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Screening of the microbial community of sewage sludge of Beni-Suef Wastewater Treatment Plant and identification of a novel actinomycetes strain

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ABSTRACT

The current study aimed to isolate and identify a novel bacterial and actinomycetes strains from sewage sludge (SS) at Beni-Suef Wastewater Treatment Plant. Eight strains were isolated from SS under aerobic conditions. Among the colonies appeared on the plates, 4 bacterial isolates, and 4 actinomycetes isolates and their ability in the biological treatment of SS tested previously in other study. The bacterial isolates were identified by their morphological and biochemical characteristics. The bacterial population was dominated by *Dermacoccus nishinomiyaensis* (95% probability), *Kocuria rosea* (95% probability), *Streptococcus parasanguinis* (95% probability), *Kocuria varians* (97% probability). On the other hand, the two potent actinomycetes strains which gave the best results among all isolated strains in the treatment in previous work were identified by 16S rRNA and phylogenetic analysis and they were defined as *Nocardiopsis lucentensis* and *Saccharomonospora azurea*, *Saccharomonospora azurea* proposed as a new species.

INTRODUCTION

Sewage sludge is now becoming a worldwide environmental problem because of its increasing production and its high contents of organic waste and pathogens, also heavy metals and xenobiotics (Elsayed *et al*. 2017).This waste may cause the environment and human as well as animal health exposed to tremendous threat if not being treated or disposed properly (Ahring 2003). Municipal solid waste has to be stabilized prior to discharge to reduce biological activity and slow the release of harmful chemicals into the environment and reduce of our production. The consortium microorganisms (CM) suggested as a new technology to assist in the disposal of sewage sludge, conforming to strict environmental regulations (Ishak *et al*. 2011). A lot of microorganisms were isolated from the activated sludge of municipal and industrial wastewater (Mitchel 1976; Mendes and Nascimento 1991; Zorenzana *et al*. 1992; Hozzein *et al*. 2011; Ishak *et al*. 2011). Different microorganisms including bacteria, fungi and yeasts are known for their ability to degrade hydrocarbons and alter or break the sludge constituents (Thangaraj *et al*. 2007; Morelli *et al*. 2005; Van Hamme *et al*. 2003; Chaillan *et al*. 2004). The aerobic degrading bacteria in organo-polluted site include the genera *Pseudomonas*, *Acinetobacter*, *Alcaligenes*, *Flavobacterium*, *Cytophaga*, *Xanthomonas*, *Nocardia*, *Mycobacterium*, *Corynebacterium*, *Arthrobacter* and *Bacillus* (Fritsche and Hofrichter 2005). (Omer 2012) used *Pseudomonas putida*, *Pseudomonas flouresence* and *Azotobacter vinelandii* in degrading phenols and production of organic biofertilizer from olive mill waste water. Effective microorganisms are being applied to stimulate plant growth

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and nutrient cycling.(Shaheen *et al*. 2017) reported that mixing of effective microorganisms with organic wastes stimulate the growth yield and quality of spinach .

Kamaluddeen *et al*.(2016) assessing sewage sludge as a source of nutrient for backing microorganisms to utilize the crude oil (hydrocarbon chain) as the primary source of carbon in bioremediation activity. actinomycetes were found to be active in the decomposition of organic materials in the wastewater bioreactors (McCarthy 1987). Also actinomycetes in the wastewater treatment plants are able to use several growth substrates varying from sugars to high molecular weight polysaccharides, proteins and aromatic compounds (Lemmer and Kroppenstedt 1984; Lemmer 1986). Partial sequence analysis of 16S rRNA is a very powerful tool now for actinomycete classification (Stackebrandt *et al*. 1981a and b; Embley *et al*. 1994). It is becoming clear that 16S rRNA sequences should form part of the minimal description of actinomycete species (Chun *et al*. 1997; Kim *et al*. 1999 and 2000).

The aim of the present work was to isolate and identify novel bacterial and actinomycetes strains and test its ability in the treatment of sludge in the wastewater treatment plant at Beni-Suef city in the middle Egypt.

Materials And Methods Sampling:

The Wastewater Treatment Plant (WWTP) in Beni-Suef is working by the extended activated sludge system, where samples of sludge for isolation were collected manually from drying beds (DS) and from the outlet after thickener tanks (WS) during the Summer (March, April and May 2014).

Isolation of the sewage sludge bacteria and actinomycetes:

Serial dilutions of sludge sample were adopted for isolation the bacterial and actinomycetes strain. The isolated organisms were isolated according to (Johnson *et al*. 1959). The nutrient agar (Williams and Cross 1971) was used for taxonomical determination of the main physiological groups of microorganisms. Appropriate aliquots (0.1 ml) of each dilution were spread over the surface of the isolation plates which were then incubated for 14 days at 30 ºC. After incubation, 15 isolates were selected from the plates and repeatedly streaked onto the same medium to obtain pure cultures. The pure isolates were preserved as stock suspensions in 20% glycerol at -20 °C; then, 8 morphologically different isolates (bacteria and actinomycetes) were selected for further studies.

Characterization of the sewage sludge bacterial isolates:

The 4 selected bacterial isolates were recognized and initially characterized by standard methods of bacterial identification by biochemical tests via strip system (BioMereux) on Vitek 2C using the GP identification card which based on established biochemical methods and newly developed substrates (Atlas 1993; Barros *et al*. 2001; Bille *et al*. 1992; Collins *et al*. 1984a; Collins *et al*. 1984b; Collins and Lawson 2000; Collins *et al*. 2001; Coykendall 1989; Devriese *et al*. 1988; Farrow *et al*. 1989; Freney *et al*. 2000; Holt *et al*. 1994; Kilpper-Bälz and Schleifer 1987; Krieg and Holt 1984; Murray *et al*. 1999; Poyart *et al*. 2002; Schlegel *et al*. 2000; Viera *et al*. 1998; Whiley *et al*. 1999).

Characterization of the sewage sludge actinomycetes:

Actinomycetes isolates were characterized by their cultural and morphological characteristics after microscopic observations. The optical microscopy was performed with an Olympus model BH2 microscope. The 4 selected actinomycetes isolates were recognized and initially characterized by their morphological characteristics through the coverslip culture technique (Kawato and Shinobu 1959). Characterization of the isolates was based on the guide described in Bergey's manual of determinative bacteriology (Holt *et al*. 1994). The potent two actinomycetes isolates in treatment were characterized by 16S rRNA gene by Macrogen (Macrogen, Inc.).

Phylogenetic analysis:

The 16S rRNA gene sequences of related taxa were obtained from Gene Bank. Multiple sequence alignments were performed using Clustal W version 2.0 (Larkin *et al*. 2007) with default settings. Phylogenetic analyses were constructed by the maximum likelihood (ML) method with maximum parsimony (MP) using MEGA version 6.0 (Tamura *et al*. 2013). The bootstrap values illustrated on the phylogenetic trees were generated with 1000 replications.

Results:

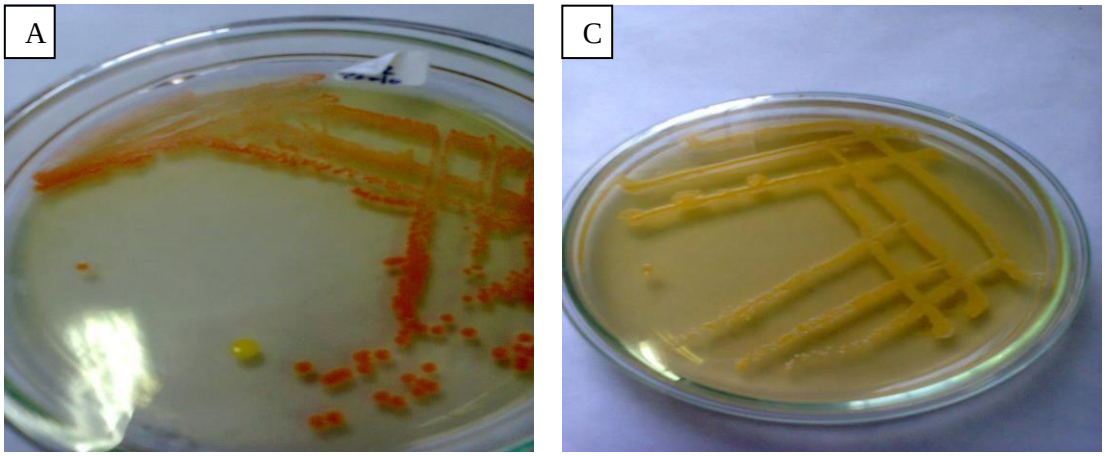
Isolation of the sewage sludge bacteria and actinomycetes:

Samples of sludge were collected from drying beds (DS) and from the outlet after thickener tanks (WS) during the Sumer (March, April and May 2014). Four bacterial strains and four actinomycetes strains were isolated from Wastewater Treatment Plant at Beni-Suef Governorate, Egypt. Figure1, **C** and **A** represent some pure isolates of bacteria, Figure 2, **C** and **D** represent some pure isolates of actinomycetes. The data in Table 1,

showed the morphological characteristics of isolated bacteria, the isolate no **A** appeared as circular bright orange , smooth surface colony with entire margin. The isolate **B** appeared as circular off white smooth surface colony with entire margin. The isolate **C** appeared as circular yellow surface colony, while isolate **D** appeared as circular orange surface colony. The data also indicated that gram stain procedure resulted in all bacterial strains are gram- positive Cocci (GPC).

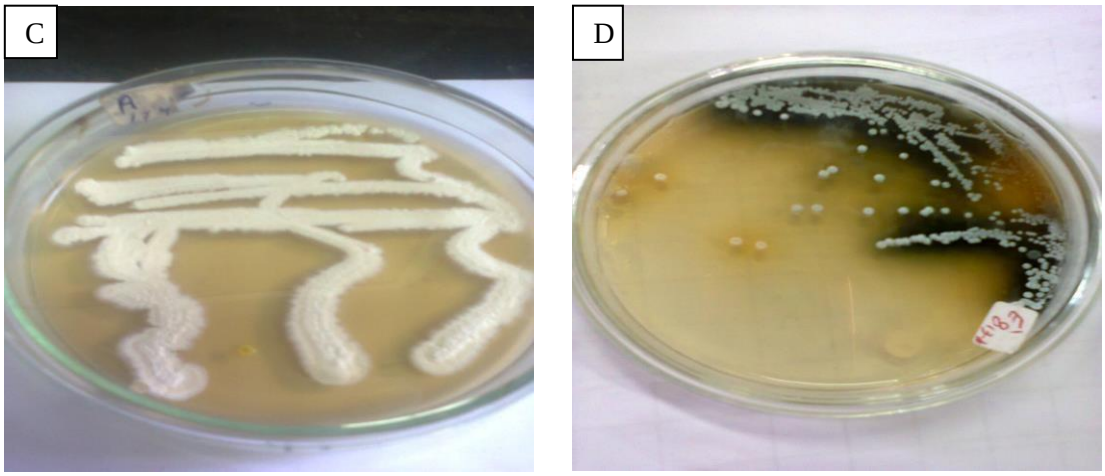
Biochemical identification of bacterial isolates:

The GP card is used for the automated identification of 115 taxa of the most significant non-spore- forming Gram-positive bacteria (primarily Cocci). The GP identification card is based on established biochemical methods and newly developed substrates there are 43 biochemical tests measuring carbon source utilization, enzymatic activities and resistance. The biochemical identification of the bacterial strains by Vitek 2C resulting in the following strains: strain (**A**) identified as *Dermacoccus nishinomiyaensis* with (95% probability), strain (**B**) identified as Streptococcus *parasanguinis* with (95%probability), strain (**C**) identified as *Kocuria rosea* with (95%probability), strain(**D**) identified as *Kocuria varians* with (97%probability).



(A) ***Dermacoccus nishinomiyaensis*** (C) ***Kocuria rosea***

Fig. 1: Some isolated bacterial strains



(C) *Nocardiopsis lucentensis* **(D)** *Saccharomonospora azurea*

Fig. 2: Some isolated actinomycetes strains

Table 1: Characterization of the bacterial isolates on nutrient agar media

Isolate No	Shape of colonies					Shape of cells	Gram reaction
	Colour	Form	Elevation	Margin	Surface		
A	Bright orange	Circular	Slightly convex	Entire	smooth	Coccus	+ve
B	Off white	Circular	Slightly convex	Entire	smooth	Coccus	+ve
C	Yellow	Circular	Slightly convex	Entire	smooth	Coccus	+ve
D	Orange	Circular	Flat	Entire	smooth	Coccus	+ve

Table 2: Characterizations of the selected actinomycetes isolates on nutrient agar media

Character	A	B	C	D
Growth	good	good	Abundant	Abundant
Aerial mycelium	Yellowish white	gray	white	bluish white (azure)
Substrate mycelium	brownish yellow	brownish yellow	Yellowish brown	brown
Soluble pigments	None	None	brown	Greenish blue

Characterizations of the selected actinomycetes:

The results in Table 2 revealed that, strain (**A**) showed good growth on nutrient agar media. The aerial mycelium color varied from white to yellow and the substrate mycelium color varied from yellow to yellowish brown. No soluble pigments were produced on the used media. The data indicated also that strain (**B**) showed good growth on nutrient agar media. Its aerial mycelium color varied from white to grayish white. The substrate mycelium color varied from yellowish white to yellowish brown. No soluble pigments were detected. Strain (**C**) showed abundant growth on nutrient agar media with aerial mycelium color varied from white to yellowish white. The substrate mycelium color of this strain varied from yellowish white to brown. Soluble pigments were brown. Long chains of spores were formed on the aerial hyphae which was zig-zag shaped before maturation and a sort of fragmentation of the substrate mycelium was observed. On the other hand, strain (**D**) showed abundant growth on nutrient agar media with aerial mycelium color varied from grayish white to blue. The substrate mycelium color of this strain varied from yellowish white to brown. Soluble pigments were greenish blue. Single spores were formed on the long aerial hyphae with no fragmentation of hyphae were observed.

Identification of the two potent actinomycetes:

The identification of the two potent actinomycetes in the treatment was obtained after a stepwise phylogenetic analysis of the 16S rRNA gene sequence with the closely related similar sequences. It was found that strain **C** belongs to genus *Nocardiopsis* as obvious from the phylogenetic tree (Fig. 3). The similarity values between strain **C** and the selected closely related *Nocardiopsis* species ranges from 99.9 to 98.8%. It is evident also from the tree that strain **C** closely related to *Nocardiopsis lucentensis.*

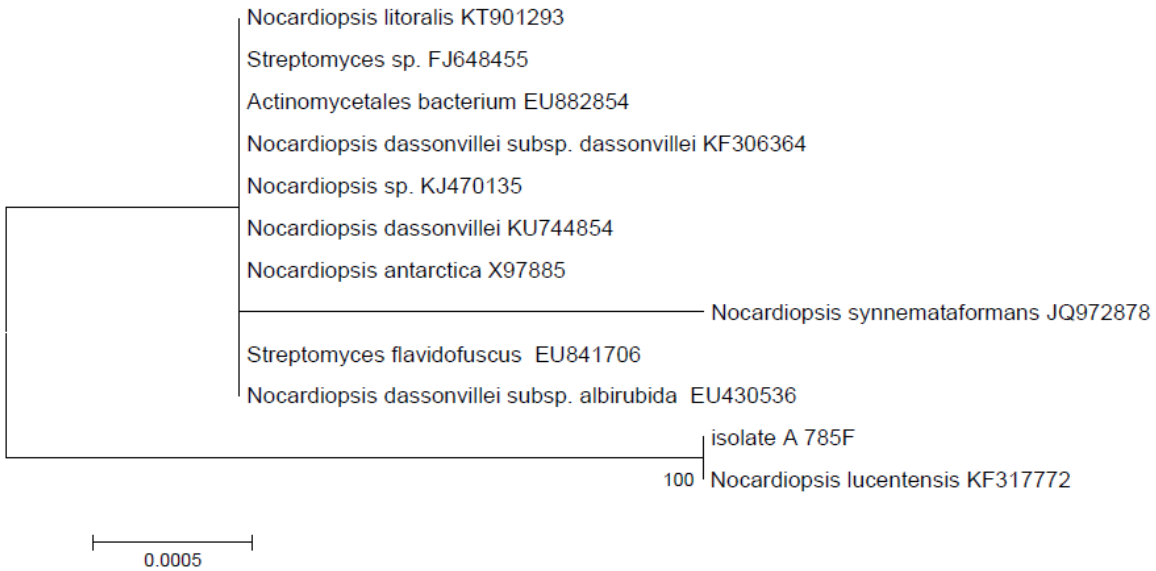


Fig. 3: Unrooted phylogenetic tree based on nearly complete 16S rDNA sequences showing relationships between strain **C** and members of the genus *Nocardiopsis* using Maximum Likelihood method with 1000 bootstrap replications.

On the other hand, Phylogenetic tree (Figure4), showed that strain **D** belongs to the genus *Saccharomonospora*. The similarity values between strain **D** and the selected closely related *Saccharomonospora* species ranges from 99.7 to 98.5%. It is evident also from the tree that strain **D** closely related to *Saccharomonospora azurea* with bootstrap support (91%) and it could represent a new species of the genus *Saccharomonospora*.

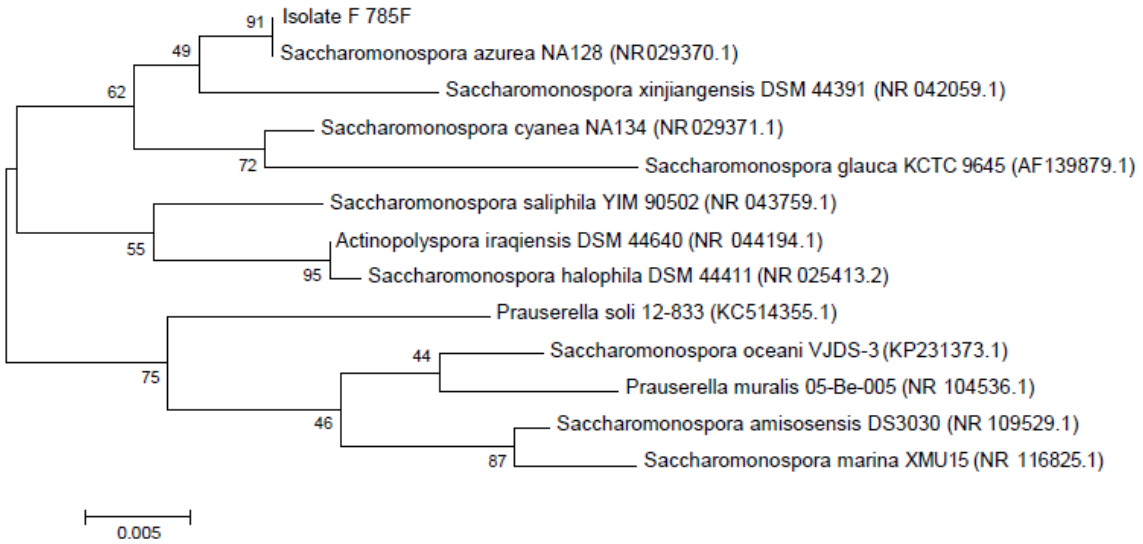


Fig. 4: Unrooted phylogenetic tree based on nearly complete 16S rDNA sequences showing relationships between strain **D** and members of the genus *Saccharomonospora* using Maximum Likelihood method with 1000 bootstrap replications.

Discussion:

A widely numerous applications of bacterial community isolated from wastewater sludge are employed. For de-nitrification and polyphosphate accumulation in biological removal of nutrient about 165 denitrifying bacteria were isolated from activated sludge (JØrgensen. *et al*. 1995). Several authors have been studied on biodiversity of bacterial community isolated from activated sludge (Barberio *et al*. 2001; Picard *et al*. 2000). *Nocardiopsis alba* isolated from different compost facilities (Paściak *et al*. 2014). All those are in agree with our results of isolation of different strains of bacteria and actinomycetes from SS and using them in the biological treatment. Actinomycetes are gram-positive mycelia (soil bacteria) having ability to synthesize a wide variety of antibiotics and biologically active compounds as well as they produce extracellular hydrolytic enzymes to obtain nutrients and energy by solubilizing polymeric compounds in soil (Stamford *et al*. 2001). Hozzein *et al*. (2011) isolated 10 strains of actinomycetes and record their ability in the treatment. All previous references are in agree with our results which indicated that the two potent actinomycetes isolated from SS have high ability in degrading and treatment of SS. The microbial species associated with the degrading polymers were identified as bacteria (*Pseudomonas, Streptococcus, Staphylococcus, Micrococcus, Moraxella*), fungi (*Aspergillus niger, Aspergillus glaucus*)). *Actinomycetes* sp. and *Saccharomonospora* genus (Swift 1997). The genus *Nocardiopsis* was created by (Meyer 1976) to harbor *Actinomadura dassonvillei* on the basis of morphological and chemotaxonomic properties. The genus currently comprises seven validly described species, namely *Nocardiopsis alba*, *Nocardiopsis dassonvillei*, *Nocardiopsis halophila*, *Nocardiopsis listeri*, *Nocardiopsis lucentensis*, *Nocardiopsis prasina* and *Nocardiopsis synnemataformans* (Al-Tai and Ruan 1994; Kroppenstedt 1992; Yassin *et al*. 1993, 1997).The members of *Nocardiopsis* are phylogenetically coherent and form a monophyletic clade that is equated with the family *Nocardiopsaceae* (Rainey *et al*. 1996). The genus name *Saccharomonospora* was derived from the Greek words for sakchâr, sugar, monos, single or solitary, and spora, a seed or spore, meaning the sugar (-containing) single spored (organism) (Euzéby 1997). The species epithet was derived from the Latin adjective azurea, azure, referring to the color of the areal mycelium (Runmao 1987).

Runmao *et al*. (1987) reported that *Saccharomonospora azurea* is a member of the genus *Saccharomonospora*, which is in the family *Pseudonocardiaceae* and thus far poorly characterized genomically and also Strain NA-128T is a strain of the species *Saccharomonospora azurea*. The formation of single spores mainly on the aerial mycelium places strain NA-128T in the *Saccharomonospora* genus. Two species, *S. viridis* and *S. yunnanensis* have been described previously (Chenglin *et al*. 1985 ; Nonomura and Ohara 1971) as species of the *Saccharomonospora* genus. These two species, however, principally differ from strain NA-128T as the two previously described species from aerial mycelia having a greenish color; only strain NA- 128T forms an azure aerial mass (Runmao 1987**)**. Also (Garrity 2010) indicated that *Saccharomonospora azurea* one of nine species currently in the genus *Saccharomonospora* . This genus forms a distinct clade within the evolutionary radiation encompassed by the family *Pseudonocardiaceae* (Kim *et al*. 1995). Members of the genus *Saccharomonospora* are of interest because they may play a role in the primary degradation of plant material by attacking hemicellu- lose also they originate from diverse habitats, such as compost, leaf litter, manure, the surface of peat (Göker *et al*. 2012). All mentioned reviews are in greed with our results.

Conclusion:

A novel bacterial and actinomycetes strains can be isolated from sewage sludge (SS) under aerobic conditions. The ability of all isolates to treatment sewage sludge tested previously. The two potent actinomycetes in the treatment were identified by 16S rRNA and phylogenetic analysis and they were defined as *Nocardiopsis lucentensis* and *Saccharomonospora azurea*, *Saccharomonospora azurea* proposed as a new species.

Compliance with ethical standards:

Conflict of interest:

The authors declare that they have no conflict of interest.

REFERENCES

Ahring, B.K., 2003. Perspectives for anaerobic digestion. In: Advances in Biochemical Engineering/Biotechnology, T. Scheper (ed.), Springer-Verlag, Berlin Heidelberg, (81).

Al-Tai, A.M., J.S. Ruan, 1994. *Nocardiopsis halophila sp*. nov.a new halophilic actinomycete isolated from soil, Int J Syst Bacteriol, (44): 474-478.

Atlas, R.A., 1993. Handbook of Microbiological Media. CRC Press, Ann Arbor.

Barberio, C.L., D.C. Pagliai, R. Fani, 2001. Biodiversity and horizontal gene transfer in culturable bacteria isolated from activated sludge enriched in nonylphenol ethoxylates. Res.Microbiol, (152): 105-112.

Barros, R.R., G.S. Carvalho, J.M. Peralta, R.R. Facklam, L.M. Teixeira, 2001. Phenotypic and Genotypic Characterization of Pediococcus Strains Isolated from Human Clinical Sources. J. Clin. Microbiol, (39): 1241-1246.

Bille, J., B.B. Catimel, E. annerman, C. Jacquet, M.N. Yersin, I. Caniaux, D. Monget, J. Rocourt, A.P.I. Listeria, 1992. a New and Promising One-Day System to Identify Listeria Isolates. Appl. Environ. Microbiol, (58): 1857-1860.

Chaillan, F., A. Le Fleche, E. Bury, Y. Phantavong, P. Grimont, A. Saliot, J. Oudot, 2004. Identification and biodegradation potential of tropical aerobic hydrocarbon degrading microorganisms. Research in Microbiology, (155): 587-595.

Chenglin, J., X. Lihua, 1985. A study on aquatic actinomycetes in the Plateau Lakes in Yunnan. V: A new species of genus *Saccharomonospora*. Acta Microbiol. Sin., (25): 363-365.

Chun, J., H.D. Youn, Y.I. Yim, H. Lee, M.Y. Kim, Y.C. Hah, S.O. Kang, 1997. *Streptomyces seoulensis sp*. nov. Int. J. Syst. Bacteriol, (47): 492-498.

Collins, M.D., J.A.E. Farrow, V. Katic, O. Kandler, 1984. Taxonomic studies on *streptococci* of serological groups E, P, U and V: description of *Streptococcus porcinus sp*. nov. Syst. Appl. Microbiol, (5): 402-413.

Collins, M.D., R.A. Hutson, L. Hoyles, E. Falsen, N. Nikolaitchouk, G. Foster, 2001. *Streptococcus ovis sp*. nov. isolated from sheep. Int. J. Syst. Evol. Microbiol, (51): 1147-1150.

Collins, M.D., D. Jones, J.A.E. Farrow, R. Kilpper-Bälz, K.H. Schleifer, 1984. *Enterococcus avium nom*. rev., comb. nov.*; E.casseliflavus* nom. rev., comb. nov.; *E. durans* nom. rev., comb. nov.; *E. gallinarum* comb. nov.; and *E. malodoratus sp*.nov. Int. J. Syst. Bacteriol, (34): 220-223.

Collins, M.D., P.A. Lawson, 2000. The genus *Abiotrophia* is not monophiletic: proposal of *Granulicatella* gen. nov., *Granulicatella adiacens* comb.nov., *Granulicatella elegans* comb. nov.and *Granulicatella balaenopterae* comb. nov. Int. J. Syst. Evol. Microbiol, (50): 365-369.

Coykendall, A.L., 1989. Classification and Identification of the *Viridans Streptococci*. Clin. Microbiol. Rev., (2): 315-328.

Devriese, L.A., R. Kilpper-Bälz, K.H. Schleifer, 1988. *Streptococcus hyointestinalis sp*. nov. from the gut of swine. Int. J. Syst. Bacteriol, (38): 440-441.

Elsayed, K.N.M., T.A. Kolesnikova, A. Noke, G. Klöck, 2017. Imaging the accumulated intracellular microalgal lipids as a response to temperature stress, 3 Biotech, (7): 41. DOI: 10.1007/s13205-017-0677-x

Embley, T.M., E. Stackebrandt, 1994. The molecular phylogeny and systematics of the actinomycetes. Ann. Rev. Microbiol, (48): 257-289.

Farrow, J.A.E., R.R. Facklam, M.D. Collins, 1989. Nucleic acid homologies of some *vancomycin*- resistant *leuconostocs* and description of *Leuconostoc citreum sp*. nov. and *Leuconostoc pseudomesenteroides sp*. nov. Int. J. Syst. Bacteriol, (39): 279-283.

Freney, J., F. Renaud, W. Hansen, C. Bollet, 2000. Précis de bactériologie clinique, ESKA, Paris, France.

Fritsche, W., M. Hofrichter, 2005. Aerobic degradation of recalcitrant organic compounds by microorganisms. In: Jördening, H.J., Winter, J. (Eds.), Environmental Biotechnology, Concepts and Applications. Wiley-VCH, Weinheim, Germany, (32): 203-227.

Garrity, G., NamesforLife, 2010. Browser Tool takes exper-tise out of the database and puts it right in the browser. Microbiol Today, (37): 1-9.

Göker John, C., Detter, C. Nikos, Kyrpides, T. Woyke, 2012. Genomesequence of the soil bacterium *Saccharomonospora azurea* type strain (NA-128T). Standards in Genomic Sciences, (6): 220-229.

Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley, S.T. Williams, 1994. Bergey's Manual of Determinative Bacteriology, 9th ed. Williams and Wilkins, Baltimore, Maryland.

Hozzein, W.N., M.B. Ahmed, M.S.H. Abdel Tawab, 2011. Efficiency of some actinomycete isolates in biological treatment and removal of heavy metals from wastewater. African Journal of Biotechnology, 11(5): 1163-1168.

Ishak1, W.M.F., S. Jamek, N.A. Jalanni, N.F. Mohd Jamaludin, 2011. Isolation and Identification of Bacteria from Activated Sludge and Compost for Municipal Solid Waste Treatment System, (24): 450-454.

Johnson, L.F., E.A. Curl, J.H. Bond, H.A. Fribourg, 1959. Methods for studying soil 287 microflora- plant disease relationships, Burgess, Minnegpolis.

Jørgensen, K.S., A.S.L. Pauli, 1995. Polyphosphate accumulation among denitrifying bacteria in activated sludge. Anaerobe, (1): 161-165.

Kamaluddeen, Kabir, Yerima, Bello M, Abu, Rimi T, Deeni, Yusuf, 2016. Biostimulatory Effect Of Processed Sewage Sludge In Bioremediation Of Engine Oil Contaminated Soils, (5): 203-207.

Kawato, N., R. Shinobu, 1959. On *Streptomyces herbaricolor*, nov. sp. Supplement: A simple technique for the microscopic observation. Memoirs of the Osaka University of the Liberal Arts and Education, B. Nat. Sci., (8): 114- 119.

Kilpper-Bälz, R., K.H. Schleifer, 1987. *Streptococcus suis sp*. nov., nom. rev. Int. J. Syst. Bacteriol, vol. (37): 160-162.

Kim, B., A.M. Al-Tai, S.B. Kim, P. Somasundaram, M. Goodfellow, 2000 *Streptomyces thermocoprophilus sp*. nov., a cellulase-free endo-xylanase-producing streptomycete. Int. J. Syst. Evol. Microbiol, (50): 505- 509.

Kim, B., N. Sahin, D.E. Minnikin, J. Zakrzewska-Czerwinska, M. Mordarski, M. Goodfellow, 1999. Classification of thermophilic *streptomycetes* including the description of *Streptomyces thermoalcalitolerans*. Int. J. Syst. Bacteriol, (49): 7- 17.

Kim, S.B., 1995. Polyphasic taxonomic studies on the genus *Saccharomonospora* and related taxa. Ph.D thesis. University of Newcastle upon Tyne,Newcastle upon Tyne, United Kingdom.

Krieg, N.R., J.G. Holt, 1984. Bergey's Manual of Systematic Bacteriology, volume 1. William & Wilkins, Baltimore, Maryland.

Kroppenstedt, R.M., 1992. The genus *Nocardiopsis*. In The Prokaryotes, Edited by A. Balows, H. G. Tru$ per,M. Dworkin,W. Harder and K. H. Schleifer. New York: Springer, pp: 1139-1156.

Larkin, M.A., G. Blackshields, N.P. Brown, R. Chenna, P.A. McGettigan, H. McWilliam, F. Valentin, I.M. Wallace, A. Wilm, R. Lopez, J.D. Thompson, T.J. Gibson, D.G. Higgins, 2007. Clustal W and Clustal X version 2.0. Bioinformatics, (23): 2947-2948.

Lemmer, H., 1986. The ecology of scum causing actinomycetes in sewage treatment plants. Water Res., (20): 531-535.

Lemmer, H., R.M. Kroppenstedt, 1984. Chemotaxonomy and physiology of some actinomycetes isolated from scumming activated sludge. Syst. Appl. Microbiol, (5): 124-135.

McCarthy, A.J., 1987. Lignocellulose-degrading actinomycetes. FEMS Microbiol Rev., (46): 145-163.

Mendes, B., M. Nascimento, 1991. Zentralbl. Hyg. Umweltmed, (190): 471-473.

Meyer, J., 1976. Nocardiopsis, a new genus of the order Actinomycetales, Int J Syst Bacteriol, (26): 487-493.

Mitchel, R., 1976. Microbiology of polluted water, Moskva: Medicina (in Russian).

Morelli, I.S., M.T. Del Panno, G.L. De Antoni, M.T. Painceira, 2005. Laboratory study on the bioremediation of petrochemical sludge contaminated soil. International Biodeterioration and Biodegradation, (55): 271-278.

Murray, P.R., E.J. Baron, M.A. Pfaller, F.C. Tenover, R.H. Yolken, 1999. Manual of Clinical Microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

Nonomura, H., Y. Ohara, 1971. Distribution of actinomycetes ih soil. X. New genus and species of *monosporic actinomycetes*. J. Ferment. Technol, (49): 895-903.

Omer, A.M., 2012. Production of Organic Biofertilizer from Olive Mill Waste Wate .Australian Journal of Basic and Applied Sciences, 6(10): 654-663.

Picard, C.D., F. Cello, R. Ventura, Fani and A. Guckert, 2000. proposal of *Nocardiopsaceae* fam. nov, Int J Syst Bacteriol, (46): 1088-1092.

Poyart, C., G. Quesne, P. Trieu-Cuot, 2002. Taxonomic dissection of the *Streptococcus bovis* group by analysis of manganese-dependent superoxide dismutase gene (sodA) sequences: reclassification of *Streptococcus infantarius subsp*. *coli* as *Streptococcus lutetiensis sp*.nov. and of *Streptococcus bovis* biotype II.2 as *Streptococcus pasteurianus sp* nov. Int. J. Syst. Evol. Microbiol, (52): 1247-1255.

Rainey, F.A., N. Ward-Rainey, R.M. Kroppenstedt, E. Stackebrandt, 1996. The genus *Nocardiopsis represents* a phylogenetically coherent taxon and a distinct actinomycete lineage: Runmao H. *Saccharomonospora azurea sp*. nov., a new species from soil. Int J Syst Bacteriol 1987; (37): 60-61.

Runmao, H., 1987. *Saccharomonospora azurea sp*. nov., a new species from soil. *Int J Syst Bacteriol*, (37): 60-61.

Schlegel, L., F. Grimont, M.D. Collins, B. Regnault, P.A.D. Grimont, A. Bouvet, 2000. *Streptococcus infantarius sp.* nov., *Streptococcus infantarius subsp* infantarius subsp. nov. and *Streptococcus infantarius subsp coli subsp*. nov., isolated from humans and food. Int. J. Syst. Evol. Microbiol, (50): 1425-1434.

Shaheen, S., M. Khan, M.J. Khan, S. Jilani, Z. Bibi, M. Munir, M. Kiran, 2017. Effective Microorganisms (EM) co-applied with organic wastes and NPK stimulate the growth, yield and quality of spinach (Spinacia oleracea l.). Sarhad Journal of Agriculture, 33(1): 30-41.

Shirling, E.B., D. Gottlieb, 1966. Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol, (16): 313- 340.

Stackebrandt, E., C.R. Woese, 1981a. Towards a phylogeny of actinomycetes and related organisms. Curr. Microbiol, (5): 131-136.

Stackebrandt, E., C.R. Woese, 1981b. The evolution of prokaryotes. In Carlile, M.J. Collins, J.F. and Moseley, B.E.B. (eds.), Molecular and Cellular Aspects of Microbial Evolution, Cambridge University Press, Cambridge, pp: 1- 31.

Stamford, T.L.M., N.P. Stamford, L.C.B.B. Coelho, J.M. Araújo, 2001. Production and characterization of a thermostable α-amylase from *Nocardiopsis sp*. endophyte of yam bean. Bioresour Technol, (76): 137-141.

Swift, G., 1997. Non-medical biodegradable polymers: environmentally degradable polymers. In: Handbook of biodegradable polymers. Hardwood Academic, Amsterdam, pp: 473-511.

Tamura, K., G. Stecher, D. Peterson, A. Filipski, S. Kumar, 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Mol. Biol Evol., (30): 2725-2729.

Thangaraj, K., A. Kapley, H.J. Purohit, 2007. Characterization of diverse *Acinetobacer* isolates for utilization of multiple aromatic compounds. Bioresource Technology. doi:10.1016/j.biortech.04.053.

Van Hamme, J.D., A. Singh, O.P. Ward, 2003. Recent advances in petroleum microbiology. Microbiology and Molecular Biology Review, (67): 503-549.

Viera, V.V., L.M. Teixeira, V. Zahner, H. Momen, R.R. Facklam, A.G. Steigerwalt, D.J. Brenner, A.C.D. Castro, 1998. Genetic relationships among the different phenotypes of *Streptococcus dysgalactiae* strains. Int. J. Syst. Bacteriol, (48): 1231-1243.

Whiley, R.A., L.M.C. Hall, J.M. Hardie, D. Beighton, 1999. A study of small colony beta hemolytic, Lancefield group C *streptococci* within the anginosus group: description of *Streptococcus constellatus* subsp. pharynges subsp.nov., associated with the human throat and pharyngitis. Int. J. Syst. Bacteriol, (49): 1443-1449.

Williams, S.T., T. Cross, 1971. Actinomycetes. In Booth, C. (ed.), Methods in microbiology. Academic Press, London, (4): 295-335.

Yassin, A.F., E.A. Galinski, A. Wohlfarth, K.D. Jahnke, K.P. Schaal, H.G. Trueper, 1993. A new actinomycete species, *Nocardiopsis lucentensis sp*. nov, Int J Syst Bacteriol, (43): 266-271.

Yassin, A.F., F.A. Rainey, J. Burghardt, D. Gierth, J. Ungerechts, I. Lux, P. Seifert, C. Bal, K.P. Schaal., 1997. Description of *Nocardiopsis synnemataformans sp*. nov., elevation of *Nocardiopsis alba subsp.* prasina to *Nocardiopsis prasina* comb. nov., and designation of *Nocardiopsis antarctica* and *Nocardiopsis alborubida* as later subjective synonyms of *Nocardiopsis dassonvillei*, Int J Syst Bacteriol, (47): 983-988.

Zorenzana, Z.M., E. Becates, M.Z. Zopez, 1992. 6th International Symposium on Microbiology and Ecology, 6-11 Sept., Barcelona, Spain, abstracts, 224.