JOURNAL OF PURE AND APPLIED MICROBIOLOGY, June 2014. Vol. **8**(3), p. 2283-2290

Preliminary Studies on Actinomycetes Diversity Isolated from Afghanistan Using Water Agar

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(Received: 06 April 2014; accepted: 18 May 2014)

Three soil samples were collected from different locations in Afghanistan. The actinomycetes were isolated in tap water agar (TWA) medium. Colony forming unit was calculated for the samples per ml and color grouping was noted. 50 actinomycetes isolates were obtained with a wide range color groups including pale white. Yellow white, dark gray, pale brown, white, pink, yellow and yellow brown. Five strains have been selected for the preliminary study for different biological characteristics based on the appearance and color group. The isolates were characterized culturally, physiologically and biochemicallyand shown to be different from each other. Antibacterial test was done against seven human pathogenic microorganisms which were *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus*ATCC 25923, *salmonella suis* ATCC 13076, *Pseudomonas aeruginosa* ATCC 27583, *Escherichia coli* ATCC 25922 and *Shigellasonnei* ATCC 11060 and one microorganism was human pathogenic fungus: *Candida albicans* ATCC 10231. One strain of the five turned out to be active against one pathogenic bacterium. Further molecular and chemical studies will contribute to the science that many strains isolated from Afghanistan soil might be new species and their secondary metabolites would be the potential values in the biotechnological company.

Key words: Actinomycetes, *Streptomyces*, color diversity, Afghanistan, antibacterial activity.

Actinomycetes are one of the microorganismsgroupsthat is widely distributed in nature and noted mostly to inhabit the soil[1]. This group isone of the most attractive families of industrial bacteria on account of their superior potential for producing valuable secondary metabolites including antibiotics, anti-cancer drugs, immunosuppressors and enzyme inhibitors[2,3]. These bacteria are Gram positive, free living, saprophytic bacteria, widely distributed in soil, water and colonizing plants. Their population has been identified as one of the major group of soil population. The actinomycetes are noteworthy known as antibiotic producers, making three quarters of all known products. The species belonging to the genus *Streptomyces* are especially prolific and can produce many types of antibiotics and other class of biologically active secondary metabolites.

Streptomyces constitute 50% of the total population of soil actinomycetes and 75-80% of the commercially and medicinally useful antibiotics have been derived from this genus[4]. The list of novel microorganisms and products derived from poorly explored areas of the world like China, Australia, Antarctica and Jordan suggests that a careful exploration of new habitats might continue to be useful[3]. The search for new antibiotics continues to be most important in research

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programs around the world [5]because the increase of the resistant pathogens and toxicity of some used antibiotics. Among microorganisms actinomycetes are one of the most investigated groups particularly members of the genus *Streptomyces* from which, a large number of antibiotics was obtained and studied[6]. The vast majority of actinomycetes have originated from soil[7] and their isolation method deal almost exclusively with those suitable for *Streptomyces* species which grow rapidly on soil dilution plates.

Afghanistan is a country located in south-central Asia, it is a high, landlocked country bordered on the west by Iran and on the east and south by Pakistan. Its northern neighbors are Turkmenistan, Uzbekistan, and Tajikistan and China lies to the northeast (Figure 1).

There is wide variation in the country's geography, it is known for its mountainous terrain. The huge Hindu Kush Mountains from a barrier between the Northern provinces and the rest of the country. This mountain range has also divided Afghanistan into three very different geographical regions known as: the Central Highlands, the Northern Plains and the Southwestern Plateau. The altitude, climate and soil condition in Afghanistan varies greatly on where in the country a person is (http://www.afghan-web.com/geography/lr.html).

Climate of Afghanistan is immensely varied. The country experiences extremes of climate. Afghanistan on the whole is dry, falling under desert classification. Summers are hot and dry, while winters are cold and snowy. Roughly snow season falls in mountains in October-April. However, it varies with elevation. Various regions of the country have significant regional variations. North-east region has subarctic climate with dry, cold winters, while the areas adjacent to Pakistan border are influenced by Indian monsoons. In south-west, strong winds blow during summer (http://afghanistan.saarctourism.org/geography.html).

Afghanistan has been through many wars with different forces and each of those used their own developed weapons which among were the nuclear weapons that jeopardized the ecosystem of the country and specially the soil since it absorbs most of the particles. Having this fact in mind, the soil habitat could be changed in the phenotypic and genotypic backgrounds. This preliminary study focused on the actinomycetes diversity in Afghanistan and their antimicrobial activity to find new and unique bioactive compounds.

MATERIALS AND METHODS

Source of soil samples

Three soil samples were collected in clean sterile plastic bags from three different geographical locations in Afghanistan. (Figure 1a andb). Several diverse habitats in different areas were selected for the isolation of actinomycetes isolates. These habitats included the rhizosphere of plants and agricultural soil. The samples were taken from up to 20 cm depth, after removing approximately 3 cm of the soil surface.

Preparation of samples and actinomycetes isolation

The soil samples were sieved and air-dried at room temperature for seven days. The actinomycetes were isolated from the air dried soil samples by soil dilution plate technique on the modified TWA medium and incubated at 30^{o}C for 4 weeks. The actinomycetes colonies were picked up and purified on yeast extract- starch agar (YSA) medium without adding any kind of antifungal or antibacterial agents[8].

Colony forming unit (CFU)

Plate count technique was used to estimate the viable cells number, Actinomycetes counts in the soil samples ranged from 108x10^{2}cfu/g to 220x10^{2}cfu/g.

Actinomycetes color groups found in the soil samples

All the selected actinomycetes isolates were purified using Yeast extract-Soluble starch agar (YSA). The isolates were incubated for 7 days at 30^{o}C. Then the physiological characteristics and color groups of each selected isolates were observed on YSA agar medium (Table 1) and (Figure 2).

Physiological and biochemical test

Growth on NaCl

Sodium chloride tolerance level of the studied strains was evaluated on yeast extract agar medium supplemented with graded doses of sodium chloride (7% and 10%)[9,10]. An inoculum of each of the fiveactinomycetes strains under the study were cultured on the previously mentioned media and incubated at 30^{o}C for 7days.

Gelatin hydrolysis test

Gelatin is solid at room temperature; some bacteria have the ability to hydrolyze gelatin and turn it in to liquid. For this test Nutrient gelatin (NG) tubes were prepared containing [120 g gelatin, 3 g beef extract, 10 g peptone, 1 L distilled water] for each strain which made a total of 5 tubes including one as control tube. Each gelatin tube was inoculated by stabbing with a needle containing some of the stock culture. The tubes were incubated at room temperature (about 20º C)[11]. The tubes were gently placed in the refrigerator for 15 minutes every few days. The incubation of the cultured gelatin tubes continued for two weeks which after all tubes were examined for gelatin hydrolysis.

Catalase test

In this test two drops of 3% Hydrogen peroxide (H_20_2) were added to the bacterial culture, bubble appearance would indicate a positive result[12].

Casein hydrolysis

Skim Milk Agar (SMA) media plates were prepared containing; [5 g skimmed milk, 2 g agar and 300 ml distilled water]. The plates were labeled with the sample name, initial and date. Aseptic technique was used to inoculate the skim milk agar plates thru streaking a short line of the desired strains on the SMA surface with a loop. The plates were inoculated at 30º C for 7 days[13]. After incubation, all plates were examined for clear zone around the bacterial growth on the plate.

Urea hydrolysis

For this test Urea broth (UB) tubes were prepared containing [(0.1 g yeast extract, 9.1 g potassium phosphate monobasic, 9.5 g potassium phosphate dibasic, 20 g urea, 0.01 g phenol red and 1 L distilled water)]. An inoculum from a pure culture of the selected strains was transferred aseptically to sterile tubes of urea broth. The inoculated tubes were incubated at 30º C for 7 days then the results were determined[11].

Citrate utilization

Simmons Citrate broth (SCB) medium was prepared containing (24.5 g simmons citrate in 1 L of distilled water). The broth was distributed in tubes which were later inoculated using a fresh culture. The selected cultures were transferred to the SCB tubes. The tubes were incubated at 30º C for 7 days[9].

Kligler Iron Agar (KIA) media in test tubes was used for this experiment; [containing 55 g kligler iron agar in 1 L distilled water]. An inoculum from a pure culture was transferred aseptically to the sterile tubes containing the medium. The inoculated tubes were incubated at 30º C for 7 days and the results were determined[9]. The presence of hydrogen sulfide was also determined by observing the KIA tubes after incubation[9]. The formation of hydrogen sulfide gas which is black mass indicates a positive result.

Scanning electron microscopy for morphological characterization

Bacterial cells were prepared for SEM (scanning electron microscopy) (JEOL, JSM, 3060) at King Saud University Central Laboratory, Saudi Arabia according to the initial fixation and dehydration steps previously published[14]. The cells were fixed at 24°C overnight with 3% glutaraldehyde (Sigma-Aldrich ChemieGmbh, Steinheim, Germany), washed with distilled water 3 times each for 10 minutes , fixed again with 1% Osmium tetroxide overnight , washed once more with distilled water as before ,dehydrated with a serial ascending concentrations of ethanol as follows (30% for10 min, 50% for 10 min, 60% for 10 min, 70% for 10 min, 80% for 10 min, 90% for 10 min, 100% for 10 min, 100% again overnight), and then dried on a critical point dryer (HCP-2; Hitachi Co.). The dried cell samples were gold coated using a gold sputter (JEOL, JFC 1600) and examined using a SEM (JEOL, JSM, 3060)[15].

Thin layer chromatography (TLC)

Each sample was applied as 3 µl on the base line of a cellulose TLC plate (20 cm X 20 cm) (Merck No.126827) using a capillary tube. As a standard, 1 µl of 10mg (0.01g) DL-2, 6-Diaminopimelic acid (DL-DAP) (Sigma Chemical Co., St. Louis Mo., USA) was also applied. This authentic material is a mixture of DAP isomers. TLC was developed with the solvent system methanol-water-6N HCl-pyridine (80:26:4:10 v/v) in a lid covered tank.

Development took approximately 4 h. The spots were visualized by spraying with 0.2% ninhydrin solution containing (0.2g ninhydrin (Merck, GERMANY), 100ml Acetone (Winlab, UK) (See Appendix II) followed by heating at 100oC for 5 min. DAP isomers appeared as violet color spots with R,0.29 (LL-isomer) and 0.24 (meso- and DD-isomer)[16]

RESULTS

Isolation of actinomycetes

Total 50 colorful diverse actinomycetes strains were obtained from isolation on TWA and five of those were selected for this biological and chemical study.

Colony count

The colony count calculation resulted due to the numbers of Colony Forming Units (CFU) per 1 ml of soil. Three dilutions were used for the isolation of actinobacteria. Among these, dilution 1 was too many colonies to count, so it was excluded. Later, dilution 2 and 3 were used for all the experiments and in Table 2 shows the CFUs for dilutions 2 and 3.

Color grouping

Color grouping of the mature culture

All fifty strains were purified on YSA and the color groups were noted after full growth at 30°C for 2 weeks. Among the 50 isolates, five strains were chosen based on the different color groups of the aerial mycelium for preliminary studies.

Antimicrobial activity

The five selected actinomycetes isolated were tested for their antimicrobial activity against seven pathogenic microorganisms which were: *S. aureus* ATCC 13076, *B. subtilis* ATCC 6633, *S. sonnei* ATCC 11060, *E. coli* ATCC 2592, *P.aeruginosa* ATCC 27583, *S. suis* ATCC 13076 and *C. albicans* ATCC 10231. From these five isolates only one showed antimicrobial activity against one pathogenic microorganism and these five strains were labeled as AK 13, AS 13, AS 14, AS 17 and AP1. These antimicrobial activity (zone of inhibition

Table 1. The actinomycetescolor groups from the Afghanistan's soil samples isolates

No	Colony color (with aerial mycelium color)	Percentage
1	Pale white	14%
2	Yellow white	18%
3	Dark gray	8%
6	Light yellow	2%
7	Whitish gray	6%
8	Whitish brown	4%
9	Yellow	6%
10	White	20%
11	Pale brown	2%
12	Brownish white	4%
13	Whitish yellow	2%
14	Gray	2%
15	Orange brown	4%
16	Yellow brown	2%
17	Reddish brown	2%
18	Pink	2%

Table 2. Colony Forming Unit (CFU) calculated from soil dilutions collected from Afghanistan

Isolation sites	10^{-1}	10^{-2}	10^{-3}
Karghah	To many colonies to count	170×10^2 CFU/ml	40×10^3 CFU/ml
Policharkhi	To many colonies to count	108×10^2 CFU/ml	35×10^3 CFU/ml
Shpula	To many colonies to count	220×10^2 CFU/ml	105×10^3 CFU/ml

Table 3. The antimicrobial test results against seven pathogenic microorganisms using agar well diffusion method.

Code no. of the Isolates (methanol extract)	Pathogenic microorganisms						
	Gram negative bacteria			Gram positive bacteria Yeast			Yeast
	(9) *S. suis* ATCC 13076	(10) *E. coli* ATCC 2592	(11) *S. sonnei* ATCC 11060	(12) *P.aeruginosa* ATCC 27583	(13) *B. subtilis* ATCC 6633	(14) *S. aureus* ATCC 13076	(15) *C. albicans* ATCC 10231
AK13	-	-	-	-	-	-	-
AS 13	-	-	-	-	-	-	-
AS 14	-	-	-	-	-	-	-
AS 17	-	-	-	-	-	-	-
AP 1	-	-	-	-	-	15mm	-

Table 4. Cultural characteristics, diffusible pigments andcolor groups of the selectedactinomycetes strains

No	Strain's code	Growth rate	Arial mycelium	Substrate mycelium	Pigmentation description	Growth
1	AK 13	++	Blackish white	Black	Brown	Spread
2	AS 13	++	Chalky white	Black	Brown	Compact
3	AS 14	+	Red	Red	Dark red	Compact
4	AS 17	+	Whitish black	Black	Light Brown	Compact
5	AP 1	++	Grayish white	Creamy	-	Spread

Table 5. The physiological test results of the five selective actinomycetes strains in the study

Strain No	NaCl Conc. 1	7	10	Gelatin	Catalase hydrolysis	Urea hydrolysis	Citrate Utilization	Sugar fermentation Glucose	Lactose	H_2O production
AK13	+	-	-	+	+	+	+	+	-	-
AS13	+	-	-	+	-	-	+	+	-	-
AS14	+	-	-	+	-	-	+	+	-	-
AS17	+	-	-	+	+	-	-	-	-	-
AP1	+	+	-	+	-	+	-	-	-	-

is 15 mm) of the selected strain is shown in Figure 3 and Table 3.

Cultural Characteristics

Cultural characteristics were determined on YSA showing the culture's shape, color, growth rate and pigmentation diffusion for the selected five isolates obtained from the soil of Afghanistan. The results are shown in (Table 4)

Physiological and biochemical characteristics

Growth on NaCl

Among the five selected strains, the strain AP1 grown on YSA agar media with 7% NaCl but not the YSA media with 10% NaCl.

Gelatin hydrolysis test

This test was prepared to study the activity of the gelatinase enzyme. The tubes were observed after incubation for two weeks. The result showed that all five strains had the ability to hydrolyze gelatin completely.

Catalase test

The positive result to the catalase test is exhibited in the form of bubbles appearance in the surface of strain after adding a drop of hydrogen peroxide H_2O_2 to it. Among the five strains AK13 and AS17 have shown positive result to the catalase test while the strains AS13, AS14 and AP1 were negative.

Casein hydrolysis

The appearance of clear zone on Skim Milk agar (SMA) media around the bacterial growth area is an indicator for the presence of proteolyticactivity . Two of the five isolates under this study had shown proteolytic activity in the SMA plates which can be seen from the appearance of clear zones around the strain colonies which were AP1 and AK13, the three others were not active as there was no zone of clarity around them which were AS13, AS14 and AS17.

Urea hydrolysis

Four out of five strains have shown positive result to this test which were AS13, AS14, AS 17 and AP1, the negative strain was AK13.

Citrate utilization

The change of color from green to blue indicates a positive result. In this test three of the fiveactinomycetes strains showed a positive result after two weeks of incubation which were AK 13, AS13 and AS14, the other two were negative to the test as the color of the Simmons citrate broth remained green.

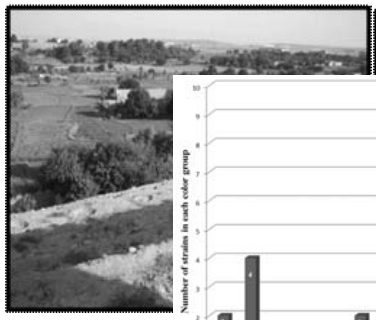
Glucose and Lactose fermentation test

Three strains out of five were positive to glucose fermentation test which were AS 17, AS14 and AK13 and the two others were negative to the test. In lactose fermentation test all five strains

have shown negative result.

Hydrogen sulfide (H2S) production test

All strains were negative to this test as there was no any indicator of hydrogen sulfide gas in the KIA tubes. The results are shown in table 4.



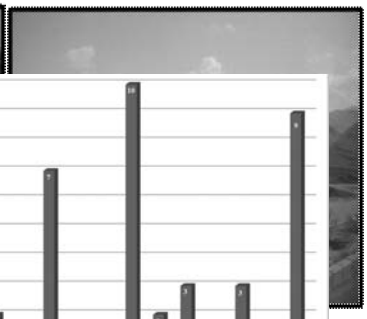


Fig. 1(a). Map of Afghanistan showing the borders and an

Fig. 2. Actinomycetes color group diversity in Afghanistan's soil

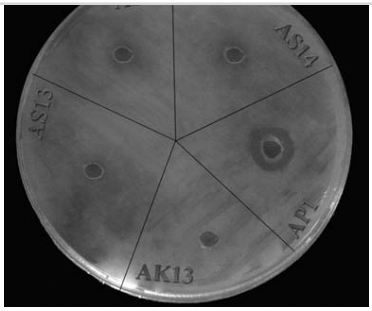


Fig. 3. The antimicrobial activity (zone of inhibition 15 mm) test showing the positive result for the strain AP1 against human pathogenic bacteria *S. aureus* ATCC 13076

DISCUSSION

Gram-positive, filamentous, actinomycetes group was isolated from Afghanistan; our data indicated that it is an eminently suitable ecosystem for diverse actinobacteria group. Actinomycetes counts ranged from 50×102 cfu/g to 700×102 cfu/g in the soil samples collected in Afghanistan. A total of 50 actinomycetes were isolated using simple dilution technique with distilled water on TWA medium.

The isolates exhibited a range of diverse aerial mycelium colors (such as: grey, dark grey, pale grey, white grey, grey white, white, off white, chalk white, blackish grey, pink white, red white, brown red, dark brown, brown white, cream white, yellow and yellow white) on yeast extract-starch soluble agar (YSA) medium.

The highest number of actinomycetes group was found in soil samples collected from Shpula area in Afghanistan which is known for its agricultural soil. In all the three soil samples about 17 actinomycetes color series were observed and among them, white, yellow white and grey series represented the most dominant color group in the study. These diverse color groups showed high possibility of the different streptomycetes strains in the soil samples. Our finding is in concordance with Dhanasekaran et al. (2008) and they reported high diversity of Streptomyces population isolated from agricultural soils.

The colony forming unit count supports that the agricultural soil of Shpula area is rich in the actinomycetes diversity as the count ranged from 105 x 103 CFU/g to 220 x 102 CFU/g which is much more when comparing with the two other areas of Karghah (40 x 103 CFU/g to 170 x 102 CFU/g) and Policharkhi (35 x 103 CFU/g to 108 x102 CFU/g).

The antimicrobial test results were unexpected as only one of the five tested strains which was AP1 turned out to be active against only one pathogenic bacteria known as Staphylococcus aureus ATCC 13076. But when examining the physiological and biochemical test results, it appeared that this strain differentiated in the results compared to other strains (pls show the table of physiological and biochemical and other characters together we can see all characters) results it was positive for proteolytic, growth on different pHs, 45°C heat tolerant and gelatin hydrolysis test and negative for all other tests. The war conditions and contamination of the country's soil could probably be the reason for some of the unexpected results obtained in this study.

Based on the colony color, aerial mycelium, growth intensity, diffusible pigmentation, substrate mycelium color, fermentation broth color, light microscopy of spore chain shape, biochemical characteristics (such as gelatin hydrolysis, catalase test, urea hydrolysis, citrate utilization, H^2S production, physiological parameters (such as growth on 7% NaCl, growth at 45°C), antimicrobial activities and cell wall diaminopimelic acid tests showed that all the selected strains are more or less different than each other. Further molecular and chemical studies will contribute to the science that many strains isolated from Afghanistan soil might be new species and their secondary metabolites would be the potential values in the biotechnological company.

CONCLUSION

The pioneer study showed that TWA medium could be one of the good isolation medium for actinomycetes and that Afghanistan soil is a rich source of different color groups of these bacteria. But as an area of war the soil ecosystem could be affected in different ways. More intensive study will be conducted further in this region explaining the reasons on some major differences in the isolated actinomycetes strains from Afghanistan and to way is the TWA medium selected as one of the best isolation Medias for actinomycetes in this study (data not shown). The habitats in Afghanistan could be considered as an unexploited resource for biotechnology that has not been well studied at all. The country has high actinomycetes diversity; more intensive study in a wider range needs to be conducted on the isolates from Afghanistan's soil to understand the reasons for so many diversities with minimized antimicrobial activity. .

ACKNOWLEDGEMENTS

The research project was supported by a grant from the "Research Center of Female Scientific

and Medical Colleges", Deanship of Scientific Research, King Saud University.

REFERENCES

1. Oskay, M., Tamer A.U., Azeri, C. Antibacterial activity of some actinomycetes isolated from soils of Turkey. *Afr. J. Biotechnol*., 2004; **3**(9): 441–446.
2. Olano, C., Me´ndez, C., Salas, J.A. Antitumor compounds from marine actinomycetes. *Mar. Drugs*., 2009; **7**: 210-248.
3. Zitouni, A., Boudjella, H., Mathieu, F., Sabaou. N., Lebrihi, A. Mutactimycin PR, a new anthracycline antibiotic from *Saccharothrix* sp. SA 103. I. Taxonomy, fermentation, isolation and biological activities. *J. Antibiot*., 2004; **57**: 367-372.
4. Lazzarini, A., Cavaletti, L., Toppo, G., Marinelli, F. Rare genera of Actinomycetes as potential producers of new antibiotics. *Antonie van Leeuwenhoek*, 2000; **78**: 399-405.
5. Takahashi, Y. Exploitation of new microbial resources for bioactive compounds and discovery of new actinomycetes. *Actinomycetologica*, 2004; **18**: 4-61.
6. Gupta, M.D., Kalkarni, P.R. A study of antifungal antibiotic production by *Streptomyces chattanoogensis* MTCC 3423 using full factorial design. *Lett. Appl. Microbiol*., 2004; **35**: 22-26.
7. Shearer, M.C. Methods for the isolation of non *Streptomycete actinomycete*. *J. Ind. Microbiol*., 1997; **28**: 1- 98.
8. Luedemann, G.M. Micromonospora purpureo chromogenes (Waksman and Curtis 1916) comb. nov. (Subjective synonym. Micromonospora fusca Jensen 1932). ; *Int. Assoc. Microbiol. Soc*., 1971; **21**(3): 240-247.
9. Reddy, N.G., Ramakrishna, D.P.N., Raja Gopal, S.V. A morphological, Physiological and Biochemical Studies of Marine *Streptomyces rochei* (MTCC 10109) showing Antagonistic Activity against Selective Human Pathogenic Microorganisms. *Asian J. Biol. Sci*., 2011; **4**: 1-14.
10. Halima, B., Imen, N., Mohammed, I.H., Naima, E.G., Maher, G., Fikri, B. Phenotypic and genotypic characterizations of rhizobia isolated from root nodules of multiple legume species native of Fez, Morocco. *Afr. J. Microbiol. Res*., 2012; **6**: 5314-5324.
11. Subhankari, P.C., Santanu, K.M., Somenath, R. Biochemical characters and antibiotic susceptibility of *Staphylococcus aureus* isolates; *Asia Pac. J. Trop. Biomed*., 2011; 212-216.
12. Ajay, K., Amit, K., Shikha, D., Sandip, P., Chandani, P., Sushila, N. Isolation, screening and characterization of bacteria from Rhizospheric soils for different plant growth promotion (PGP) activities: an in vitro study. *Recent Res. Sci. Technol*., 2012; **4**: 01-05.
13. Katekan, D., Shannaphimon, W., phichaya, T., Prapaporn, B., Arunee, A., Panupong, S., Ekachai, C. Comparative study of proteolytic activity of protease-producing bacteria isolated from Thua Nao. *Maejo Int. J. Sci. Technol*., 2009; **3**: 269-276.
14. Moore, C.W. , Del, R. , Valle, J., McKoy, A., Pramanik, Gordon, R.E. Lesions and preferential initial localization of [S-methyl- 3 H] bleomycin A2 on *Saccharomyces cerevisiae* cell walls and membranes. *Antimicrob. Agents Chemother*., 1992; **36**: 2497-2505.
15. Tamura, T., Nakagaito, Y., Nishii, T., Hasegawa, T., Stackebrandt, E., Yokota, A. A new genus of the order Actinomycetales, Couchioplanes gen. nov., with descriptions of Couchioplanesc aeruleus (Horan and Brodsky 1986) comb. nov. and *Couchioplanes caeruleus* subsp. *azureus* subsp. nov. *Int. J. Syst. Bacteriol*., 1994; **44**: 193-203.
16. Staneck, J.L., Roberts, G.D. Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. ; *Appl. Microbiol*., 1974; **28**: 226-231.