].

318 Different actinomycetes species were isolated from the Red Sea Coast in Sharmel- Sheikh,
319 Egypt. Especially *Nocardiosis* sp. EGY22 displayed antimicrobial activity against various
320 microorganisms, including *Streptococcus pyogenes*, *Staphylococcus haemolyticus*, *Salmonella*
321 *typhi*, *P. aeruginosa*, and *C. tropicalis*. The crude extract of this strain also showed significant
322 cytotoxic activity against the MDA- MB- 231 breast cancer cell line [63]. More recently, a
323 study explored seawater and coral microbial communities in the Red Sea in Egypt, including
324 the *Nocardiosis* genera, for their anti- quorum sensing and anti- biofilm activities. Among
325 the tested strains, 67% showed anti- quorum sensing activity against *Aeromonas hydrophila*,
326 *P. aeruginosa*, and *Vibrio alginolyticus*. The 16S rRNA gene sequencing of the active isolates
327 identified one of the strains as *N. dassonvillei*, suggesting its potential as a source of bioactive
328 substances involved in countering quorum sensing [64]. Finally, three strains were identified
329 by 16S rDNA sequencing as members of the genera *Micromonospora*, *Streptomyces*, and
330 *Nocardiosis*, isolated from the Red Sea soft coral *Sarcophyton glaucum*. The chemical
331 diversity and biological activities of these selected strains were investigated using various
332 approaches, including the OSMAC (one strain many compounds) method, complemented by
333 LC- MS- based metabolomics and molecular modeling techniques. The evaluation of
334 antitrypanosomal activity was against *Trypanosoma brucei*. The results showed that
335 *Micromonospora* sp. UR 17 and *Nocardia* sp. UR27 exhibited interesting inhibitory activity

336 against *T. brucei*. However, *Nocardiosis* sp. UR17 did not show significant
337 antitrypanosomal activity [65].

338 2.2.2 Bioactive Strains With Characterized Molecules

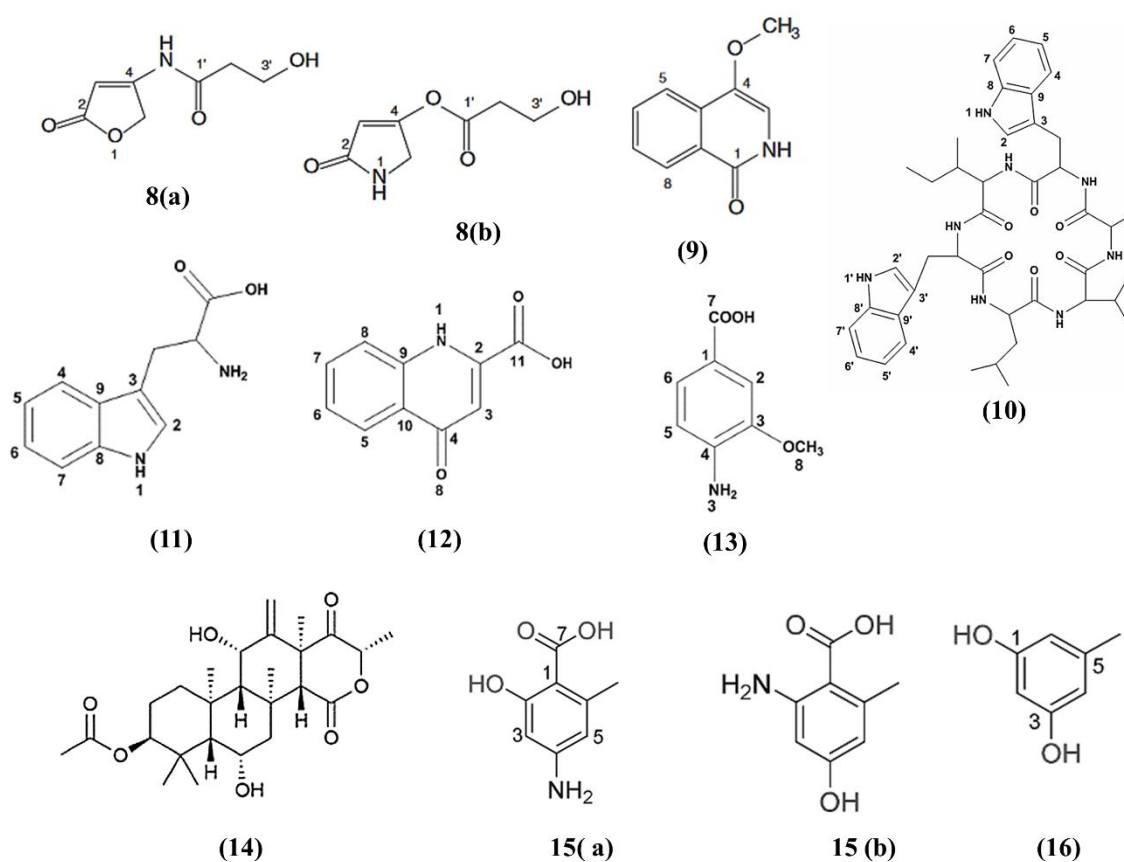
339 Chemical investigation of other *Nocardiosis* strains from Egyptian biotopes led to the
340 isolation of several promising compounds. In a study conducted by Eliwa et al., a new marine
341 actinomycete named *Nocardiosis lucentensis* sp. ASMR2 was isolated from marine plants
342 collected from the Red Sea, Hurghada, Egypt, at a depth of 30m [41]. This actinomycete was
343 found to produce numerous compounds, including two novel compounds identified as
344 3'- hydroxy- N- (2- oxo- 2,5 dihydrofuran- 4- yl) propenamide (**Compound 8a**) and the
345 naturally new 4- methoxy- 2H- isoquinolin- 1- one (**Compound 9**). Their chemical
346 structures were achieved by 1D and 2D NMR in combination with EI- HR (Electron
347 Ionization- High Resolution) mass spectrometry. Additionally, eight known compounds were
348 also described: indole- 3- carboxylic acid, indole- 3- acetic acid, indole- 3- acetic acid
349 methyl ester, furan- 2,5 dimethanol, tyrosol, glycerol linoleate, cyclo- (Tyr, Pro), and
350 adenosine. The antimicrobial activities of the crude extract and those of newly purified
351 compounds were evaluated using the agar diffusion technique. The methanol extract obtained
352 from the strain grown on rice medium demonstrated potent activity against *C. albicans*,
353 exhibiting a zone of inhibition measuring 14 mm. It also displayed moderate activity ranging
354 from 9 to 11 mm against *S. cerevisiae*, as well as the Gram- positive bacteria *B. subtilis* and
355 *S. aureus*. However, Compounds 8 and 9 did not show any antimicrobial activity against the
356 microorganisms tested, nor did they exhibit cytotoxic activity against the human cervix
357 carcinoma cell line (KB- 3- 1) and its multidrug- resistant subclone (KBV1) [38].

358 A further study on the strain *Nocardiosis* sp. UR67 isolated from a marine sponge called
359 *Callyspongia* sp. collected from the Red Sea revealed a new cyclic hexapeptide, nocardiotide
360 A, labeled **Compound 10**, along with three known compounds: tryptophan (**Compound 11**),
361 kynurenic acid (**Compound 12**), and 4- amino- 3- methoxy benzoic acid (**Compound 13**).
362 These compounds were identified using NMR spectroscopy and high resolution electrospray
363 ionization mass spectrometry (HR- ESI MS). Furthermore, to determine the absolute
364 stereochemistry of all the amino acid components of nocardiotide- A, Marfey's method was
365 employed. The molecular formula of the new hexapeptide was identified as C₄₂H₅₆N₈O₆
366 through HRMS analysis. The purified compounds were evaluated for their cytotoxic activities
367 against human cell lines. In particular, Nocardiotide- A has provided significant cytotoxic
368 properties with IC₅₀ values of 8, 11, and 12μM/mL against human MM.1S multiple

369 myeloma, human HeLa cervix carcinoma, and murine CT26 colon carcinoma, respectively
370 [39]. In a different study, the strain *Nocardiosis* sp. UR67 underwent a metabolomics
371 analysis. The production of metabolites was carried out using two methods: in suspension and
372 immobilized in calcium alginate bead cultures. Ethyl acetate extracts obtained from the broth
373 were named EG- 49 and J- 48g, respectively. The chemical profiling of the raw extract
374 revealed the presence of 16 major chemical compounds, primarily polyketides, macrolides,
375 and peptides. Additionally, extracts EG- 49 and J- 48g demonstrated antifungal activity
376 against *C. albicans*, with IC50 values measured at 8.1 and 8.3mg/mL, respectively. They also
377 exhibited a significant cytotoxic effect against H1650 lung adenocarcinoma, with IC50 values
378 of 12.6 and 13.7mg/mL, respectively. However, it is worth noting that only EG- 49 displayed
379 notable activity against trypanosomes at a concentration of 7.5 mg/mL [66].

380 In the study of Hamed et al., a new highly oxidized and unique tetracyclic
381 6- hydroxymero-terpenoid, Terretonin N (**Compound 14**), was isolated from *Nocardiosis* sp.
382 LGO5, along with seven known compounds. This strain was obtained from sediment samples
383 collected from a lake in Helwan, Egypt. The chemical structures were elucidated by
384 spectroscopic analysis, including 1D NMR, 2D NMR, and ESI HR MS. The antimicrobial
385 properties of both the crude extract and the isolated compound were assessed using the agar
386 diffusion method against various microorganisms. The findings revealed significant activity
387 of the crude extract against *P. aeruginosa* and *S. cerevisiae*, with inhibition zones of 13 and
388 14mm, respectively. However, a lower level of activity was observed against *E. coli* DSMZ
389 1058, *B. subtilis* DSMZ 704, *Pseudomonas agarici* DSMZ 11810, *M. luteus* DSMZ 1605,
390 *Staphylococcus warneri* DSMZ 20036, *S. aureus*, and *C. albicans*. Regarding the
391 antimicrobial potential of compound 14, it demonstrated strong activity against *S. warneri*
392 with an inhibition zone of 15mm and showed lower activity (7 and 8mm) against
393 *Pseudomonas agaraci* and *E. coli*. Contrastingly, neither the bacterial extract nor the purified
394 compound, terretonin N, showed significant cytotoxicity against the human cervix carcinoma
395 cell line KB- 3- 1 [40]. Finally, 4- amino- 6- methylsalicylic acid (**Compound 15**), a
396 phenolic acid derivative, was described for the first time as a natural product by Eliwa et al. It
397 was isolated along with 5- methylresorcinol (**Compound 16**) and linoleic acid from
398 *Nocardiosis* sp. AS23C, isolated from the marine Alga *Sargassum arnaudianum* at the
399 Hurghada Coast in the Red Sea. The chemical structures of isolated compounds were
400 determined by 1D and 2D NMR and mass spectroscopy techniques. The antimicrobial activity
401 of the crude extract obtained from *Nocardiosis* sp. strain AS23C was evaluated against a set

402 of pathogenic microorganisms using the agar diffusion technique with paper platelets. It
 403 showed good antibacterial activity against *S. aureus* (17mm) and *B. subtilis* ATCC 6051
 404 (17mm), while exhibiting moderate activity against *Streptomyces viridochromogenes* Tü 57
 405 (12mm). The antimicrobial potential of the isolated compounds was not evaluated. The
 406 cytotoxic potential of compounds 15 and 16 was tested against the human cervical carcinoma
 407 cell line KB- 3- 1. No significant cytotoxic activity was observed for these compounds [41].
 408 Figure 2 illustrates all the compounds that were isolated from *Nocardiopsis* strains originating
 409 from various Egyptian ecosystems. In addition, Table 1 details all the compounds isolated
 410 from North African biotopes and their biological properties.



411
 412 **Figure 2.** Chemical structures of purified compounds obtained from *Nocardiopsis* strains
 413 isolated from Egyptian environments.

414

Table 1. Summary of isolated compounds derived from *Nocardiosis* strains collected from North African biotopes and their associated biological activities

Producer strain	Isolation site	Isolated compound	Biological activity of pure compounds	Reference
<i>Nocardiosis</i> sp. HR-4	Algerian salt-lake soil located in Sahara desert, Algeria	Compound 1: 8- <i>O</i> -methyltetrangomycin [C ₂₀ H ₁₆ O ₅]	Antibacterial activity against: <i>S. aureus</i> ATCC 25923, <i>S. aureus</i> (MRSA) ATCC 43300, <i>E. faecalis</i> ATCC 29212 and <i>M. luteus</i> ATCC 4698.	[36]
		Compound 2: (-)-7-deoxy-8- <i>O</i> -methyltetrangomycin: [C ₂₀ H ₁₈ O ₅]		
<i>Nocardiosis</i> CG3 (DSM 106572)	Saltpan of Kenadsa, in southern Algeria	Compounds of Kenalactams A–E (3–7);		[37]
		3(A): [C ₂₃ H ₂₉ NO ₃]	Inactive.	
		4(B): [C ₂₃ H ₂₉ NO ₃]	Not evaluated.	
		5(C): [C ₃₀ H ₃₈ N ₂ O ₂]	Moderate antimicrobial, antifungal activity, and weak cytotoxicity. Weak antiviral activities against HCV.	
		6(D): [C ₃₃ H ₄₂ N ₂ O ₂]	Moderate antibacterial activity and weak cytotoxicity. Weak antiviral activities against HCV.	
		7(E): [C ₃₆ H ₄₆ N ₂ O ₂]	Moderate antimicrobial activity. Weak antiviral activities against HCV. Cytotoxic against human prostate cancer PC-3 cells, IC ₅₀ value of 2.1 μM.	
<i>Nocardiosis</i> <i>lucentensis</i> sp. ASMR2	Marine plants collected in the Red Sea, Hurghada, Egypt	Compound 8a: 3'-hydroxy- <i>N</i> -(2-oxo-2,5-dihydrofuran-4-yl) propenamide.	No antimicrobial activity.	[38]
		Compound 9: 4-methoxy-2 <i>H</i> -isoquinolin- 1-one.	No cytotoxic effect.	

<i>Nocardiopsis</i> <i>sp. UR67</i>	marine sponge Callyspongia sp. from the Red Sea, Egypt	Compound 10: Cyclic hexapeptide: [C ₄₂ H ₅₆ N ₈ O ₆]. <hr/> Compound 11: tryptophan Compound 12: kynurenic acid Compound 13: 4-amino-3-methoxy benzoic acid	Cytotoxic effect with IC50: 8, 11 and 12 [39] μM/mL against the human MM. 1S multiple myeloma, human HeLa cervix carcinoma, and murine CT26 colon carcinoma, respectively. <hr/> No cytotoxic effect.
<i>Nocardiopsis</i> <i>sp. LGO5</i>	sediment sample collected from a lake in Helwan, Egypt	Compound 14: (Terretonin N) Tetracyclic 6-hydroxymero-terpenoid.	Active against <i>S. warneri</i> DSMZ 20036 with 15 mm of inhibition zone. Low activity (7–8 mm) against <i>P. agaraci</i> [40] DSMZ 11810 and <i>Escherichia coli</i> DSMZ 1058. No significant cytotoxic effect.
<i>Nocardiopsis</i> <i>sp. AS23C</i>	marine Alga <i>Sargassum</i> <i>arnaudianum</i> in the Red Sea at Hurghada coast, Egypt	Compound 15 a: 4-amino-6- methylsalicylic acid [C ₈ H ₉ NO ₃]. Compound 16: 5-methylresorcinol.	No noticeable cytotoxic activity the human [41] cervix carcinoma cell line (KB-3-1).

415

416

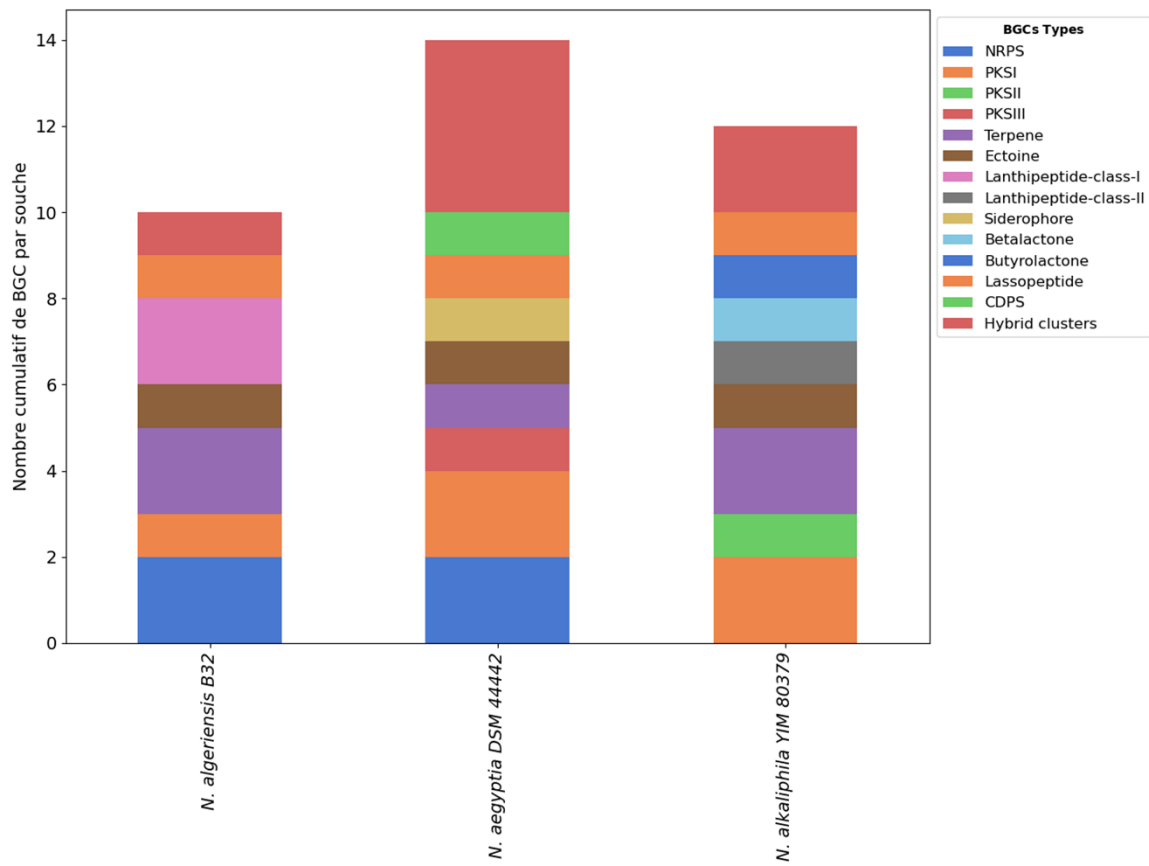
417 2.3 Research of Bioactive Actinomycetes in Other North African Countries

418 Except for those isolated from Algeria and Egypt, no novel isolated molecules from the genus
419 *Nocardiopsis* have been discovered in other North African countries. Nevertheless, interesting
420 studies have been conducted on actinobacteria, mainly *Streptomyces*. In Tunisia, research was
421 performed on actinobacteria and their bioactive potential. Notably, the *Streptomyces* TN256 strain
422 was obtained from Tunisian Saharan soil. This strain yielded four active compounds: (1:
423 N- [2- (1H- indol- 3- yl)–2 oxo- ethyl] acetamide, an “alkaloid” derivative; 2: di- (2- ethylhexyl)
424 phthalate, a phthalate derivative; 3:1- nonadecene, and 4:cyclo(L- Pro- L- Tyr), a diketopiperazine
425 derivative). The antimicrobial potential of these compounds was evaluated by disk diffusion assay
426 against a range of pathogenic bacteria and fungi, including *M. luteus* LB14110, *S. aureus* ATCC
427 6538, *S. enterica* ATCC 43972, *E. coli* ATCC 8739, and *Fusarium sp.* According to the findings, all
428 four of the identified compounds showed significant activity against the targeted microorganisms
429 [67]. Another notable strain, *Streptomyces cavourensis* TN638, was isolated from Tunisian soil
430 contaminated with industrial wastewater. This strain yielded a total of seven bioactive compounds
431 upon isolation: Cyclo- (Leu- Pro) (1), Cyclo- (Val- Pro) (2), Cyclo- (Phe- Pro) (3), nonactin (4),
432 monactin (5), dinactin (6), and trinactin (7). Both the crude extract and the purified compounds of *S.*
433 *cavourensis* TN638 displayed antimicrobial activity against various phytopathogenic
434 microorganisms. This suggests that this strain could potentially be used for the management of
435 diseases caused by plant pathogens [68]. In Moroccan ecosystems, diverse strains of actinobacteria
436 belonging to various genera, such as *Streptomyces*, *Saccharomonospora*, and *Actinomadura*, have
437 been extensively investigated. Their biological potential has been evaluated in various contexts,
438 including combating grapevine gray mold [69], acting as insecticides [70], functioning as
439 antimicrobials [71–73], and serving as plant growth promoters [74]. Another study focused on
440 Actinobacteria isolated from the marine waters of the Moroccan Atlantic Coast. This study unveiled
441 the strains' activity against multidrug- resistant bacteria by using the plate diffusion method,
442 primarily identifying active strains within the *Streptomyces* genus [75]. Additionally, screening for
443 the antimicrobial activity of 44 actinomycete isolates from three soil samples collected at three
444 extremely cold sites in Morocco was conducted. Primary and secondary screenings were performed
445 against *C. albicans* ATCC 60 193, *E. coli* ATCC 25 922, *S. aureus* ATCC 25 923, *B. cereus* ATCC
446 14 579, other multidrug- resistant clinical bacteria, and 13 phytopathogenic fungi. This study led to
447 the selection of 11 active isolates for further investigation. Moreover, numerous extracts exhibited
448 high antimicrobial and antioxidant activities [76].

449

450 **3 Genome Mining Analysis of *N. algeriensis* B32, *N. alkaliphila* YIM 80379, and *N. aegyptia*** 451 **DSM 44442**

452 Draft- genome sequences of three *Nocardiopsis* strains, previously mentioned in the literature survey
453 and isolated from the study area, are currently available in public databases. Analyses of gene
454 clusters involved in the biosynthesis of various secondary metabolites on these genomes, as well as
455 the prediction of the chemical structures of bioactive compounds that may be produced, were
456 performed using the web tool AntiSMASH version 7.1.0 with the option “strict” [77], which is
457 available at [https://antismash.
secondarymetabolites.org#!/start](https://antismash.secondarymetabolites.org#!/start). AntiSMASH employs a
458 probabilistic algorithm based on hidden Markov models (HMMs) to identify BGC regions within
459 genomes. It utilizes the PFAM protein families database in this process [78]. The genomes of *N.*
460 *algeriensis*, *N. alkaliphila* YIM 80379, and *N. aegyptia* are available on the NCBI database with the
461 following accession numbers: JACHJO000000000.1, NZ_ANBD000000000.1, and
462 JACCFS000000000.1, respectively. A total of 36 putative BGCs were predicted by AntiSMASH,
463 encompassing various classes of secondary metabolites, as illustrated in Figure 3. In this study, these
464 clusters are referred to as clusters 1–36. Of these, there are 10 BGCs for *N. algeriensis* B32T, 14 for
465 *N. alkaliphila* YIM 80379, and 12 for *N. aegyptia* DSM 44442. These clusters included multiple
466 groups containing NRPS (4 clusters), Type I polyketide synthases (PKS I, 5 clusters); Type II
467 polyketide synthases (PKS II, 1 cluster); Type III polyketide synthases (PKS III, 1 cluster). Also,
468 tRNA- dependent cyclodipeptide synthases (CDPS, 1 cluster), as well as clusters putatively coding
469 for lanthipeptides; Type I lanthipeptides (2 clusters), Type II lanthipeptides (1 cluster), Lasso peptides
470 (3 clusters), Betalactone (1 cluster), and Butyrolactone (1 cluster). Most of these clusters are
471 described as encoding bioactive compounds [79–82]. Additionally, one cluster associated with a
472 siderophore was predicted, three with ectoine, and five with terpene BGCs. Cluster 4, in particular,
473 was predicted to be responsible for the biosynthesis of geosmin, a compound contributing to the
474 earthy odor of soils [83]. In addition, seven hybrid metabolic clusters were identified. Interestingly,
475 seven clusters showed no similarity to known clusters (clusters 3, 10, 18, 20, 23, 28, and 30),
476 suggesting that they may be associated with novel natural products. All predicted BGCs and
477 characterized functions are summarized in Table 2. The genome mining analysis of the three
478 genomes has revealed the metabolic biodiversity of the genus *Nocardiopsis*, with many of them
479 described for their biological activities. Additionally, exploring orphan BGCs could offer the
480 potential for discovering novel bioactive compounds, thus opening up new avenues in the field of
481 drug discovery.



482

483 **Figure 3.** Distribution of secondary metabolite biosynthetic gene clusters in the genomes of the three
 484 *Nocardiosis* strains.

485

486 **Table 2.** Summary of anti-SMASH 7.1.0 secondary metabolite prediction of *Nocardioopsis* strains

Genome strain	Cluster no.	Contig. Number	Metabolite class	Most similar known metabolite (%)	Span [nt]	
					From	To
<i>N. algeriensis</i> B32	Cluster 1	Region 1.1	NRPS	friulimicin A / B / C / D (21%)	353,704	413,717
	Cluster 2	Region 2.1	Terpene	isorenieratene (100%)	59,970	85,480
	Cluster 3	Region 3.1	Lanthipeptide-class-I	unknown	3,176	27,079
	Cluster 4	Region 4.1	Terpene	geosmin (100%)	24,662	46,926
	Cluster 5	Region 4.2	Lasso peptide	albusnodin (50%)	68,322	90,414
	Cluster 6	Region 7.1	NRPS	anthramycin (64%)	77,575	121,978
	Cluster 7	Region 7.2	PkSI	<u>actinoalloyide A</u> (30%)	216,906	274,364
	Cluster 8	Region 15.1	Hybrid: NRPS, NRP-metallophore	fuscachelin A / B / C (100%)	1	47,145
	Cluster 9	Region 16.1	Ectoine	ectoine (75%)	17,067	27,465
	Cluster 10	Region 20.1	Lanthipeptide-class-I	unknown	1	17,662
<i>N. alkaliphila</i> YIM 80379	Cluster 11	Region 18.1	CDPS	albonoursin (33%).	1	9,824
	Cluster 12	Region 26.1	NRPS	Q6402A (5%)	1	27,172
	Cluster 13	Region 31.1	Hybrid; NRPS, PKS I	azinomycin B (46%)	989	71,548
	Cluster 14	Region 47.1	Ectoine	ectoine (75%)	9,766	20,167
	Cluster 15	Region 51.1	Hybrid; T2PKS, ectoine, thiopeptide	kosinostatin (66%)	60,713	115,713
	Cluster 16	Region 72.1	Terpene	isorenieratene (100%).	108,721	134,264
	Cluster 17	Region 103.1	Hybrid; Ectoine, Lasso peptide	ikarugamycin (8%)	24,943	47,281
	Cluster 18	Region 105.1	PKSIII	unknown	1	12,164

	Cluster 19	Region 115.1	PKSI	nigericin (36%)	54,981	87,274
	Cluster 20	Region 116.1	PKSI	unknown	1	18,467
	Cluster 21	Region 119.1	lassopeptide	dudomycin A (21%)	1	21,589
	Cluster 22	Region 141.1	NRPS	omnipeptin (9%)	1	17,636
	Cluster 23	Region 143.1	Hybrid; LAP, Thiopeptide	unknown	1	22,601
	Cluster 24	Region 152.1	NI-siderophore	desferrioxamine (100%)	E 10,978	40,786
<i>N. aegyptia</i> DSM 44442	Cluster 25	Region 1	Lasso peptide	albusnodin (50%)	955,982	978,242
	Cluster 26	Region 2	PKSI	carrimycin (22%)	1,785,952	1,843,754
	Cluster 27	Region 3	Ectoine	ectoine (75%)	1,444,751	2,455,167
	Cluster 28	Region 4	Betalactone	unknown	3,212,791	3,237,530
	Cluster 29	Region 5	Butyrolactone	asukamycin (3%)	3,826,183	3,837,163
	Cluster 30	Region 6	Lanthipeptide-classII	unknown	3,860,066	3,890,384
	Cluster 31	Region 7	PKSI	coelimycin P1 (25%)	4,026,656	4,092,404
	Cluster 32	Region 8	Hybrid; lanthipeptide-class-III, NRP metallophore, NRPS-	scabichelin (80%)	4,132,863	4,225,265
	Cluster 33	Region 9	PKSII	actinorhodin (45%)	4,444,393	4,516,860
	Cluster 34	Region 10	Terpene	isorenieratene (87%)	5,097,248	5,122,840
	Cluster 35	Region 11	Terpene	legonindolizidine (12%)	A6 5,490,019	5,511,383
Cluster 36	Region 12	Hybrid; NRPS, PKSI	kedarcidin (1%)	6,297,344	6,355,077	

487 **4 A Critical Overview of the Methodologies Followed in the Study Area and Possible Research** 488 **Directions**

489 The field of natural product- based drug discovery continues to reveal new and innovative
490 molecules, and several new approaches have recently been introduced in this area. Many of these
491 approaches are supported by in silico tools, including databases of genomic, chemical, or spectral
492 analysis data [84], the integration of various information- containing data sets such as multi- Omics
493 analysis tools [85], and machine learning applications [86]. Despite the significant divergence
494 between the predicted biosynthetic potential and the rate of discovery of new compounds, targeted
495 genetic engineering efforts guided by genome mining and metabolomics analyses promise innovative
496 breakthroughs in the discovery of natural products.

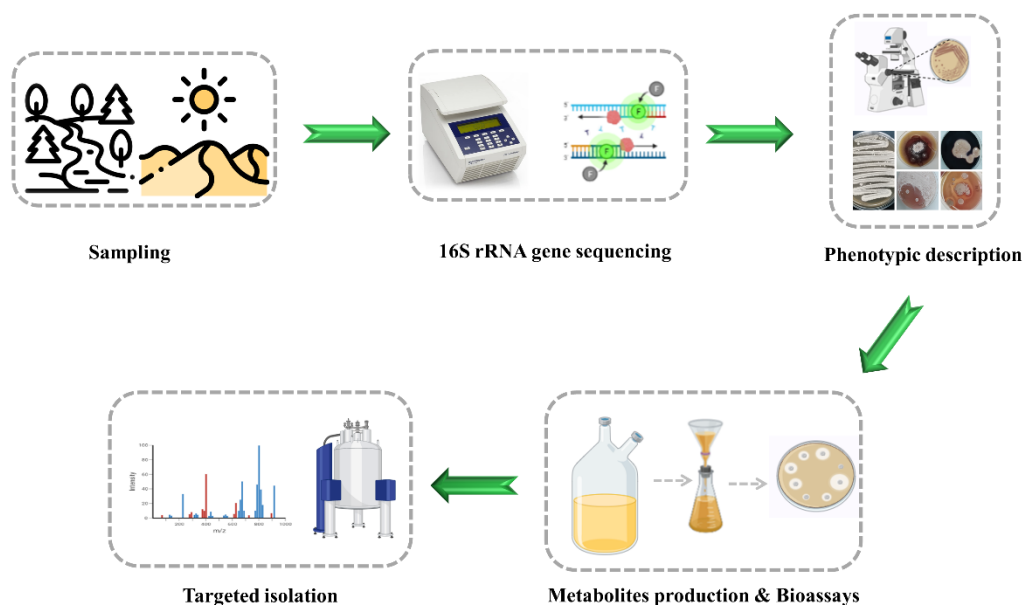
497 In recent years, Omics Sciences have witnessed the emergence of a new term, “metabologenomics,”
498 which involves the combination of genomic and metabolomic data [87]. Many new natural products
499 with therapeutic potential have been derived from this combination, and new insights have been
500 revealed to highlight genomic features that distinguish potent antibiotic producers from weaker ones.
501 As a result, the combination of multi- omics approaches can also provide a more comprehensive
502 picture of microbial communities and their metabolism [85, 88]. This integrated approach can also
503 serve as an alternative means to identify potentially valuable cryptic natural products from
504 microorganisms, provide a better understanding of regulation and specialized metabolism, and
505 improve the ability to prioritize and induce the expression of secondary metabolite BGCs [89]. In
506 essence, this combined strategy has accelerated the discovery of novel natural drugs and
507 cost- effectively, reducing the rate of rediscovery and paving the way for a new phase of discovery.
508 Two recent reviews that stand out in this context are those by [90, 91].

509 The bulk, if not all, of the studies reviewed here, adopted traditional design approaches, as illustrated
510 in Figure 4. These approaches involve several key stages, starting with the isolation of bacteria,
511 followed by polyphasic identification and screening by bioassay tests. Subsequently, the production
512 of metabolites takes place, and the compounds are characterized using NMR and HRMS methods,
513 among others. It is worth noting that some of the documented studies have led to the isolation of
514 known compounds. Furthermore, the prediction of the putative BGCs of the three sequenced
515 *Nocardiosis* strains has revealed the potential of this genus for the biosynthesis of secondary
516 metabolites. These genomes could be promising for further studies and provide valuable insights into
517 the field of drug discovery. This type of analysis has the potential to considerably expedite the
518 understanding of metabolite biosynthesis, both those that have already been identified and those that

519 are suspected to be new products of the genus *Nocardiopsis*. An in- depth investigation using omics
520 approaches can potentially reveal the extensive ability of *Nocardiopsis* to generate bioactive natural
521 products beyond what is typically identified by conventional methods. Additionally, a discernible
522 pattern emerges from the observation of bioactive isolates originating from highly diverse
523 environments in this region, spanning from deserts to marine settings (see Figure 5). Therefore,
524 adopting a broader strategy that involves the application of metagenomics on isolates from diverse
525 ecological niches could improve the prospects of discovering bioactive compounds and provide a
526 more detailed description of the chemical diversity within this bacterial genus.

527 Overall, the search for bioactive molecules in North Africa remains restricted by the limited range of
528 methodologies employed. Despite the biodiversity noted within the *Nocardiopsis* species, including
529 new species recorded, research efforts are largely limited to preliminary investigations, and there is
530 no in depth follow- up from the biosynthetic potential of the genome to the description of the
531 metabolome. To challenge this, we recommend a more thorough exploration of the potential within
532 previously identified strains by leveraging the in silico techniques mentioned above. These
533 approaches not only offer the opportunity to overcome the isolation of already- known molecules
534 but also to accelerate drug discovery pipelines. While it is recognized that certain state- of- the- art
535 methods may pose financial constraints for some laboratories in Africa, collaboration with
536 well- equipped laboratories and training personnel in these analyses could present a promising
537 opportunity for advancement. This collaboration could significantly increase the likelihood of
538 discovering new molecules and contribute to broader access to the world's biodiversity.

539

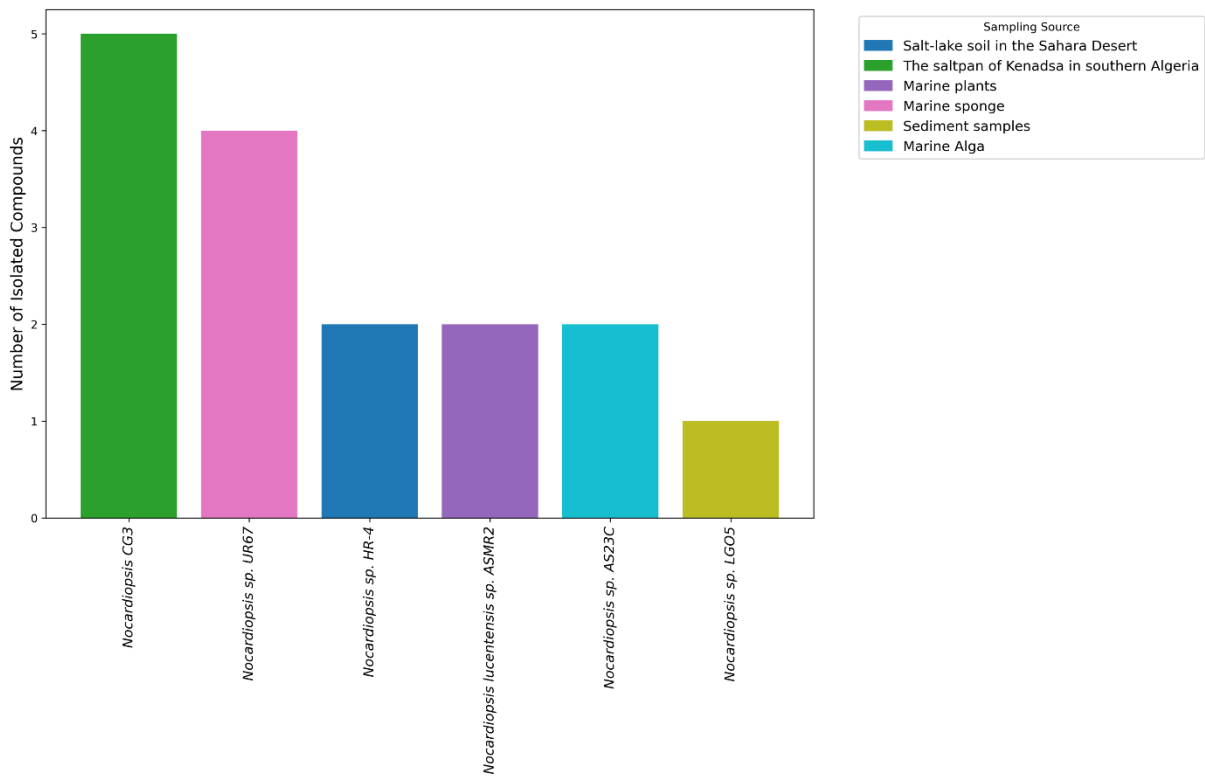


540
541 **Figure 4.** Summary of the experimental design followed in the Studies Under Review.

542

543 **5 Conclusion**

544 Actinobacteria, and the *Nocardiosis* genus in particular, remain highly promising for the discovery
 545 of bioactive compounds. While numerous authors have extensively discussed the remarkable
 546 potential of *Nocardiosis* in generating a diverse array of natural products, this genus has received
 547 less attention and remains underexplored in North Africa. Our literature survey revealed that the
 548 study area has yielded promising *Nocardiosis* strains with interesting biological potential, including
 549 the identification of new species within this genus. The published isolated compounds are limited to
 550 those from Algeria and Egypt. A total of 16 molecules, either known, new, or described for the first
 551 time as natural products, were isolated from *Nocardiosis* isolates collected from diverse
 552 environments (Figure 5). Some of these compounds showed interesting biological potential, along
 553 with several known compounds with a wide range of pharmacological activities (Table 1). The
 554 number of reported studies in the study area is relatively limited, and based on these studies, research
 555 on bioactive molecules from the genus *Nocardiosis* is still in its infancy. Genome mining analysis
 556 of *Nocardiosis* genomes reveals a high diversity of BGCs (Table 2), facilitating and stimulating the
 557 discovery of new drugs. In conclusion, the primary objective of this review was to provide insight
 558 into the process of discovering bioactive molecules from the *Nocardiosis* genus in North Africa.
 559 Additionally, this review aims to serve as a guide for researchers active in this field or those planning
 560 to embark on similar studies.



561

562 **Figure 5.** The number of compounds isolated from *Nocardioopsis* strains collected from North
 563 African biotopes according to producer strain and their sampling sources.

564 **Author Contributions**

565 **Rima Ouchene:** conceptualization, data curation, formal analysis, investigation, software,
566 validation, visualization, writing—original draft, writing—review and editing. **Nawel Zaatout:**
567 writing—review and editing. **Marcelino T. Suzuki:** conceptualization, supervision, validation,
568 writing—review and editing

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572 **Conflicts of Interest**

573 The authors declare no conflicts of interest.

574 **Data Availability Statement**

575 No generated experimental data in this paper and all discussed data here are from the
576 publications referred in this paper.

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