

WA52-A Macrolide Antibiotic Produced by Alkalophile *Nocardioopsis Dassonvillei* WA52

¹Mohamed I. Ali, Maged S. Ahmad and Wael N Hozzein

Botany Department, Faculty of Science, Beni Suef University, Beni Suef, ¹Cairo University,
Cairo.Egypt

Abstract: WA52-A antibiotic macrolide in nature having; anti Gram-positive and anti Gram-negative bacteria; was extracted from cell free culture of alkalophile strain which isolated from a sandy soil in Wadi Araba, Egypt. The strain has been identified as *Nocardioopsis dassonvillei* WA52 based on taxonomic studies. The active metabolite was extracted with methanol-ethyl acetate mixture (1:2) and concentrated in vacuole and the crude fraction was purified using column and thin layer chromatography. Elemental analysis give C, 61.25%; H, 9.3%; and N, 1.95%, and the empirical formula was calculated as C₃₇H₆₇NO₁₂. Ultra violet maximum absorption peak was recorded at 286 nm and a peak at 236 nm. The IR and NMR were consulted to confirm the chemical characteristics of the antibiotic. The biological activity and toxicity were investigated.

Key words: Macrolides antibiotic, Alkalophile, *Nocardioopsis*

INTRODUCTION

Many research groups have searched systematically for new bioactive compounds by chemical screening as Zahner and Maas (1972). The principle of chemical screening is, first, the isolation and detection of new metabolites by specific tests such as color reactions, using several reagents to detect functional groups present in molecules of known secondary metabolites. This step is followed by evaluation of the biological activities of the isolated metabolites. The main characteristics of this screening method are its convenience for simple detection and quick isolation of the new metabolite from microorganisms (Nakagawa, 1992).

Another trend is the use of extreme cultural conditions for discovering new bioactive compounds. Several successful examples have already been reported, such as, pyrrolnitrin and other antibiotics produced by using high phosphate containing media (Arima *et al.*, 1965); helvolic acid and other antibiotics produced by alkalophilic and alkaline-resistant organisms (Sato *et al.*, 1983); MAZ-10, a peptide antibiotic produced by a halotolerant organism (Ahmed, 1995); Glionitrin A, a new diketopiperazine disulfide antibiotic produced from coculture of a mine drainage-derived *Sphingomonas* bacterial strain, KMK-001, and a mine drainage-derived *Aspergillus fumigatus* fungal strain, KMC-901 (Park *et al.*, 2009), and many other antibiotics produced under thermophilic conditions.

The most popular trends in antibiotics research, is the genetic manipulation in *Streptomyces*, which has been established by Hopwood and his coworkers (Hopwood *et al.*, 1985). So far, treatment with mutagens and the screening of resulting clones has been repeated and adopted as a procedure for the breeding of antibiotic producing microorganisms and has produced good results. New hybrid antibiotics, named mederrhodins A and B were produced by genetically engineered strain from the transformants that incorporated recombinant plasmids (Omura *et al.*, 1986). Modern genetic engineering has opened door both to production of novel hybrid antibiotics and to greatly increased production of known types (Ikeda, 1992).

We assume that Wadi Araba, Egypt characterized by unique flora and fauna could also harbor new microorganisms with potentially useful biological activities. So, the aim of this work was to isolate and describe the, purification and identification of a new bioactive metabolite from selective alkaliphilic actinomycete isolate.

MATERIALS AND METHODS

Collection of Soil Samples:

Thirty soil samples from six different localities represented Wadi Araba, Egypt were collected. Each soil

Corresponding Author: Maged.S.ahmad, Beni-suef University,Egypt

sample was taken at depth of 5-20 cm with a collecting spatula. Most of the collected samples were obtained from the rhizosphere of the dominant plants. About 1kg of soil was collected from each site in clean sterilized bags and then stored in a refrigerator at 4°C. Samples were analyzed as quickly as possible for actinomycetes producing antimicrobial compounds.

Isolation of Alkalophilic and Alkaline-resistant Microbe:

Soil dilution technique was used for such a purpose. Many authors have described the basic procedure and a detailed description was given by Johnson *et al.* (1959). The isolation medium is medium "A", which is recommended by Sato *et al.* (1983) for isolation of microorganisms on high alkaline pH.

Testing the Antimicrobial Activities:

The isolates were inoculated onto Sato medium "A" and then incubated for 3 days on a rotary shaker of 200 rpm at 30°C. One ml suspension of each culture was transferred aseptically to the modified starch-nitrate medium (Tadashi, 1975) used for antibiotic production, then another incubation 3 days on a rotary shaker of 200 rpm at 30°C. Testing of the antimicrobial activities were carried out by using the classical diffusion method (Cooper, 1963 and 1972). In general, this method is based on the observation of inhibition zone of microbial growth on agarized media.

The following microbial cultures were used as test organisms for such a purpose: *Bacillus subtilis* NCTC 10400, *Bacillus pumilus* NCTC 8214, *Staphylococcus aureus* NCTC 7447, *Micrococcus luteus* ATCC 9341, *Escherichia coli* NCTC 10416, *Pseudomonas aeruginosa* ATCC 10145, *Klebsiella pneumonia* NCIB 9111, *Saccharomyces cerevisiae* CBS 1171, *Candida albicans* IMRU 3669, *Penicillium chrysogenum* ATCC 12960 and *Aspergillus flavus*. These test organisms were kindly provided by Botany and Microbiology Dept., Faculty of Science, Al-Azhar University, Cairo, Egypt.

Taxonomic Studies:

Micro-morphological studies were carried out with a phase contrast microscope on culture grown at 30°C for 10 days on yeast extract-malt extract agar, oatmeal agar, glycerol asparagine agar medium, inorganic salts-starch agar and Sato medium A. Spore surface examinations were observed with a transmission electron microscope.

Cultural and physiological characteristics were examined on media as described by Waksman (1961), International Streptomyces Project (ISP) (Shirling and Gottlieb, 1966), Williams *et al.* (1983) and Bergey's Manual (1989 and 1994). Whole cell sugars were identified by the method of Lechevalier and Lechevalier (1980) and diaminopimelic acid isomers were analyzed by the method of Backer *et al.* (1964). Chemical analysis of cell wall was performed according to the method of Becker *et al.* (1964), and Lechevalier and Lechevalier (1980).

Fermentation:

A loopful of the most potent alkalophile isolate was inoculated into a 250 ml conical flask containing 60 ml of sterile seed medium and incubated at 30°C for 4 days on rotary shaker (180 rpm). The seed medium composed of (g/100 ml); glucose, 2.5; NaNO₃, 0.2; K₂HPO₄, 0.12; MgSO₄·7H₂O, 0.05; KCl, 0.06 and FeSO₄·5H₂O, 0.0008, and the pH of the medium was adjusted at 9. The seed culture was transferred into other conical flasks containing 100 ml of a producing medium (the same composition of seed medium). The fermentation was carried out at 30°C for 3 days under agitation of 200 rpm.

Extraction and Purification of the Antibiotic:

The selection of suitable organic solvents using the bioautographic technique (Weinstein and Wagman, 1978) at different pH's indicate that the active metabolites was extracted with methanol – ethyl acetate mixture in the ratio of 1:2 respectively, pH 8.5. The methanol – ethyl acetate extract was evaporated in rotary evaporator under vacuum. Thin layer and column chromatography purified the crude extract. Silica gel 80 column was used and eluted with methyl alcohol and the elute was collected in 18 ml fractions. The individual fractions were examined by bioassay. The elute was collected and dried in vacuum and redissolved in 30 ml methanol – ethyl acetate mixture. Bioautography was used again to confirm the purity of the active metabolite for spectral analysis.

Acid and Alkaline Hydrolysis of the Active Compounds:

Samples (10 mg) were hydrolyzed with 1ml of 6N HCl at 100°C for 24 h. in sealed tube and dried with washing many times using distilled water on water bath and evaporated to release the HCl residue and

redissolved in 5 ml isopropanol (10%). The hydrolysate was spotted on Whatman No. 1 paper chromatographic sheet and on silica gel G plates with authentic amino acids and chromatographed descendingly with the developing solvent system consisting of methanol: water: 10N HCl: pyridine (80:17.5:2.5:10, v/v/v/v). Ninhydrin was used as spray reagent employed for the detection of amino acids.

Behaviour of the Active Compound Towards Different Color Reactions:

The behaviour of the active compound towards the following color reactions was conducted: Molish's, Sakaguchi, Ninhydrin, Ehrlich, Nitroprusside, Million's, Ferric chloride, Fehling, Meyer's and lead sulfide reactions.

Spectroscopy of the Pure Active Compound:

Ultra violet and visible spectra of the active compound using spectroscopic grade methanol with Shimadzu UV-1601 PC, UV-visible spectrophotometer was investigated. Infra red (IR) spectra of the active compound was obtained in KBr with Shimadzu FT-IR 8000 spectrophotometer, and mass spectral data were obtained by GC-MS-QP1000EX Shimadzu spectrophotometer at 70eV using the direct probe insert. The ¹H NMR and ¹³C NMR spectra were recorded at 300MHz in DMSO with Varian mercury (with an Oxford magnet).

Determination of MIC and LD₅₀

The minimum inhibitory concentration (MIC) of the active compound using several genera of bacteria, yeasts and filamentous fungi as test organisms was determined by using the agar diffusion method. The mammalian toxicity (LD₅₀) was determined after administering the antibiotic orally (Abdel Baset, 1978).

RESULTS AND DISCUSSION

Isolation of Alkalophilic and Alkaline-resistant Microorganisms:

The number of microorganisms grown on high alkaline medium was counted, and it was estimated to be 10² ~ 10⁴ CFU per one gram of soil. In general, among colonies on Sato medium A, 67% of the colonies were bacteria; 30% were actinomycetes and the remaining 3% were fungi.

By using Sato medium A, we isolated 117 alkalophilic and alkaline-resistant microorganisms from 30 soil samples from different six localities in Wadi Araba, Egypt. Among of them were 73 isolates bacteria, 40 isolates actinomycetes and 4 isolates fungi.

Antimicrobial activity:

After testing the antimicrobial activities of the isolated alkalophilic and alkaline resistant microorganisms by the paper disk method, the strains having antimicrobial activities were 23 isolates of bacteria, 22 isolates of actinomycetes and two fungal isolates.

Antimicrobial substances produced by bacterial isolates were not further examined because most of these substances were unstable. Also, the two antimicrobial active fungal isolates showed low activity and so, we choose to continue work with the isolated alkalophile actinomycetes.

It was found that most potent actinomycete isolate (coded WA52) having activity against Gram-positive and Gram-negative bacteria, and recorded the largest diameter of clear zone activity among other actinomycete isolates.

Characterization of the Most Potent Alkalophile Actinomycetes Isolate WA52.

Actinomycete isolate WA52 showed excellent growth and abundant aerial mycelium formation at pH 9-10 and much less growth and aerial mycelium formation at lower pH values on the same medium. The color of the aerial mycelium was white to pale pink. The vegetative mycelium was almost colorless. A red to deep orange soluble pigments were formed on some media especially Sato medium A, yeast extract-malt extract and oatmeal agar media. The color of the reverse substrate mycelium was red to deep orange on most agar media used. The macroscopic growth characteristics are shown in table (1).

The microscopic observations showed that the aerial mycelium fragmented into long spore chains. The sporophore morphology was belonging to the section Rectus-Flexibilis (RF)(Fig.1), and spore surface was smooth (Fig. 2). Meanwhile, physiological properties showed that no melanoid pigments were produced on any media used, and exhibited good growth on potassium nitrate, threonine, serine, phenylalanine and arginine. The isolate could utilized glucose, arabinose, xylose, mannose, mannitol, fructose, galactose, lactose, rhamnose, sucrose, trehalose and sodium citrate. It produced H₂S, protease, lipase, catalase, rease and gelatinase.

Isolation and purification of the active metabolite was carried out according to the scheme given in Fig. (3). The bioautography of the active compound with different solvent systems revealed that the antibiotic WA52-A migrated as a single spot, whose R_f values in each of them are presented in Table (2).

The ultraviolet absorption spectrum of the antibiotic solution in methanol (Fig. 4) exhibited maximum absorption band at 286 nm and a peak at 236 nm. No absorption maxima were found in the region of the visible spectrum. The infra-red absorption spectrum of the antibiotic WA52-A (Fig. 5) showed peaks at 3394 cm^{-1} , 2970 cm^{-1} , 1735 cm^{-1} , 1689 cm^{-1} , 1465 cm^{-1} , 1380 cm^{-1} and 1172 cm^{-1} . The mass spectrum revealed that molecular weight was 716 m/z (Fig. 6). The prominent ion peaks in the spectrum were at m/z 158, 383 and 558. The proton nuclear magnetic resonance spectra (^1H NMR and ^{13}C NMR) are illustrated in Figs. (7 and 8). Table (3) summarizes the physicochemical properties and spectroscopic analysis of the antibiotic.

The elemental analysis of purified antibiotic was recorded as follows (%): C, 61.25; H, 9.3; N, 1.95; and O, 27.5. Consequently, its empirical formula was calculated as $\text{C}_{37}\text{H}_{67}\text{NO}_{12}$. The color reactions were positive with Molish and Fehling tests while they were negative towards neutral ferric chloride, Ehrlich's, nitroprusside, Millon's, ninhydrin, Meyer's. Also, it was found that amino acid not detected in the acid hydrolysate of the antibiotic.

The antibacterial and antifungal activity of the purified substance was carried out and exhibited activity against all the tested Gram-positive bacteria and *Klebsiella pneumoniae* NCIB 9111 from the Gram-negative bacteria but no activity was detected against the tested fungi. The minimum inhibitory concentrations (MIC) of the compound are presented in table (4). The median lethal dose of the antibiotic under study equals 230 mg/kg, table (5).

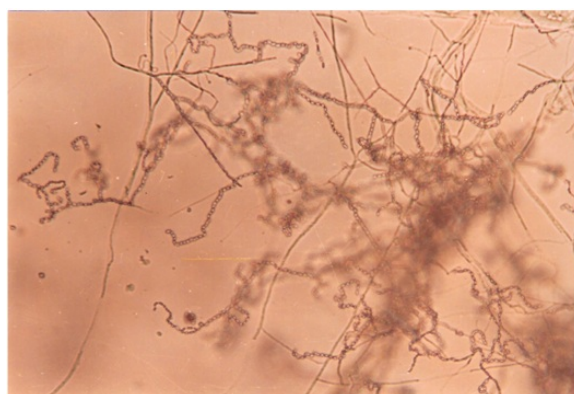


Fig. 1: A microscopic photograph showing the sporulating aerial mycelium of the alkalophile actinomycete isolate WA52 (14 days old culture on yeast extract-malt extract (ISP), X400).

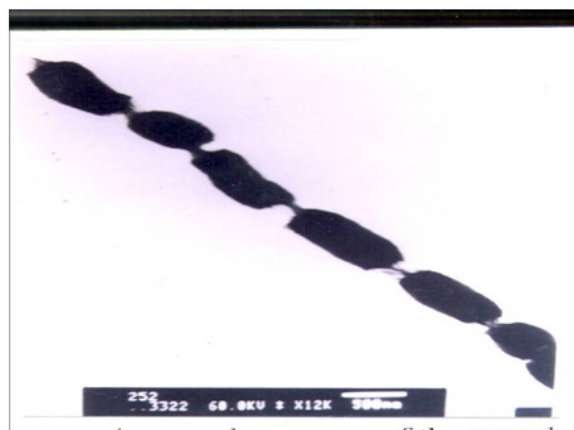


Fig. 2: Electron micrograph of the sporulating aerial hyphae of the alkalophile actinomycete isolate WA52 showing the smooth spore surface (14 days old culture on Sato medium A, X12000).

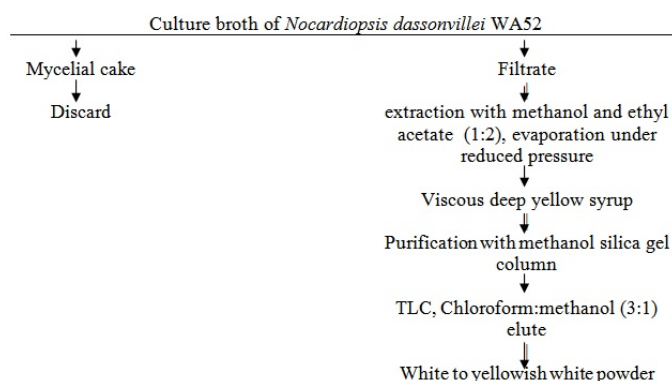


Fig. 3: A simplified scheme for the extraction, isolation and purification of the antibiotic WA52-A biosynthesized by *Nocardioopsis dassonvillei* WA52.

Table 1: Cultural characteristics of the most potent alkaliphilic actinomycete isolate WA52 on different culture media.

Medium	Growth	Aerial mycelium color	Substrate mycelium color	Soluble pigment
Yeast Extract- Malt extract (ISP2)	Abundant	Yellowish pink (41 ypk)	Red (18 red)	Reddish gray (22 r gray)
Oatmeal (ISP3)	Moderate	Pinkish gray (10 pk-gray)	Dark orange (72-d-oy)	Yellow (70 o-y)
Inorganic salts- starch (ISP4)	Good	White (263 white)	Yellowish white (92 y white)	-ve
Glycerol-asparagine (ISP5)	Moderate	White (263 white)	Yellowish white (92 y white)	-ve
Sato medium A	Abundant	Yellowish pink (41 ypk)	Red (18 red)	Reddish gray (22 r gray)
Starch-nitrate	Abundant	White (263 white)	Grayish red (46 gy-r)	Yellowish pink (41 ypk)
Czapek Dox	Moderate	Yellowish white (92 y white)	Yellowish white (92 y white)	-ve
Nutrient agar	Good	Yellowish white (92 y white)	Yellow (70 o-y)	Yellowish pink (41 ypk)

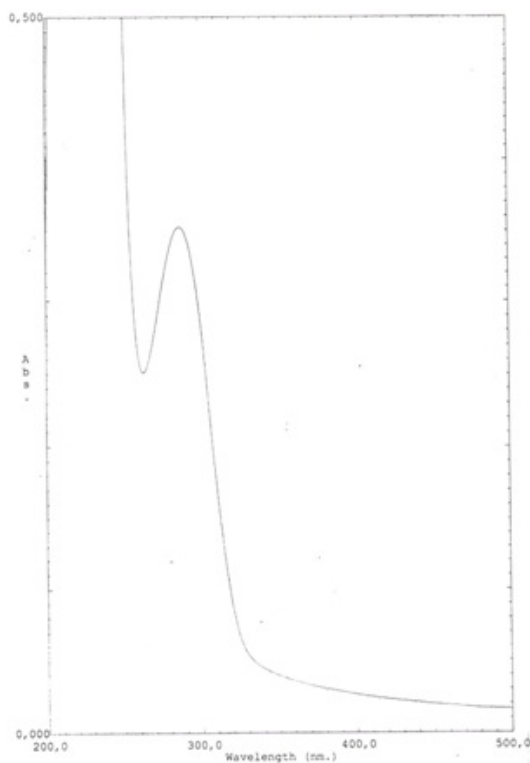


Fig. 4: The UV absorption spectrum of the purified antimicrobial compound produced by the alkaliphile isolate *Nocardioopsis dassonvillei* WA52.

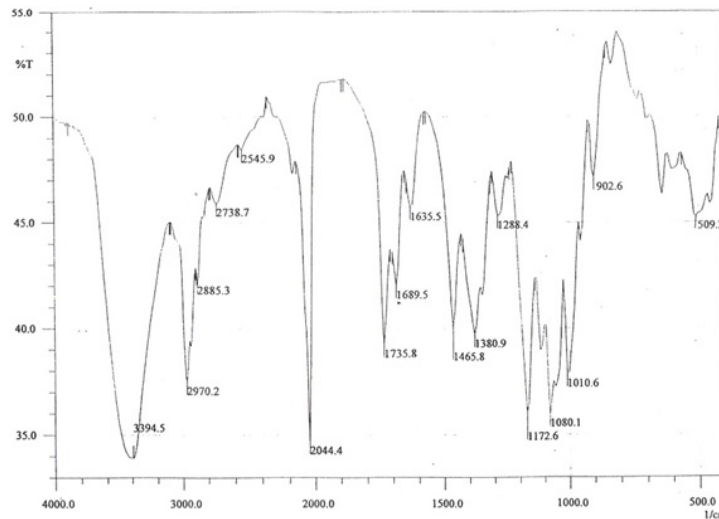


Fig. 5: The IR spectrum of the purified antimicrobial compound produced by the alkalophile isolate *Nocardiopsis dassonvillei* WA52.

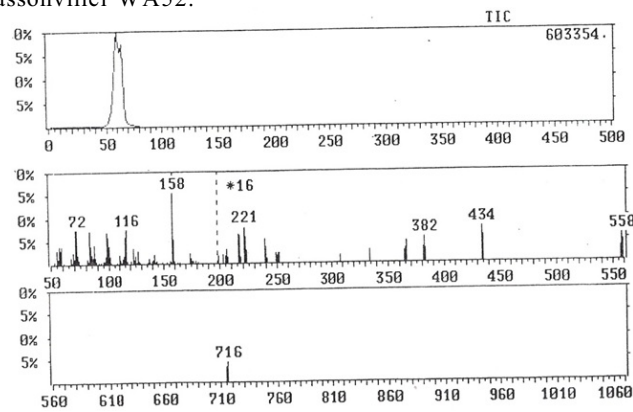


Fig. 6: The Mass spectrum of the purified antimicrobial compound produced by the alkalophile isolate *Nocardiopsis dassonvillei* WA52.

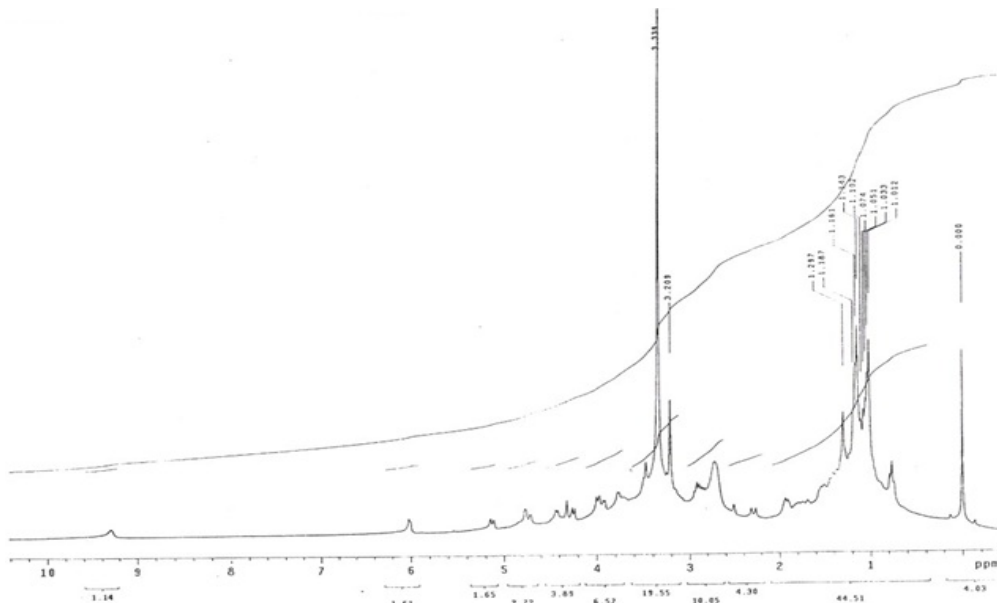


Fig. 7: The ¹H NMR spectrum of the purified antimicrobial compound produced by the alkalophile isolate *Nocardiopsis dassonvillei* WA52.

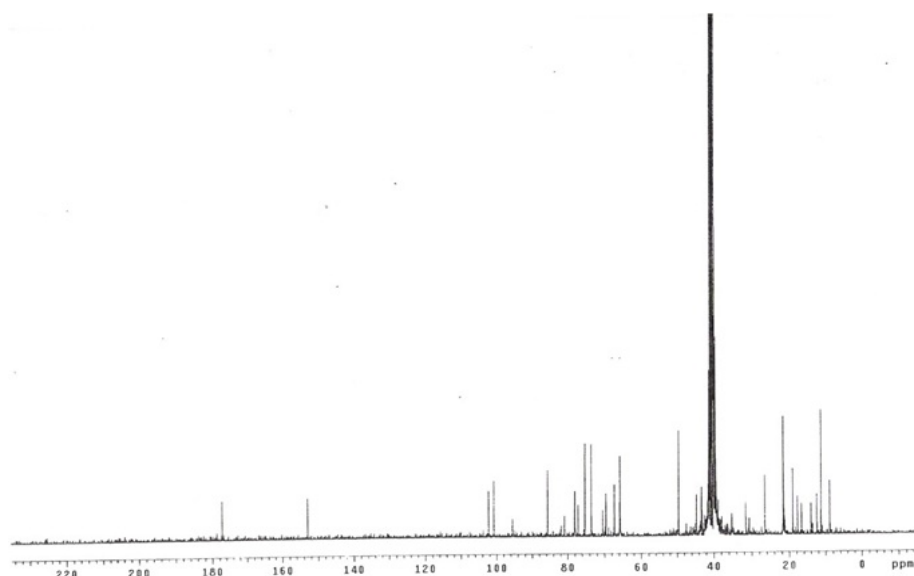


Fig. 8: The ^{13}C NMR spectrum of the purified antimicrobial compound produced by the alkalophile isolate *Nocardiopsis dassonvillei* WA52.

Table 2: Bioautography and migration (R_f values) of the purified active compound isolated from broth culture of *Nocardiopsis dassonvillei* WA52 (SA-M12) with various developing solvents.

Developing solvent system	R_f values
Petroleum ether	0.00
Benzene (saturated with water)	0.00
Chloroform (saturated with water)	0.86
Methanol	0.96
n-Butanol (saturated with water)	0.85
Acetone	0.45
Diethyl ether	0.50
Ethyl acetate	0.92
Amyl acetate	0.44
n-Butanol : pyridine : water (1:0.6:1)	0.87
n-Butanol : Acetic acid : water (2 : 1 : 1)	0.83
Distilled water	0.35
Methylene chloride : water (1:1)	0.20

Table 3: Physicochemical properties of antibiotic SA-M12.

Melting point $^{\circ}\text{C}$	179 $^{\circ}\text{C}$
Appearance	White to yellowish white powder
UV λ_{max} nm (E)	286 and 236
Molecular formula	$\text{C}_{17}\text{H}_{16}\text{NO}_{12}$
Molecular weight	716
IR ν_{max} (cm^{-1})	3394 cm^{-1} hydroxyl-OH 1689 cm^{-1} aromatically C-C
Color reaction	Positive with: Molish and Fehling Negative with: nitroprusside, FeCl_3 , Meyer's, ninhydrin, Ehrlich's and Millon's

Table 4: The antimicrobial spectrum and MIC of the purified compound isolated from *Nocardiopsis dassonvillei* WA52 against different test organisms.

Test organism	MIC ($\mu\text{g/ml}$)
<i>Bacillus subtilis</i> NCTC 10400	1.56
<i>Bacillus pumilus</i> NCTC 8214	3.12
<i>Staphylococcus aureus</i> NCTC 7447	6.25
<i>Micrococcus luteus</i> ATCC 9341	6.25
<i>Escherichia coli</i> NCTC 10416	>100
<i>Pseudomonas aeruginosa</i> ATCC 10145	>100
<i>Klebsiella pneumonia</i> NCIB 9111	50
<i>Saccharomyces cerevisiae</i> CBS 1171	>100
<i>Candida albicans</i> IMRU 3669	>100
<i>Penicillium chrysogenum</i> ATCC 12960	>100
<i>Aspergillus flavus</i>	>100

Table 5: The median lethal dose (LD₅₀) of the purified compound WA52-A isolated from *Nocardioopsis dassonvillei* WA52.

Dose (mg/kg)	N ^o of animals/group	N ^o of dead animals/group	(d)	(z)	(z x d)
200	6	0	10	-	-
210	6	1	10	0.5	5
220	6	2	10	1.5	15
230	6	3	10	2.5	25
240	6	4	10	3.5	35
250	6	5	10	4.5	45
260	6	6	10	5.5	55

$$LD_{50} = T - \sum (z \times d)/n$$

$$LD_{50} = 260 - 180/6 = 230 \text{ mg/kg}$$

Discussion:

The list of novel microorganisms and products found in microbiology poorly explored areas of the world stresses the advantage of investigated new habitats (Nolan and Cross, 1988). So, we collected soil samples from Wadi Araba in the Eastern Desert of Egypt as a microbiologically poorly explored area.

The physicochemical characteristics of the soil samples show that all of them are sandy soils and varied from non-saline to slightly saline according to Jackson (1967). Microorganisms that prefer unusual extreme conditions to normal conditions offer an important research tool for investigating the relationship and interactions between environment factors and microbial life (Gould and Corry, 1980). In general, microorganisms that proliferate and grow under the most extreme conditions are obligatory adapted to their particular environment (Horikoshi and Grant, 1991), and fail to grow at lower intensities of the same environmental factor.

The number of microbial colonies grown on high alkaline medium from the different soil samples varied from 10² CFU g⁻¹ to 10⁴ CFU g⁻¹. On a neutral pH agar plate with sodium carbonate omitted from Sato medium A, 10⁵~10⁷ colonies appeared in one gram of the same soil. Therefore, the numbers of colonies capable of growing on a high alkaline medium seemed to decrease by about 10³ of that on a neutral pH medium. We think that, this count is far from the actual count and in general it is lower than the real count. This is because of the efficiency of the dilution plate technique used is markedly influenced by the composition of the nutrient medium (Williams and Cross, 1971).

The presence of meso-diaminopimelic acid, absence of diagnostically important sugars in whole cells hydrolysate indicated that the alkalophile isolate WA52 belonged to cell wall type III and sugar pattern type C (Lechevalier and Lechevalier, 1970). It was found that this isolate is a member of the genus *Nocardioopsis*, in accordance with the characteristic features of genus *Nocardioopsis* reported by Meyer (1976).

According to the key of McCarthy (1989) for the species differentiation of the genus *Nocardioopsis*, the alkalophile isolate WA52 is easily differentiated from *N. africana*, *N. coeruleofusca*, *N. longispora* and *N. mutabilis* by its aerial mycelium and substrate mycelium colors. It is also differed from *N. flava* by physiological characteristics. Therefore, the used alkalophile isolate WA52 was closely related to *Nocardioopsis dassonvillei* (Meyer, 1976). Furthermore, according to Hensyl (1994) the species *Nocardioopsis dassonvillei* has two subspecies; *Nocardioopsis dassonvillei* subsp. *dassonvilli* and *Nocardioopsis dassonvillei* subsp. *prasina*. The used alkalophile isolate WA52 is differed from *Nocardioopsis dassonvillei* subsp. *dassonvilli* (Meyer, 1976) by its inability to utilize maltose as sole carbon source, by its pale pink aerial mycelium and red substrate mycelium; and by its optimum pH at 9-10. The alkalophile isolate WA52 under study is also differed from the alkalophile *Nocardioopsis dassonvillei* subsp. *prasina* (Miyashita *et al.*, 1984) by its ability to utilize rhamnose as sole carbon source and its aerial mycelium color.

Therefore, on the basis of results presented above we propose that the alkalophile isolate WA52 isolated from Wadi Araba, Egypt may be considered as a new subspecies of *Nocardioopsis dassonvillei* which was given the name *Nocardioopsis dassonvillei* WA52. It differed from other strains of *Nocardioopsis dassonvillei* by its substrate mycelium color and some other characters. However, to confirm our ranking as a new subspecies of *Nocardioopsis dassonvillei* WA52; it needs further studies of 16S rRNA and sequencing with reference strains. Bioautographic technique (Goodall and Levi, 1946; Wagman and Weinstein, 1973) was used for the detection of the active spot and to determine the R_f values of the antibiotic on paper strip chromatograms using different solvent systems as suggested by Blinov and Khokhlove (1970). One definite inhibition zone was detected in each paper strip chromatogram, which indicates that the obtained antibiotic is one pure component.

The antibiotic gives positive reactions with Molish and Fehling indicate the presence of a sugar moiety in its structure while other tests were negative which means that it does not contain amino acids and/or peptides. This is confirmed by the absence of any amino acids in its acid hydrolysate. The maximum peak at 286 nm of UV spectrum indicated a ketone absorption. This ketone absorption is also confirmed by the absorption at 1690 cm⁻¹ in the IR spectrum. Furthermore, IR spectrum indicated absorption at 3394 cm⁻¹ stretching -OH, and 2970 and 1735 cm⁻¹ stretching lactone (C=O).

Concerning the antimicrobial activity of antibiotic WA52-A, it was active against Gram-positive but not Gram-negative bacteria (except *Klebsiella pneumoniae* NCIB 9111), yeast and fungi. Similarly, Irschik *et al.* (2007), Xie *et al.* (2007), and Sohng *et al.* (2008) reported on isolation of thuggacin (1), MFTZ-1 and nargenicin new macrolide antibiotics have only anti Gram-positive activity *in vitro* and *in vivo*, respectively.

The antibiotic WA52-A was then subjected to an identification process using keys of antibiotic identification (Umezawa, 1967; Berdy, 1980 and Betina, 1983). These investigations revealed that it has similar characters to the members of the macrolide antibiotics particularly erythromycins. This suggestion agrees with the molecular formula, color reactions, spectroscopic analysis and most of the physicochemical properties. The presence of erythronolide (aglycone) is inferred from the fragment ion at m/z 383 (Martin *et al.*, 1982) and a weak UV absorption at 286 nm (Kibwage *et al.*, 1987). The prominent ions attributed to desosamine and cladinose, the sugar fragments in erythromycin, at m/z 158 and 159 respectively are also present. The presence of desosamine is also shown by the characteristic N(CH₃)₂ signal at about 2.26 ppm in its ¹H NMR spectrum (Kibwage *et al.*, 1987).

To our knowledge, only one alkalophilic species of the genus *Nocardiopsis*, *Nocardiopsis* sp. M119, has been reported as antibiotic producer of 16-membered macrolide antibiotics, the M119 complex (Tanba *et al.*, 1985). The alkalophile *Nocardiopsis dassonvillei* WA52 isolated from Wadi Araba, Egypt was thus found to be a new producer of one of the erythromycins.

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