Current Trends in Natural Sciences
Vol. 11, Issue 21, pp. 339-345, 2022
https://doi.org/10.47068/ctns.2022.v11i21.037
Current Trends in Natural Sciences (on-line)
ISSN: 2284-953X
ISSN-L: 2284-9521
Current Trends in Natural Sciences (CD-Rom)
ISSN: 2284-9521
ISSN-L: 2284-9521

NEW THERMOPHILIC *THERMOBIFIDA* STRAIN KB-T3 FROM ALGERIAN SAHARAN SOIL: ISOLATION AND POLYPHASIC TAXONOMY

Nacera Benoussaid [1,2], Dalila Boubetra [2], Affaf Laassami [2], Khaoula Bouznada [2], Michael D. Holtz [4], Noureddine Bouras [2,3], Atika Meklat [2*]

[1] Laboratoire de Protection et Valorisation des Ressources Biologiques (LPVRB), Faculté SNV, Université de Blida 1, Algeria
[2] Laboratoire de Biologie des Systèmes Microbiens (LBSM), Ecole Normale Supérieure de Kouba, Algiers, Algeria
[3] Département de Biologie, Faculté des Sciences de la Nature et de la Vie et Sciences de la Terre, Université de Ghardaia, BP 455, Ghardaïa 47000, Algeria
[4] Field Crop Development Centre, Alberta Agriculture and Forestry, 5030-50 Street, Lacombe, AB T4L 1W1, Canada

Abstract
During a screening for the diversity of actinobacterial strains from Saharan soil samples collected from Béchar region (Algeria), one strain designated KB-T3 was isolated by dilution technique on chitin-vitamins agar medium. The taxonomic position of this strain was determined by using a polyphasic approach. Morphological and chemical characteristics of the KB-T3 strain were consistent with those of the genus Thermobifida. The KB-T3 strain had a white aerial mycelium with dictomically branched sporophores carrying coccoid secluded spores. The substrate mycelium was pale yellow, sterile, and non-fragmented. The strain is characterized by the presence of meso-diaminopimelic acid in the cell wall, the galactose in whole-cell, and phosphatidylethanolamine in the cell membrane. The unique characteristic of this strain was its abundant growth with the absence of NaCl and in temperature ranging from 40 to 65 °C, its capacity to decompose acetate, and its ability to use fructose, glucose and xylose as sole carbon source. Phylogenetic analysis based on 16S rRNA gene sequence revealed that the strain KB-T3 should be classified in the genus Thermobifida and exhibited 99.79 % gene sequence similarity to Thermobifida fusca NBRC 14071^T.

Keywords: Thermophilic actinobacteria, Thermobifida, Saharan soil, Taxonomy.

1. INTRODUCTION

Actinobacteria are a distinct group of bacteria that are widely distributed in nature. They are one of the major soil populations where they play an essential role in the cycling of organic compounds (Hazarika et al., 2020). They have a wide range of habitats, including extreme geographical locations such as deserts, hot springs, salt lakes, caves, and deep-sea (Lee et al., 2012, Quin et al., 2016, Law et al., 2018). Actinobacteria from extreme habitats represent not only extensive taxonomic diversity but also interesting potential to produce valuable natural compounds (Boubetra et al., 2013; 2015; Meklat et al., 2013; Khebizi et al., 2018).

*Corresponding author, E-mail address: atika.meklat@g.ens-kouba.dz

Current Trends in Natural Sciences
Vol. 11, Issue 21, pp. 339-345, 2022
https://doi.org/10.47068/ctns.2022.v11i21.037
Current Trends in Natural Sciences (on-line)
ISSN: 2284-953X
ISSN-L: 2284-9521
Current Trends in Natural Sciences (CD-Rom)
ISSN: 2284-9521
ISSN-L: 2284-9521

The genus *Thermobifida* (Zhang et al., 1998), which belongs to the family *Nocardiopsaceae* (Kroppenstedt and Evtushenko, 2006) of the order *Streptosporangiales* (Goodfellow, 2015), currently contains only four validly named species, including *Thermobifida alba*, *T. fusca* (Zhang et al., 1998), *T. cellulosilytica* (Kukolya et al., 2002) and *T. halotolerans* (Yang et al., 2008). This genus constitutes a promising source of valuable secondary metabolites, mainly enzymes, such as cellulases, xylanases, mannanase, mannosidase, chitinase, cutinase and amylase (Yang et al., 2004, Chen et al., 2010, Gomez del Pulgar and Saadeddin, 2014, Gaber et al., 2016).
The extremobiosphere is nowadays becoming the target ecosystem for searching rare actinobacteria because of the uniqueness of environmental conditions. The Sahara is one of the extreme environments on Earth and constitutes an unexplored source of thermophilic actinobacteria. Exploration of thermophilic actinobacteria from Algerian Saharan soils yielded the isolation of strain KB-T3 with morphological structures typical of the genus *Thermobifida*. However, as far as we know, no reports are available on the isolation of the members of *Thermobifida* from Saharan soils. The present study aims to describe this new strain of actinobacteria using a polyphasic approach based on morphological, physiological, chemotaxonomic, and molecular investigations.

2. MATERIALS AND METHODS

2.1. Isolation of strain

During an investigation of actinobacterial diversity in Saharan soils, a strain named KB-T3 was isolated from non-rhizospheric soil sample collected from Béchar region (31° 37′ 00″ N, 2° 13′ 00″ E). The serial dilution method was used, and the isolation was performed on chitin-vitamin B agar medium, supplemented with cycloheximide to suppress micro-fungal growth. After the incubation of the plates at 55°C for 4 days, the strain was purified and maintained on nutrient agar medium.

2.2. Phenotypic characteristics

A morphological, biochemical, and physiological characterization approach was adopted to determine the taxonomic status at the genus level of the KB-T3 isolate.
The macromorphological characterization of the actinobacterial isolate was based on cultural characteristics, including aerial and substrate mycelium color and production of diffusible pigment for a culture grown for 10 days on ISP2, ISP4 (Shirling and Gottlieb, 1966), nutrient agar, and R8 (Amner et al., 1998) culture media. Spores and mycelia were examined by a light microscope (Motic, B1 Series) as reported by Goodfellow and Haynes (1984).
For chemotaxonomic study, diaminopimelic acid and whole-cell sugars were analyzed according to the procedures of Becker et al. (1964) and Lechevalier and Lechevalier (1970), respectively. Phospholipid types were determined according to the method of Minnikin et al. (1977).
Several physiological tests were used to characterize the actinobacterial strain. The degradation of different organic compounds was evaluated as described by Goodfellow (1971). Carbohydrate utilization was performed by using ISP9 medium supplemented with different carbohydrate compounds. Lysozyme sensitivity and resistance to some chemical compounds such as chloramphenicol, erythromycin, kanamycin, streptomycin and penicillin were examined according to the methods of Gordon and Barnett (1977). Growth at different temperatures (30, 40, 45, 50, 55, 60, 65 and 70°C), at different pH values (5, 6, 7, 8 and 9) and at different NaCl concentrations (0, 3, 5 and 10% w/v) was determined on nutrient agar.

*Corresponding author, E-mail address: atika.meklat@g.ens-kouba.dz

Current Trends in Natural Sciences
Vol. 11, Issue 21, pp. 339-345, 2022
https://doi.org/10.47068/ctns.2022.v11i21.037
Current Trends in Natural Sciences (on-line)
ISSN: 2284-953X
ISSN-L: 2284-9521
Current Trends in Natural Sciences (CD-Rom)
ISSN: 2284-9521
ISSN-L: 2284-9521

2.3. 16 rRNA Gene Sequencing and Phylogenetic Analysis

Genomic DNA of the KB-T3 strain was extracted according to the method of Liu et al. (2000). PCR amplification of the 16S rRNA gene was performed as described by Rainey et al. (1996) using a primer pair 10-30F (5′-GAGTTTGATCCTGGCTCA-3′) and 1500R (5′-AGAAAGGAGGTGATCCAGCC-3′). The PCR products were analyzed by agarose gel electrophoresis and then submitted to Genewiz (United Kingdom) for purification and sequencing. The 16S rRNA gene sequence of strain KB-T3 was deposited in the GenBank data library under accession number OM475721 and was compared with corresponding sequences of the type strains found on the EzBioCloud server (https://eztaxon-e.ezbiocloud.net) (Yoon et al., 2017). Phylogenetic analysis was conducted using MEGA 7.0 software (Kumar et al., 2016) according to the method described by Li et al. (2019). The phylogenetic dendrogram was constructed using the neighbor-joining method of Saitou and Nei's (1987) tree-making algorithm. The evolutionary distance model of the Kimura 2-parameter (Kimura 1980) was used to generate evolutionary distance matrices for the neighbor-joining algorithm. The topology of each tree was evaluated by bootstrap analysis (Felsenstein, 1985) with 1000 replications.

3. RESULTS AND DISCUSSIONS

Morphological observation revealed good growth of strain KB-T3 on R8 end nutrient agar (NA) agar media, but no growth was observed on ISP 2 and ISP4 media. The aerial mycelium was observed with white color. However, the substrate mycelium was observed to be a light yellow on the same culture media. The aerial hyphae carry coccoid and single spores which are borne on short dichotomously branched sporophores (Figure 1). The substrate mycelium is sterile and non-fragmented. The diffusible pigments were not produced on R8 and NA media.

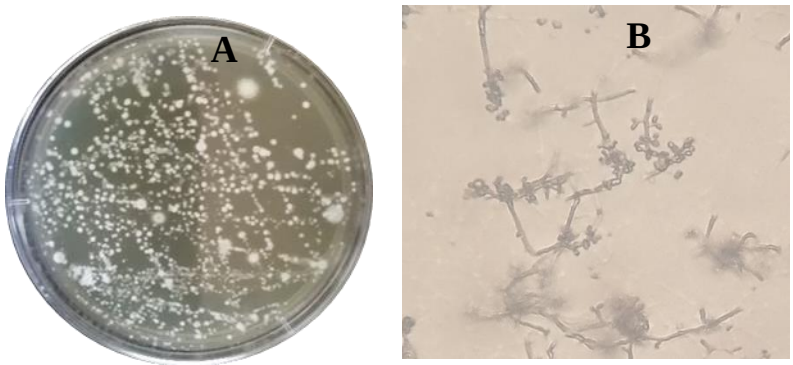

Figure 1. Morphological caracteristics of KB-T3 strain of Thermobifida on nutrient agar medium after 6 days of incubation at 55 °C. A. Cultural features. B. Microscopic observation under light microscope (400X).

Chemotaxonomic properties of strain KB-T3 showed that cell-wall hydrolysate contained the *meso*-diaminopimelic acid isomer, but not glycine. Whole-cell hydrolysates were found to contain galactose, which is typical of cell-wall type III and whole-cell sugar pattern type C (Lechevalier and Lechevalier 1970). Strain KB-T3 was found to possess phosphatidylethanolamine corresponding to phospholipid type PII (Lechevalier et al., 1977). The morphological and chemotaxonomic properties of strain KB-T3 are consistent with those shared by members of the genus *Thermobifida* (Zhang et al., 1998).

*Corresponding author, E-mail address: atika.meklat@g.ens-kouba.dz

Current Trends in Natural Sciences
Vol. 11, Issue 21, pp. 339-345, 2022
https://doi.org/10.47068/ctns.2022.v11i21.037
Current Trends in Natural Sciences (on-line)
ISSN: 2284-953X
ISSN-L: 2284-9521
Current Trends in Natural Sciences (CD-Rom)
ISSN: 2284-9521
ISSN-L: 2284-9521

The KB-T3 strain uses a low amount of sugar and organic acid (acetate). Only three sugars (fructose, glucose and xylose) out of 23 tested can be used by the strain as a carbon source. The amino acids tested as a source of nitrogen were not used.

Table 1. Differential phenotypic properties of strain KB-T3 and the type strains of Thermobifida species Strains: 1, KB-T3; 2, T. fusca DSM 43792^T; 3, T. alba DSM 43795^T; 4, T. cellulosilytica DSM 44535^T; 5, T.halotolereans YIM 90462^T.
+ Positive, – negative, Nd not determined.

Characteristics	**Strains**				
	1	**2**	**3**	**4**	**5**
Arial mycelium on ISP2	-	-	-	+	-
Temperature range for growth (°C)	35-65	28-55	20-50	28-55	20-50
pH range for growth	6-7	6-9	6-9	6-10	6-9
Maximum NaCl concentration (%, w/v)	5	5	3	3	10
Utilization of					
D-Arabinose	-	-	+	-	+
D-Fructose	+	+	-	+	-
Glycerol +	-	+	+	-	+
Lactose, Maltose and D-Mannose	-	+	-	+	-
L-Rhamnose	-	-	+	+	-
D-Ribose	-	-	-	+	-
Adonitol, Erythritol and Salicine	-	Nd	Nd	Nd	Nd
Cellobiose	-	+	+	+	+
Galactose and Xylose	+	+	+	+	+
Inositol and Sorbitol	-	-	-	-	-
Mannitol	-	-	-	Nd	Nd
Melezitose	-	+	+	+	Nd
Melibiose	-	-	-	Nd	Nd
Raffinose	-	+	+	+	+
Saccharose	-	+	+	Nd	Nd
Glucose	-	+	+	-	+
Degradation of					
Casein, Acetate and Starch	+	Nd	Nd	Nd	Nd
Gelatin	-	-	-	+	-
Tyrosine, Guanine, Hypoxanthine and Xanthine	-	-	-	Nd	Nd
Tween 80	-	+	+	+	+
Alanine, Benzoate, Butyrate, Citrate, Oxalate, tartrate, Propionate, Testosterone, Proline, Pyruvate, Serine and Succinate	-	Nd	Nd	Nd	Nd
Growth in the presence of					
Chloramphenicol and Kanamycine	-	-	-	-	Nd
Erythromycine	-	Nd	Nd	+	Nd
Streptomycine	-	Nd	Nd	-	Nd
Penicilline	-	-	-	Nd	Nd
Lysozyme	-	Nd	Nd	Nd	Nd

Temperature and pH ranges for growth are 40–65 °C and pH 6.0–8.0, with optima at 50–60 °C and pH 7.0. The NaCl concentration range for growth is 0–5 %, with optimal growth occurring at 0%.

*Corresponding author, E-mail address: atika.meklat@g.ens-kouba.dz

Current Trends in Natural Sciences
Vol. 11, Issue 21, pp. 339-345, 2022
https://doi.org/10.47068/ctns.2022.v11i21.037
Current Trends in Natural Sciences (on-line)
ISSN: 2284-953X
ISSN-L: 2284-9521
Current Trends in Natural Sciences (CD-Rom)
ISSN: 2284-9521
ISSN-L: 2284-9521

The optimum temperature range of the strain KB-T3 let classify it as strictly thermophilic actinobacterium (Jiang and Xu,1993; Yang et al., 2008). The organism was found to be sensitive to kanamycin (5 mg ml^{-1}), erythromycin (10 mg ml^{-1}), streptomycin (10 mg ml^{-1}), penicillin (25 mg ml^{-1}), chloramphenicol (25 mg ml^{-1}), and lysozyme (0.005 % w/v). The biochemical results showed that the strain KB-T3 is physiologically different from the recognized *Thermobifida* species as can be seen from the differential physiological characters given in Table 1. Regarding the use of sugars as a carbon source, we noticed that there is variability in the response spectrum. The KB-T3 strain degrades a small amount of compounds compared with the *T. fusca* DSM 43792^T.
The 16S rRNA gene sequence analysis indicated that the strain KB-T3 is closely related to *Thermobifida fusca* with 99.79% of similarity. The strain KB-T3 was clustered in the same clade with *Thermobifida fusca* (Figure 2).

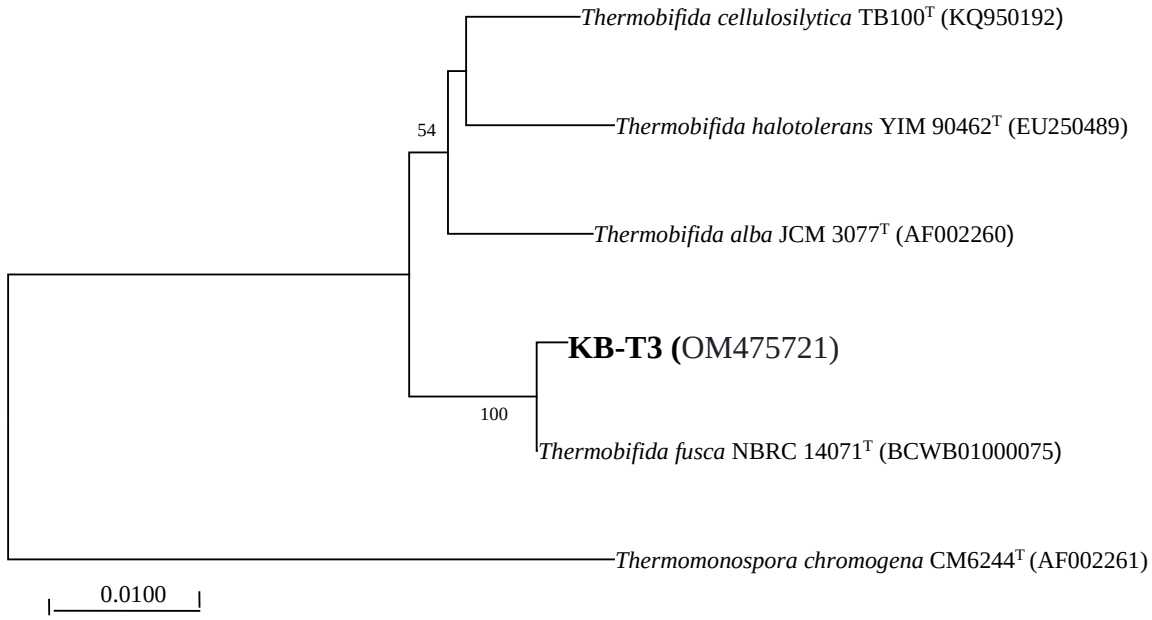

Figure 2. Phylogenetic tree showing the relationship between the isolate KB-T3 and their phylogenetic neighbors based on 16S rRNA gene sequences. The neighbor-joining method (Saitou and Nei 1987) was used to construct the phylogenetic tree. Bootstrap values (50 %) based on 1000 resamplings are shown at branch nodes. Thermomonospora chromogena was used as the out-group. Bar, 0.0100 nt substitutions per site.

4. CONCLUSIONS

The strain KB-T3 was related to *Thermobifida fusca* with a hight percentage of similarity. Nevertheless, this strain differs from the closest species in several physiological characteristics. Hence, the deep taxonomic status at the species level of this strain should be completed. Therefore, DNA/DNA hybridization experiments analysis need to be performed between the studied strain KB-T3 and *Thermobififa* species.

5. ACKNOWLEDGEMENTS

We thank the late Prof. Nasserdine Sabaou (LBSM, Ecole Normale Supérieure de Kouba, Algiers, Algeria) for great help in isolation and taxonomic analyses.

*Corresponding author, E-mail address: atika.meklat@g.ens-kouba.dz

Current Trends in Natural Sciences
Vol. 11, Issue 21, pp. 339-345, 2022
https://doi.org/10.47068/ctns.2022.v11i21.037
Current Trends in Natural Sciences (on-line)
ISSN: 2284-953X
ISSN-L: 2284-9521
Current Trends in Natural Sciences (CD-Rom)
ISSN: 2284-9521
ISSN-L: 2284-9521

6. REFERENCES

Amner, W., Edwards, C., McCARTHY, A.J. (1989). Improved medium for recovery and enumeration of the farmer's lung organism, *Saccharomonospora viridis*. *Applied and Environmental Microbiology*, *55*(10), 2669-2674.

Becker, B., Lechevalier, M.P., Gordon, R.E., Lechevalier, H.A. (1964). Rapid differentiation between *Nocardia* and *Streptomyces* by paper chromatography of whole-cell hydrolysates. *Applied Microbiology*, *12*(5), 421-423.

Boubetra, D., Sabaou, N., Zitouni, A., Bijani, C., Lebrihi, A. Mathieu F. (2013). Taxonomy and chemical characterization of new antibiotics produced by *Saccharothrix* SA198 isolated from a Saharan soil. *Microbiological Research*, *168*(4), 223-230.

Boubetra, D., Zitouni, A., Bouras, N., Schumann, P., Sproer, C., Klenk, H.P., Sabaou, N. (2015). *Saccharothrix tamanrassetensis* sp. nov., an actinomycete isolated from Saharan soil. *International Journal of Systematic And Evolutionary Microbiology*, *65*, 1316-1320.

Chen, S., Su, L., Billig, S., Zimmermann, W., Chen, J., Wu, J. (2010). Biochemical characterization of the cutinases from *Thermobifida fusca*. *Journal of Molecular Catalysis B: Enzymatic*, *63*(3-4), 121-127.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution 39*(4), 783-791.

Gaber, Y., Mekasha, S., Vaaje-Kolstad, G., Eijsink, V.G., Fraaije, M.W. (2016). Characterization of a chitinase from the cellulolytic actinomycete *Thermobifida fusca*. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics*, *1864*(9), 1253-1259.

Gomez del Pulgar, E.M., Saadeddin, A. (2014). The cellulolytic system of *Thermobifida fusca*. *Critical Reviews in Microbiology*, 40(3), 236-247.

Goodfellow, M. (1971). Numerical taxonomy of some nocardioform bacteria. *Microbiology*, *69*(1), 33-90.

Goodfellow, M. (2015). *Streptosporangiales* ord. nov. *Bergey's Manual of Systematics of Archaea* and *Bacteria*, 1-3.

Gordon, R.E., Barnett, D.A., Handarhan, J.E., Pang, C.H.N. (1974). *Nocardia coeliaca*, *Nocardia autotrophica*, and the nocardin strains. *International Journal of Systematic and Evolutionary Microbiology*, *24* (1), 54-63.

Gordon, R.E., Barnett, D.A. (1977). Resistance to rifampicin and lysozyme of strains of some species of *Mycobacterium* and *Nocardia* as a taxonomic tool. *International Journal of Systematic Bacteriology*, *27*(3), 176-178.

Hazarika, S.N. Thakur, D. (2020). Actinobacteria. In N. Amaresan, M. Senthil Kumar, K. Annapurna, Krishna Kumar, A. Sankaranarayanan, eds, Beneficial Microbes in Agro-Ecology (pp. 443-476). Academic Press. United States.

Jiang, C., Xu, L. (1993). Actinomycete diversity in unusual habitats, *Actinomycetes*, *4*(2), 47-57.

Khebizi, N., Boudjella, H., Bijani, C., Bouras, N., Klenk, H-P, Pont, F., Mathieu, F., Sabaou, N. (2018). Oligomycins A and E, major bioactive secondary metabolites produced by *Streptomyces* sp. strain HG29 isolated from a Saharan soil. *Journal de Mycologie Medicale*, *28*(1), 150-160.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, *16*(2), 111-120.

Kroppenstedt, R.M., Evtushenko L.I. (2006). The family *Nocardiopsaceae*. In M. Dworkin, S. Falkow, E. Rosenberg, H.K. Schleifer, E. Stackebrandt, eds, The Prokaryotes: a Handbook on the Biology of Bacteria (vol.3. pp. 754-795), New York: Springer, New York.

Kukolya, J., Nagy, I., La´day, M., To´ th, E., Oravecz, O., Ma´rialigeti, K., Hornok, L. (2002). *Thermobifida cellulolytica* sp. nov., a novel lignocellulose-decomposing actinomycete. *International Journal of Systematic and Evolutionary Microbiology*,*52*(4), 1193-1199.

Kumar, S., Stecher, G., Tamura, K. (2016). Mega 7: Molecular evolutionary genetics analysis in version 7.0 for bigger datasets. *Mol Biol Evol*, *33*(7),1870-1874.

Kurapova, A. I., Zenova, G. M., Studnitsyn, I. I., Kizilova, A. K. Manucharova, Zh., Norovsuren, N. A., Zvyagintsev, D. G. (2012). Thermotolerant and thermophilic actinomycetes from soils of Mongolia Desert Steppe Zone. *Microbiology*, *81*(1), 98-108.

Law, J. W. F., Tan, K. X., Wong, S. H., Ab Mutalib, N. S., Lee, L. H. (2018). Taxonomic and characterization methods of *Streptomyces*: a review. *Progress In Microbes and Molecular Biology*, *1*(1).

Lechevalier, H.A., Lechevalier, M.P. (1970). Chemical composition as a criterion in the classification of aerobic actinomycetes. *International Journal of Systematic Bacteriology*, *20*(4), 435-443.

Lechevalier , M.P., de Bie`vre, C., Lechevalier, H.A. (1977). Chemotaxonomy of aerobic actinomycetes: phospholipid composition. Biochemical Systematics and Ecology, *5*(4), 249-260.

*Corresponding author, E-mail address: atika.meklat@g.ens-kouba.dz

Current Trends in Natural Sciences
Vol. 11, Issue 21, pp. 339-345, 2022
https://doi.org/10.47068/ctns.2022.v11i21.037
Current Trends in Natural Sciences (on-line)
ISSN: 2284-953X
ISSN-L: 2284-9521
Current Trends in Natural Sciences (CD-Rom)
ISSN: 2284-9521
ISSN-L: 2284-9521

Lee, L.H., Cheah, Y.K., Sidik, S.M., Ab Mutalib, N.S., Tang, Y.L., Lin, H.P., Hong, K. (2012). Molecular characterization of Antarctic actinobacteria and screening for antimicrobial metabolite production. *World Journal of Microbiology and Biotechnology*, *28*(5), 2125-2137.

Li, FN., Lu, Q., Liao, SL., Jin, T., Li, W., Sun, CH. (2019). *Labedella phragmitis* sp. nov. and *Labedella populi* sp. nov., two endophytic actinobacteria isolated from plants in the Taklamakan desert and emended description of the genus *Labedella*. *Systematic and Applied Microbiology*,*42*(5), 126004.

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

Meklat, A., Bouras, N., Zitouni, A., Mathieu, F., Lebrihi, A., Schumann, P., Sproer, C., Klenk, H-P, Sabaou, N. (2013). *Actinopolyspora righensis* sp. nov., a novel halophilic actinomycete isolated from Saharan soil in Algeria. *Antonie Van Leeuwenhoek*, *104*(3), 301-307.

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)*, 104-11

Qin, S., Li, W. J., Dastager, S.G., Hozzein, W.N. (2016). *Actinobacteria* in special and extreme habitats: Diversity, function roles, and environmental adaptations. *Frontiers in Microbiology*, *7*, 1415.

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 Bacteriology*, *46*(4),1088-1092.

Saitou, N., Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, *4(4)*,406-425.

Shirling, E.B, Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *International Journal of Systematic Bacteriology*,*16*(3), 313-340.

Yang, C.H., Liu, W.H. (2004). Purification and properties of a maltotriose-producing α-amylase from *Thermobifida fusca*. *Enzyme and Microbial Technology*, *35*(2-3), 254-260.

Yang, L. L., Tang, S. K., Zhang, Y. Q., Zhi, X. Y., Wang, D., Xu, L. H., Li, W. J. (2008). *Thermobifida halotolerans* sp. nov., isolated from a salt mine sample, and emended description of the genus *Thermobifida*. *International journal of systematic and evolutionary microbiology*, *58*(8), 1821-1825.

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 and whole-genome assemblies. *International Journal of Systematic and Evolutionary Microbiology*, *67*(5),1613-161.

Zhang, Z., Wang, Y., Ruan, J. (1998). Reclassification of *Thermomonospora* and *Microtetraspora*. *International Journal of Systematic Bacteriology*, *48*(2), 411-422.

*Corresponding author, E-mail address: atika.meklat@g.ens-kouba.dz