ISSN : 0974 - 7532 Volume 6 Issue 11

Trade Science Inc.

Research & Reviews in

BioSciences

Regular Paper

RRBS, 6(11), 2012 [328-334]

Taxonomy and study of antimicrobial activity of two halophilic actinomycetes AH_1 and AH_2 isolated from a saline soil of Bejaia (Algeria)

Messis Abdelaziz*, Bettache Azzeddine, Kecha Mouloud, Benallaoua Said

Laboratory of Applied Microbiology, Faculty of Nature and Life Sciences,

University Abderahmane Mira Bejaia 06000 -(ALGERIA)

E-mail : az_messi@hotmail.fr

Received: 7th August, 2012; Accepted: 9th October, 2012

ABSTRACT

In this study we are interested in the taxonomic characterization of two isolates of halophilic actinomycetes, the identification of their antimicrobial and antifungal activities, and the determination of the chemical nature of bioactive molecules. Both strains AH_1 and AH_2 were isolated from saline soil in the region of Bejaia (Algeria). Based on morphological and chemotaxonomic characteristics, these isolates AH_1 and AH_2 are related to the genus *Streptomyces*. The growth and the antibiotic production by the two isolates are evaluated on two liquid culture media (ISP_2 and M_2) at different concentrations of NaCl. The results obtained showed that the antibiotic activity (antibacterial and antifungal) is very important. Extraction of antibiotic from Crude extracts by dichloromethane and n-butanol from the culture filtrate of the isolate AH_1 and AH_2 respectively, showed good activities, also, the crude extracts with hexane for both isolates showed a very important antifungal activity. The antibiotic complexes were located by bioautgraphy, and suggest that these molecules belong to subfamilies of aminoglycosides and polyether, and these antibiotics are non-polyenic.

KEYWORDS

Actinomycetes;
Halophilic;
Streptomyces;
Antimicrobials activities;
Taxonomy.

INTRODUCTION

Actinomycetes are microorganisms that have a considerable importance in production of antibiotics[37]. In particular, they are responsible for more than 80% of the antibiotics that were produced[24]. These bacteria represent a large proportion of the soil microbial biomass. In particular, they play a role in biodegradation of organic matter and recycling of elements in soils. They help also in biological control against plant pathogens, with their great ability to produce different inhibitory substances in relation to other terrestrial organisms[22]. Among the species belonging to different genus of actinomycetes, the genus *Streptomyces*, that is the largest one and he produces two-thirds of antibiotics that are produced by all actinomycetes. Moreover, *Streptomyces* produces a variety of bioactive secondary metabolites including : antibacterial, antifungal, antitumor and enzyme inhibitors. The genus *Streptomyces* is the most widespread in the environment, especially in the soil[9]. However, it has be-

come increasingly difficult to isolate new microorganisms and bioactive metabolites in normal soil. Nevertheless, a number of new actinomycetes strains producing bioactive compounds have recently been isolated from extreme or specific ecosystems, namely marine, Sahara and saline ecosystems[8,33,36].

In this study, we are interested in the study of two strains of actinomycetes isolated from saline soil in the region of Bejaia, Algeria, to our knowledge, this is the first study carried out on these ecosystems in Algeria. The work focused on the taxonomic characterization of two isolates AH_1 and AH_2, the determination of antimicrobial and antifungal activity and the determination of the chemical nature of synthesized bioactive molecules.

MATERIALS AND METHODS

Strains of actinomycetes

Two strains of halotolerant actinomycetes (AH_1 and AH_2) isolated from a saline soil of Bejaia (northeast Algeria) have been the subject of this study.

Identification of actinomycetes strains (AH1 and AH2)

Micromorphological and cultural characters

The morphology of the colonies subcultured on culture media *'International Streptomyces Project'* ISP_1, ISP_2, ISP_3, ISP_4, ISP_6 and ISP_7[6] and the culture medium GYEA was noted after 7, 14 and 21 days of incubation at 28° C. Micromorphological characters: the arrangement of spores and fragmentation of the mycelium was observed under the microscope (magnification x 40 and x 100) fresh after 07, 14 and 21 days.

Determination of cell components

Chemical analysis of cell constituents was carried out by the determination of the isomer of diaminopimelic acid (LL or DL form) and the presence of glycine by the method of Becker et al[5]. The identification of cellular sugars and parietal mycolic acid was performed according to the methods described respectively by Lechevalier[25] and Minnikin and al[7].

Physiological and biochemical characteristics

Biochemical characterization was based on: determining the use of sugars, tested in the culture medium (ISP_9), degradation of organic compounds: tyrosine[28], milk casein[23], Tween 80[11], and the production of pigments melanoids tested in culture media ISP_6 and ISP_7[6], and testing of nitrate reductase[27].

The strains were also tested for their ability to grow on the culture medium GYEA supplemented with different antibiotics: vancomycin, gentamicin, ciprofloxacin, cefoxitin, clavulanic acid, cefepime, and inhibitory compounds such as (m / v): crystal violet, 0.001% ; sodium azide, 0.001%; potassium tellurite, 0.005% and 0.01%; phenol, 0.005% and 0.05% (Athalys et al. 1981), sodium chloride, 0%, 5 %, 7.5% and 20%, and growth at pH 3, pH 5, pH 7, pH 9 at different temperatures 28 ° C, 30 ° C, 37 ° C and 40 ° C.

Antimicrobial activities

The antimicrobial activities of the two strains AH 1 and AH 2 were measured on culture Williams and Kuster medium [10g l^{-1} amidon, 0,3g l^{-1} casein, 2g l^{-1} KNO3, 2 g l^{-1} NaCl, 2g l^{-1} K2HPO4 , 0,05g l^{-1} MgSO4 (7H2O) , 0,02g l^{-1} CaCO3, 0,01g l^{-1} FeSO4 (7H20) , 1g l^{-1} glucose , pH 7.2] by the technique of agar cylinder[16]. The zones of inhibition were measured after 24 h for bacteria and 48 hours for fungi. Eight germs were used which targets six bacteria: Gram positive *(Staphylococcus aureus, Micrococcus luteus, Bacillus subtilis)* , Gram negative *(Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*), yeast *(Candida albicans)* and a filamentous fungus *(Aspergillus Niger)* .

Production and extraction of antibiotics

The production of antibiotics was performed on two liquid culture media ISP_2 [malt extract: 10 g l^{-1} yeast extract 4 g l^{-1}, glucose 4 g l^{-1}. pH 7.2] and the culture medium Williams and kaster (M_2) Using different concentration of NaCl. 100 ml of each culture medium was inoculated with 3 ml of pre-culture and was incubated at 28 ° C for 10 days. The extraction of antibiotics was carried out from the supernatant as well as from the mycelium using the technique of Mechlinski[35]. The extraction of active substances was carried out by organic solvents with different polarity: n-hexane, ethyl acetate, dichloromethane, benzene and n-butanol in order to choose the best extraction solvent. The obtained organic extracts are concentrated dry, then taken up in 2 ml of methanol. The antimicrobial activities are tested in relation to the target bacteria by the method of the wells. The inhibition zones are measured after 48h

Regular Paper

to 72h of incubation at the growth temperature of the target germ by measuring the diameter of clear zone.

Detection for polyenes antibiotics in crude extracts

The butanol extracts were evaporated dry and taken up in methanol. The polyene antibiotics are detected in UV-Visible spectrophotometer. They are presented by three absorbance maxima characteristics between 291 and 405 nm.

Bioautography

The localization of the active spots was performed by bioautography on thin-layer chromatography plate (preparative TLC) with silica gel 60 G (Fluka). Twenty-five microliters of each extract were deposited on the silica gel plate. The migration solvent used is EAE: ethanol-ammonia-water (7:2:1, v / v). After migration, the plates are covered with Muller Hinton medium or nutrient agar (9 g / l agar) previously seeded with a target germ. Reading the results by measuring the diameter of inhibition zones and calculating their front relations. Also, the thin-layer chromatographies (TLC analysis) were performed under the same conditions, the chromatograms were revealed by: ninhydrin, anisaldehyde-H_2SO_4, the aniline diphenylamine, vanillin-H_2SO_4, ferric chloride ($FeCl_3$)[10].

RESULTS

Identification of actinomycetes strains

Micromorphological and cultural characteristics

After 7 days of incubation at 28 °C, colonies of isolate AH_1 appear circular and cambered .The diameter of the colony can reach 8 mm after 14 days of incubation. Good growth was observed on culture media M_2, ISP_3, ISP_4, ISP_7, ISP_1 and ISP_2, less growth in ISP_6 , whereas a weak growth in GYEA. The aerial mycelium is white / cream and the substrate is light yellow on each of culture media, no soluble pigment is produced.

The isolate AH_2 produced rough colonies of 2 to 6 mm in diameter colored beige cream to beige yellow and soluble pigments are colored brown or dark brown.

Growth is best on culture media M_2, ISP_3, ISP_4, ISP_7, ISP_6, ISP_1 and less growth in culture media ISP_2 and GYEA. Production of aerial mycelium was observed in all culture media, it appears creamy beige to beige

TABLE 1 : Physiological and biochemical characteristics of isolates AH_1 and AH_2

Tests	Strain AH_1	Strain AH_2
Degradation of		
- Tyrosine	+	+
- Casein	+	+
- Tween 80	+	+
- Starch	+	+
- Gélatin	+	+
- Glucose	+	+
- Galactose	+	+
- Fructose	+	+
- Xylose	+	+
- Arabinose	+/-	+/-
- Sucrose	+	+
- Mannitol	+	+
- Inositol	+	+
- Rhamnose	+	+
- Raffinose	+/-	+/-
- Sorbitol	-	+/-
- Lactose	-	+
- Ribose	-	-
- Mannose	+	+
Production of pigments		
Melanoides		
Nitrate reductase	+	+
Resistance of antibiotics		
- Vancomycin	-	-
- Gentamycin	-	-
- Ciprofloxacin	-	-
- Cefoxitin	-	+
- Clavulanic acid	+	-
- Cefepime	-	
Growth in the presence of inhibitors		
- Potassium tellurite de (0.01%)		
- Potassium tellurite (0.05%)	+	+
- Phenol (0,05%)	+	+
- Phenol (0,005%)	-	-
- sodium azide (0.01%)	-	+
- Crystal violet (0.001%)	-	-
sodium chloride	-	-
- ISP1 (0%)		
- ISP1 (5%)	-	-
- ISP1 (7, 5%)	+	+
- ISP2 (0%)	+	+
- ISP2 (5%)	-	+/-
- ISP2 (7,5%)	+	+
- GYEA (0%)	+	+
- GYEA (5%)	+/-	+
- GYEA (7, 5%)	+	+

Tests	Strain AH_1	Strain AH_2
- M2 (0%)	+	+
- M2 (5%)	+	+
- M2 (7, 5%)	++	++
- M2 (20%)	+++	+++
Growth at	+	+
- pH 3	-	-
- pH 5	+	-
- pH 7	+++	++
- pH 9	+	+
- T° 28°C :	+++	+++
- T° 30 °C :	++	+
- T° 37°C :	-	-
- T°40 °C :	-	-

Note : + : Growth ; - : no growth, +/- : growth of vegetative mycelium /Absence of aerial mycelium

yellow, the substrate mycelium is beige brown. The two isolates have a fragmented mycelium, formed with long chains of spherical spores ,type *Rectus flexibilis* (RF).

Chemical analysis of cell constituents

The analysis of cellular components showed that the two isolates AH_1 and AH_2 contain in their walls diaminopimelic acid in a form of LL and glycine. However, there is no characteristics sugar and no mycolic acids.

Biochemical and physiological analysis

The results of physiological and biochemical tests of the two strains AH_1 and AH_2 are Showed in TABLE 1

Antimicrobial activity, production and extraction of active molecules

Tests of antimicrobial activities

Hydrolysis zone was illustrated in figure 01 and the antimicrobial activities of the two strains AH1 and AH2 towards the various target germs are noted in Figuer 1 and 2

Production of antibiotic

The antibiotic activities were observed from the two used culture media (ISP_2 and M_2), despite the difference in the growth which is significantly higher in M_2 than ISP_2. The UV-visible spectra of butanol extracts of isolates on both media ISP_2 and M_2 do not exhibit the characteristic peaks of polyene molecules.

Extraction of antimicrobial substances

The active molecules in various solvents extract indicate that the antibacterial activities of isolate AH_1 were

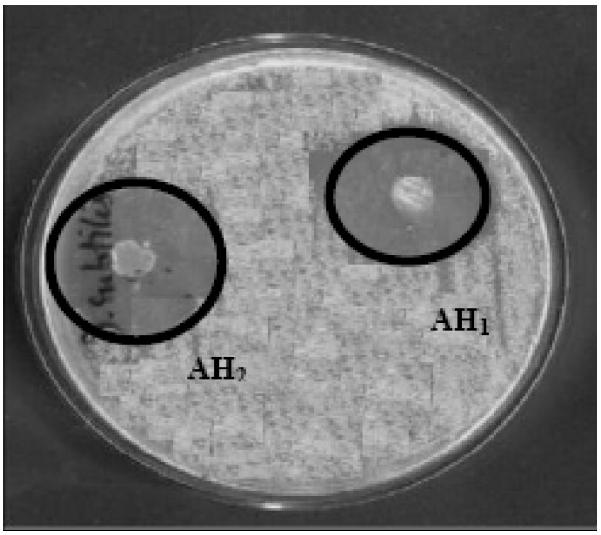

Figure 1 : Antagonistic activity of isolates AH1 and AH2 against Bacillus subtilis using the technique of agar cylinders

obtained with *n*-butanol from the M_2 culture medium, and with hexane and dichloromethane from the ISP_2 culture medium. However, the antimicrobial activities of strain AH_2 are found only in the *n*-butanol extract. Furthermore, the best antifungal activities are obtained with hexane for strains AH_1 and AH_2. The revelation of the chromatograms of butanol extracts of isolates AH_1 and AH_2 (on ISP_2 culture medium), in the E.A.W (Ethanol, Ammoniac, Water: 7.2.1 V/V) system, allowed three active fractions. These spots showed strong antibacterial activity towards *B. subtilis*.

The UV-visible spectra of the extracts of both strains AH_1 and AH_2 have maxima absorption at 300.4 nm. The absence of characteristic peaks of polyene antibiotics indicate that these molecules are not polyenes. The revelation of ferric chloride was negative, suggesting the absence of phenol and hydroxamic acid.

DISCUSSION

Actinomycetes are a potential source of secondary metabolites in antibacterial and antifungal activities. The selective isolation of some strains has led to the discovery of interesting active substances produced by the genus: *Micromospora, Streptosporangium*, Actinomadura[20]. Thus, the search for new bioactive molecules has been oriented towards the isolation of rare species from extreme ecosystems[2,30].

In our study we are interested in the characterization of two isolates of actinomycetes isolated from saline soil

Regular Paper

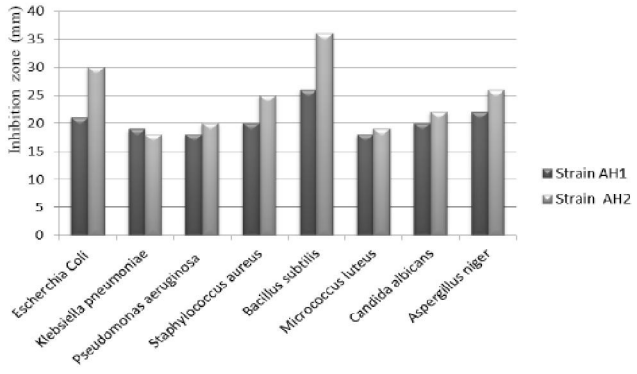

Figure 2 : Antimicrobial and antifungal activities of isolates AH_1 and AH_2 production of antibiotic

of Bejaia, Algeria. These strains were selected on the basis of the macroscopic appearance of colonies and micromorphological characters. Microscopic observation of the two strains revealed a filamentary and fragmented aspect, a predominance of spore chains type RF *(Rectus flexibilis)* was also observed. These data link the two studied isolates to the genus *Streptomyces*. Chemotaxonomic characterization of isolates AH_1 and AH_2 has shown that they have diaminopimelic acid as LL and glycine, but no mycolic acid which is the chemiotype IC according to Lechevalier and[26].

The taxonomy of actinomycetes is based on several criteria: morphological, chemical, physiological and molecular. The identification of genus is facilitated by morphological studies, while the physiological and molecular criteria separate species. In addition, the use of chemotaxonomy based on the cellular composition of amino acids[5], sugar[12], mycolic acids (unsaturated complex and parietal lipid)[14] in membrane phospholipids [25,7] and membrane menaquinones[21], combined with morphological criteria, are an essential contribution to distinguish many kinds of genus, for example, *Streptomyces*, *Nocardia*[5].

The growth of two strains AH_1 and AH_2 was carried out on several selective culture media, among them culture medium M_2 with 7.5% NaCl showed a significantly better growth. Also, the production of antibiotics was observed on the culture medium M_2 and ISP_2 with different concentration of NaCl. So, it is important to note that the NaCl content had no influence on the occurred antibiotic activity, however this had an impact on growth level. The antibacterial activity of these strains was investigated by the technique of the cylinders towards six bacteria *(Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Bacillus subtilis, Micrococcus luteus,* and *Staphylococcus aureus).* Isolates AH_1 and AH_2 showed significant antibacterial activity against all the used target germs. Similar results were observed in the study of Reghioua et al[31]. In particular, strains of actinomycetes, isolated from an arid soil of southeastern Algeria, which presented an important antibacterial activity against *Escherichi coli, Pseudomonas aeruginosa,Bacillus cereus, Streptococcus faecalis* and *Staphylococcus aureus*. Also a strain *Streptomyces sp.* isolated from a Tunisian soil showed antibacterial activity towards the gram-positive bacteria *M.luteus* and Gram-negative *E. coli*[17].

The strains AH_1 and AH_2 showed also an antifungal activity against fungi *(Aspergillus)* and yeasts *(Candida).* Similar results were reported in the study of[1], there actinomycete strains, isolated from Saharan soil of South-eastern Algeria (Biskra, El-Oued and Ourgla), presented a very significant antifungal activity against most filamentous fungi and yeasts.

Many studies have focused on the choice of extraction solvent of antibiotics. We noted in particular those of Badji[4], Boudjella et al.[13], and Zitouni et al.[3] which showed that the best extraction solvent is depending on the actinomycete strain and on the produced substance. In our work, the crude extracts in *n*-butanol and dichloromethane from the culture filtrate of isolate AH_1, and *n*-butanol from the culture filtrate of isolate AH_2 showed antibacterial activities. In addition, crude extracts with hexane of the two isolates showed a very significant antifungal activity in accordance with the work of Augustine et al.[29].

Some spots of the same Rf (front Report), were revealed positively with both ninhydrin and anisaldehyde-sulfuric acid, which indicates the existence, respectively of amine functions (free amines, amino acids or osamines) and carbohydrate functions, which suggest that these molecules belong to the subfamily of aminoglycoside according to Berdy et al.[15].

On the other hand and according to Dembitsky[34], the positively reveled spots with vanillin (alcohols, ethereal oils) and the diphenylamine-aniline (carbohydrates) belong to the family of polyethers.

The spot Rf =0.24 was revealed by bioautography, but not by other chemical revelator for the organic extract of culture filtrate of isolate AH_2 on culture medium ISP_2. Absence of polyenic antibiotics is interesting because the polyene molecules are undesirable

in *screening* programs for new antifungal molecules because they have problems related to their toxicity, their instability and their poor solubility.

REFERENCES

[1] A.Boudemagh, M.Kitouni, F.Boughachiche, H.Hamdiken, L.Oulmi, S.Reghioua, H.Zerizer, et al; Isolation and molecular identification of actinomycete microflora, of some saharian soils of south east Algeria (Biskra, EL-Oued and Ourgla) study of antifungal activity of isolated strains, J.Myco.Méd., **15**, 39–44 **(2005)**.

[2] A.Khattabi, L.Hilali, K.Dari, O.Assobhei, F.Gavini; Isolement de microorganismes d'origine marine (Maroc) antagonistes de Yersinia ruckeri et *Yersinia pseudotuberculosis*, Rev.Biol.Biotech., **2**, 28–32 **(2002)**.

[3] A.Zitouni, H.Boudjella, L.Lamari, B.Badji, F.Mathieu, A.Lebrihi, N.Sabaou; Nocardiopsis and saccharothrix genera in saharan soils in algeria: Isolation, biological activities and partial characterization of antibiotics, Res. Microbiol., **156**, 984–993 **(2005)**.

[4] B.Badji, A.Zitouni, F.Mathieu, A.Lebrihi, N.Sabaou; Antimicrobial compounds produced by Actinomadura sp. AC104 isolated from an Algerian Saharan soil, Can.J.Microbiol., **52**, 373–382 **(2006)**.

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

[6] B.Shirling, D.Gottlieb; Methods for characterization of *Streptomyces* species, Int.J.Syst.Baceriol., **16**, 3313-3340 **(1966)**.

[7] D.E.Minnikin, P.V.Patel, L.Alshamaony, M. Goodfellow; Polar lipid composition in the classification of Nocardia and related bacteria, Int.J.Syst. Bacteriol., **27**, 104-107 **(1977)**.

[8] D.Weber, O.Sterner, T.Anke, S.Gorzalczancy, V.Martino, C.Acevedo; Phomol, a new antiinflammatory metabolite from an endophyte of the medicinal plant Erythrina crista-galli, J.Antibiot., **57**, 559-563 **(2004)**.

[9] E.C.Eckwall, J.L.Schottel; Isolation and characterization of an antibiotic produced by the scab disease-suppressive Streptomyces diastatochromogenes strain Pon SSII, J.Ind. Microbiol.Biotechnol., **19**, 220–225 **(1997)**.

[10] E.Merck; Révélateurs pour la chromatographie en couches minces et sur papier, Darmstadt, 149 **(1975)**.

[11] G.Sierra; A Simple method for the detection of lipolytic activity of microorganisms and some observations on the influence of the contact between cells and fatty substrates, Antonie Van Leeuwenhoek, **23**, 15-22 **(1957)**.

[12] H.A.Lechevalier, M.P.A.Lechevalier; Critical evaluation of genera of aerobic actinomycetes, *In*: The *Actinomycetales*, H.Prauser, (Ed); G Fisher Verlag Jena, 393-405 **(1970b)**.

[13] H.Boudjella, K.Bouti, A.Zitouni, F.Mathieu, A.Lebrihi, N.Sabaou; Taxonomy and chemical characterization of antibiotics of *Streptosporangium Sg* 10 isolated from a Saharan soil, Microbiol.Res., **161**, 288-298 **(2006)**.

[14] H.Mordarska, M.Mordarski, M.Goodfellow; Chemotaxonomic characters and classification of some nocardioform bacteria, J.Gen.Microbiol., **71**, 77-86 **(1972)**.

[15] J.Berdy, A.Aszalos, K.L.Mc Nitt; CRC Handbook of antibiotic compounds, XIII. Microbial Metabolites, CRC Press Boca Raton, FL, USA, **1–3**, **(1987)**.

[16] J.J.Patel, M.E.Brown; Interactions of *Azotobacter* with rhisosphere and root-surface microflora, Plant and soil, **31**, 273-281 **(1969)**.

[17] L.Mellouli, R.Ben Ameur-Mehdi, S.Sioud, M.Salem, S.Bejar; Isolation, purification and partial characterization of antibacterial activities produced by a newly isolated *Streptomyces sp*, US24 strain, Res.Microbiol., **154**, 345–352 **(2003)**.

[18] M.Athalye, M.Goodfellow, J.Lacey, R.P.White; Numerical classification of *Actinomadura* and *Nocardiopsis*, Int.J.Syst.Bacteriol., **35**, 86-98 **(1985)**.

[19] M.Athalye, J.Lacey, M.Goodfellow; Selective isolation and enumeration of actinomycetes using rifampicin, J.Appl.Bacteriol., **51**, 289-297 **(1981)**.

[20] M.C.Shearer; Methods for the isolation of nonstreptomycetes actinomycetes, J.Ind. Microbiol., **28**, 91–98 **(1997)**.

[21] M.D.Collins, M.Goodfellow, D.E.Minnikin; Fatty acid, isoprene+id quinone and criterion in the classification of aerobic actinomycetes in: The Actinomycetales, **(1980)**.

[22] M.Goodfellow, S.T.Williams; Ecology of actinomycetes, Ann.Rev.Microbiol., **37**, 189-216 **(1983)**.

[23] M.Goodfellow; Numerical taxonomy of some nocardioform bacteria, J.Gen.Microbiol., **69**, 33-90

Regular Paper

(1971).

[24] M.G.Watve, R.Tickoo, M.M.Jog, B.D.Bhole; How many antibiotics are produced by the genus *Streptomyces?* Arch.Microbiol., **176**, 386-390 **(2001)**.

[25] M.P.Lechevalier, C.De Bievre, H.A.Lechevalier; Chemotaxonomy of aerobic actinomycetes: Phospholipid composition, Biochem.Syst.Ecol., **5**, 249-260 **(1977)**.

[26] M.P.Lechevalier, H.A.Lechevalier; Composition of whole-cell hydrolysates as a criterion in the classification of aerobic actinomycetes, In: The *Actinomycetales* H.Prauser, (Ed); G Fisher Verlag Jena, 311-316 **(1970a)**.

[27] N.Marchal, J.L.Bourdon; Milieu de culture et identification biochimique des bactéries, Ed.Doin, Paris, **(1973)**.

[28] R.E.Gordon, D.A.Barnet, J.E.Handarhan, C.Hor-Nay-Pan; *Nocardia coeliaca, Nocardia autotrophica* and norcardin strains, Int.J.Syst. Bacteriol, **24**, 54-63 **(1974)**.

[29] S.K.Augustine, S.P.Bhavsar, B.P.Kapadnis; A non-polyene antifungal antibiotic from *Streptomyces albidoflavus* PU 23, J.Biosci., **30(2)**, 201–211 **(2005)**.

[30] S.Lemriss, F.Laurent, A.Couble, E.Casoli, J.M.Lancelin, D.Saint pierre-Bonaccio, et al; Screening of non polyenic antifungal metabolites produced by clinical isolates of actinomycetes, Can.J.Microbiol., **49**, 669–674 **(2003)**.

[31] S.Reghioua, F.Boughachiche, H.Zerizer, L.Oulmi, M.Kitouni, A.Boudemagh, A.Boulahrouf; Antibacterial activity of rare actinomycetes isolated from arid soil samples of the south-east of Algeria, Antibiotiques, **8(3)**, 147-152 **(2006)**.

[32] V.Betina; Bioautography in paper and thin layer chromatography and its scope in the antibiotic field, J.Chromatogr., **78**, 41-51 **(1973)**.

[33] V.J.Mukku, M.Speitling, H.Laatsch, E.Helmke; New butenolides from two marine streptomycetes, J.Nat.Prod., **63**, 1570-1572 **(2000)**.

[34] V.M.Dembitsky; Astonishing diversity of natural surfactants: 2.Polyether glycosidic ionophores and macrocyclic glycosides, Rev.Department of Organic Chemistry and School of Pharmacy, Lipids, **40**, 219–248 **(2005)**.

[35] W.Mechlinski; The polyene antifungal antibiotics, In : Handbook of microbilogy, Cleveland OH, CRC Press, **2**, **(1978)**.

[36] Y.Lu, X.Dong, X.Liu, S.Bie; Characterization and identification of a novel marine *Streptomyces sp*. produced antibacterial substance, Mar.Biotechnol., **11**, 717–724 **(2009)**.

[37] Y.Takahashi, S.Omura; Isolation of new actinomycete strains for the screening of new bioactive compounds, J.Gen.Appl.Microbiol., **49(3)**, 141-154 **(2003)**.