ISSN: 0976-3031

Available Online at http://www.recentscientific.com

International Journal of Recent Scientific Research
Vol. 5, Issue, 12, pp.2236-2243, December, 2014

International Journal of Recent Scientific Research

RESEARCH ARTICLE

ISOLATION, IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF ACTINOMYCETE STRAIN FROM THE LATERITE SOIL OF KANDUKURU

B. Chinna Narasaiah[1], *V. Leelavathi[2], G. Sudhakar and P. Mariyadasu[3]

[1]Department of Botany & Zoology, S.V Arts & Science College, Giddaluru, A.P, India
[2]Department of Biotechnology, Acharya Nagarjuna University, Guntur, A.P. India
[3]Department of Zoology, Acharya Nagarjuna University, Guntur, A.P. India

ARTICLE INFO

Article History:

Received 6th, November, 2014
Received in revised form 16th, November, 2014
Accepted 8th, December, 2014
Published online 28th, December, 2014

Key words:

Actinomycetes, Phylogenetic tree, Scanning Electron Microscop GenBank

ABSTRACT

The laterite soil was collected from the Kandukuru of Prakasahm District and analyzed for its physico-chemical properties and pretreated and used for isolation of actinomycetes. The isolated strain was designated as CN-4.The morphological charecterisation carried with genomic (16 rDNA gene sequencing) analysis. The amplified and sequenced 16S rDNA was compared with the sequences in GenBank using BLAST and aligned with the sequences retrieved from NCBI GenBank database using the Clustal *W* method. The phylogenetic tree was constructed based on Maximum Likelihood method using bootstrap analysis and micro morphology of the strain through Scanning Electron Microscopy (SEM) was also carried out. Based on the scanning electron microscopic observation and phylogenetic tree construction the isolated strain from the laterite soil of Kandukuru was identified as *Streptomyces albus* CN-4.

© Copy Right, IJRSR, 2014, Academic Journals. All rights reserved.

INTRODUCTION

Microorganisms from environments have gained considerable attention in recent years. This is mainly due to the fact that they hold about the molecular evolution of life. Natural products are the leading sources of novel biomolecules that have been used in the pharmaceutical industries since their inception. The majority of the natural products currently used in the market as therapeutic agents or as health supplements are derived from the terrestrial organisms including plants, animals and microorganisms. Microbial secondary metabolites are the important sources of natural compounds with potential bioactivities. In general, natural products including the microbial metabolites may be practically utilized in different ways including in the fields of medicine and agriculture industries, material for subsequent chemical or microbiological modification (derivatization), lead compounds in the synthesis of new analogs or as templates in Rational Drug Design studies. Soil is the perfect laboratory for the creation of natural medicines. It holds a wide array of tiny microhabitats that creates a vast variation in the appearance and survival strategies of soil microbes. The actinomycetes which represent a transitional form between the bacteria and fungi compose a large proportion of the soil flora. They are Gram positive bacteria, filamentous and sporulating with DNA rich in G+C ranging from 55-75% (Ho *et al.*, 2002). they are generally considered as terrigenous bacteria due to their wide distribution and abundance in soil. The name actinomycetes derived from Greek aktis (a ray) and mykes (fungus) was given to these organisms from initial observation of their morphology. These members are grouped into the class Actinobacteria belonging to the division of Fermicutes of the domain Bacteria. The class Actinobacteria is categorized into five subclasses namely Actinobacteridae, Acidimicrobidae, Coriobacteridae, Sphaerobacteridae and Rubrobacteridae. Except Actinobacteridae, the other four subclasses contain only one order subsequently delineated into one family each. On the other hand, subclass Actinobacteridae consists of two orders, the Actinomycetales and Bifidobacteriales. The order Actinomycetales has ten suborders, each sub order again categorized into a total of about 38 families. The order Bifidobacteriales constitute only one family namely Bifidobacteriaceae. Classification of actinomycetes proposed by Stackebrandt *et al.* (1997). In this context, a study was conducted on the isolation of actinomycetes from the laterite soil of Kandukuru, Prekasam (Dist), A.P.,micro morphology of the strain through Scanning Electron Microscopy (SEM) as per the procedure described by the Yassin *et al*. (1997). morphological characteristics of the strain with those reported in Bergey's Manual.

The search for the pharmacological targets as well as the synthesis of modified natural compounds will keep on moving Microbiologists, Biotechnologists and Chemists in the process of new drugs discovery. In connection with the excellent track record of actinomycetes with antimicrobial activity, an attempt was made to exploit the laterite soil for the isolation of actinomycetes

* Corresponding author: **V. Leelavathi**
Department of Biotechnology, Acharya Nagarjuna University, Guntur, A.P. India

MATERIALS AND METHODS

Isolation and screening of actinomycete strains from laterite Soil sample

Laterite soil sample collected for the isolation of potent actinomycete strains was initially analysed for physico-chemical properties such as moisture content (%), pH, organic carbon (%) and total nitrogen content (%).

Soil Characteristics

Moisture content

To determine the moisture content, 10 g of soil sample was dried in a hot air oven at 105°C until a constant weight is obtained. The difference between the weights of pre-drying and post-drying was taken as the moisture content of the initial soil sample.

Soil pH

To identifying the pH of the collected soil sample was determined with the help of digital pH meter of model Di-707 (Jackson, 1973). 20 g of soil sample was taken in a 100 ml beaker and 40 ml of distilled water was added. The suspension was mixed thoroughly and allowed to settle the suspended clay particles from the suspension for about 1h before recording the pH.

Organic carbon

Organic carbon content of the soil sample was estimated by following Walkey and Black method (1934). 10 g of soil sample was taken into 500 ml Erlenmeyer flask. To this 10 ml of 1N $K_2Cr_2O_7$ was added and shaken gently to disperse the soil into the solution. 20 ml of Conc. H_2SO_4 was added followed by vigorous agitation for 1min.200 ml of distilled water was added and filtered. Few drops of *O* - phenanthroline indicator was added to the filtered solution and titrated against 0.5N $FeSO_4$**.** $7H_2O$. Sample without soil served as blank. The amount of organic carbon in the soil sample was calculated by using the following formula.

(milliequivalents of $K_2Cr_2O_7$ – milliequivalents of $FeSO_4$. $7H_2O$)x 0.003x100

Organic carbon (%) = --
----x*f* Soil weight (g)
f = correlation factor = 1.24

Total nitrogen

Total nitrogen in the soil sample was determined by Micro - Kjeldahl method.

Reagents

Conc. H_2SO_4, mercuric oxide, K_2SO_4, sodium hydroxide – sodium thiosulphate solution (60% NaOH and 5%Na_2SO_3. $5H_2O$ in distilled water), 0.02N standard HCl , 4% boric acid solution , mixed indicator solution (10ml of 0.2% methyl red in ethanol mixed with 50ml of 0.2% bromocresol green in ethanol)

Procedure

To the finely sieved soil sample (100 g) taken into a digestion flask, 2 g of K_2SO_4, 90 mg of mercuric oxide and 2 ml of Conc. H_2SO_4 were added , mixed thoroughly and kept for digestion on heater. Before carrying out of distillation process,limited addition of distilled water (5 ml) to the flask was followed by the addition of Sodium hydroxide – sodium thiosulphate solution was added. Ammonia was collected in boric
acid. The distillate (20ml) collected was titrated against 0.02N HCl. Appearance of violet colour is the end point. Blank was maintained using equal volume of distilled water instead of distillate. Total nitrogen present in the soil sample was calculated by using the formula

[{HCl (ml) in sample – HCl (ml) in blank x
normality of acidx14.01x100}]
Total nitrogen= --
Soil weight (mg)

Isolation of actinomycete strains

Laterite soil sample collected from Kandukuru, Prakasam (Dist) a depth of 5 – 8 cm was pretreated with calcium carbonate (1:1 w/w) and dried at 45oC for 1 h in order to reduce the aboundence of bacteria and fungi (El-Nakeeb and Lechhevalier, 1963). Yeast extract malt extract dextrose agar (YMD) and starch casein salts agar media were prepared, sterilized at 15 lbs pressure (120°C) for 15 min and poured into Petri plates under aseptic conditions. Both streptomycin (50 µg/ml) and nystatin (50 µg/ml) were added to the media just before pouring into Petri plate. Soil dilution plate technique was employed for isolation of actinomycetes strains (Williams and Cross, 1971). The pretreated soil (1 g) sample was suspended in 100 ml of sterile distilled water. Serial dilutions were prepared and 0.1ml of 10-3 and 10-4 dilutions were placed on media with the help of a spreader. The inoculated plates were incubated at 30°C for 7-14 days. After incubation, actinomycete colonies were isolated from soil. Streak plate method was used to purify the cultures of actinomycete strains. The colonies were picked with the loop according to the condition. The picked up specks of the colonies were streaked over YMD agar medium followed by incubation at 30oC for 7 days. Further, pure cultures were maintained on YMD agar slants and stored at 4oC for further study (Williams and Cross, 1971).

Taxonomic studies of the actinomycete strain

Cultural, morphological, biochemical and physiological characteristics together with genomic (16s rDNA gene sequencing) analysis of the strain CN-4 were studied.

Morphological studies

ISP-2 grown culture was used to observe the detailed micro morphology of the strain through Scanning Electron Microscopy (SEM) as per the procedure described by the Yassin *et al.* (1997). The culture was fixed in 2.5% gluteraldehyde prepared in 0.1 M phosphate buffer (pH 7.2) for 24 h at 4°C followed by the post fixation step in 2% aqueous osmium tetroxide for 4 h in the same buffer. The sample was then dehydrated in ethanol and then dried up to critical with the help of Electron Microscopy science CPD unit (Ruska Labs, Acharya NG Ranga University,Hyderabad,India). The dried sample was mounted on aluminum stubs covered with double-sided carbon tape. A thin layer of gold coating was applied over the sample by

using automated sputter coaster for 3 min (JEOL JFC -1600, Japan). Finally, the samples were examined under SEM at various magnifications (Model: JOEL-JSM 5600, Japan).

Molecular identification of the strain based on 16s rDNA sequence analysis

The strain was grown in YMD broth for 3 days and was centrifuged at 10,000 rpm for 20 min and the pellet was used for the extraction of DNA (Mehling *et al*., 1995). PCR mixture consisted of 2.5 µl of 10× buffer, 3.5 µl of MgCl2 (25 mM), 2 µl of dNTP (0.4 mM), 1 µl of 16S rDNA actino specific primer – forward (10 pmol/µl), 1 µl of 16S rDNA actino specific primer- reverse (10 pmol/µl), Taq polymerase (2 U/µl) and 2 µl template DNA. PCR amplification was carried out as follows: Initial denaturation step at 94 ºC for 3 min followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 65°C for 1min and extension at 72°C for 1 min, with a further 5 min extension at 72°C. The PCR product was purified with Agarose Gel DNA Purification Kit SoluteReady® Genomic DNA purification kit, PCR Master Mix, Agarose gel electrophoresis consumables and Primers from HELINI Biomolecules, Chennai, India). The 750 bp 16S rDNA sequence was determined with 16S rDNA actino specific forward and reverse primers. The 16S rRNA gene fragment was amplified using Universal Primers. (Actino specific forward Primer -5'-GCCTAACACATGCAAGTCGA-3' and Actino Specific Reverse primer - 5'-CGTATTACCGCGGCTGCTGG-5') (Nilsson and Strom,2002). The deduced 16s rDNA sequence was compared with the sequences in GenBank (http://www.ncbi. nlm.nih.gov/) using the Basic Local Alignment Search Tool (BLAST) then aligned with the related reference sequences retrieved from NCBI GenBank databases using the Clustal *W* method. Phylogenetic and molecular evolutionary analyses were conducted using Molecular Evolutionary Genetic analysis (*MEGA)* version 5.0 (Tamura *et al*. 2007).

RESULTS AND DISCUSSION

Isolation of actinomycete strains from laterite soil
Soil characters

Isolation of actinomycetes from the laterite soil, the soil sample collected from kandhukuru was tested for soil characters such as moisture content, soil pH, organic matter and soil nitrogen content following standard protocols and the results were recorded as moisture content - 10%, pH - 7.2, organic matter - 1.0% and total nitrogen - 0.12%.

Isolation of actinomycete strains

For the isolation of actinomycete strains, the soil sample amended with calcium carbonate was air - dried at 45oC for 1 h. Serial dilution plate technique was employed for the isolation of actinomycetes. Serially diluted soil sample (0.1 ml) was plated on YMD agar (ISP-2) and starch casein agar media amended with streptomycin (50 µg/ml) and nystatin (50 µg/ml) and the Petri dishes were incubated at 28±2ºC for 7-14 days. After incubation, the plates were observed for the growth of tough, leathery actinomycete colonies. Treatment of soil samples with calcium carbonate was reported to be the most efficient technique for the preferential isolation of actinomycetes (Alferova *et al.,* 1989). A total of ten actinomycete strains were isolated from laterite soil (figure 2). One of the strains designated as CN-4 was found to be more active when compared to the other isolates; hence the strain was maintained as pure culture on yeast extract, malt extract, dextrose (YMD) agar slants and preserved at 4ºC.

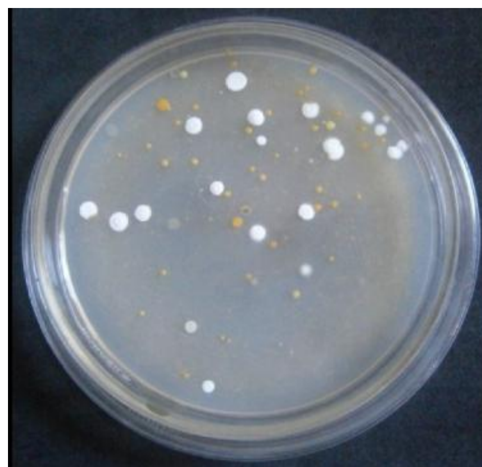

Fig 1 Isolation of actinomycetes on Starch- casein agar medium

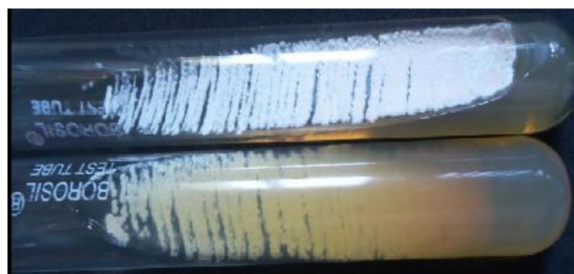

Fig 2 Growth of Streptomyces albus CN-4 on Yeast extracts malt extract- dextrose agar medium substrate and aerial mycelium.

Morphological characteristics

Slide culture technique was employed to observe the micromorphology of the strain grown on ISP - 2 medium through light microscopy and the detailed morphology of the strain was examined through Scanning Electron Microscopy (SEM). The colour of the aerial mycelium was creamy white with pale yellow substrate mycelium when grown on ISP - 2 medium. The sporophore morphology of the strain was observed as spiral with rough surface and hence placed under the spira group of the family Streptomycetaceae (Pridham *et al.,* 1958; Williams *et al.,* 1972). SEM images of the strain CN-4 are presented in figure 4.

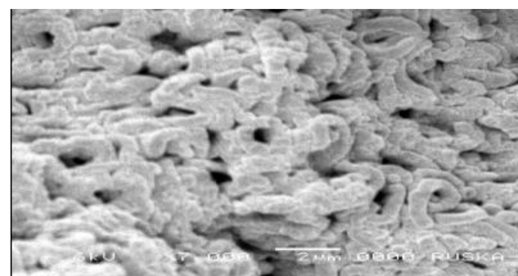

Fig 3 Scanning Electron microscopic photograph of Streptomyces albus CN4(x7000).

Phylogenetic study of the strain CN-4

The taxonomic position of the strain CN-4 was established based on 16s rDNA analysis. The 16s rDNA sequence of the strain was compared with the sequences in GenBank using BLAST and aligned with the sequences retrieved from NCBI GenBank database using the Clustal W method. The

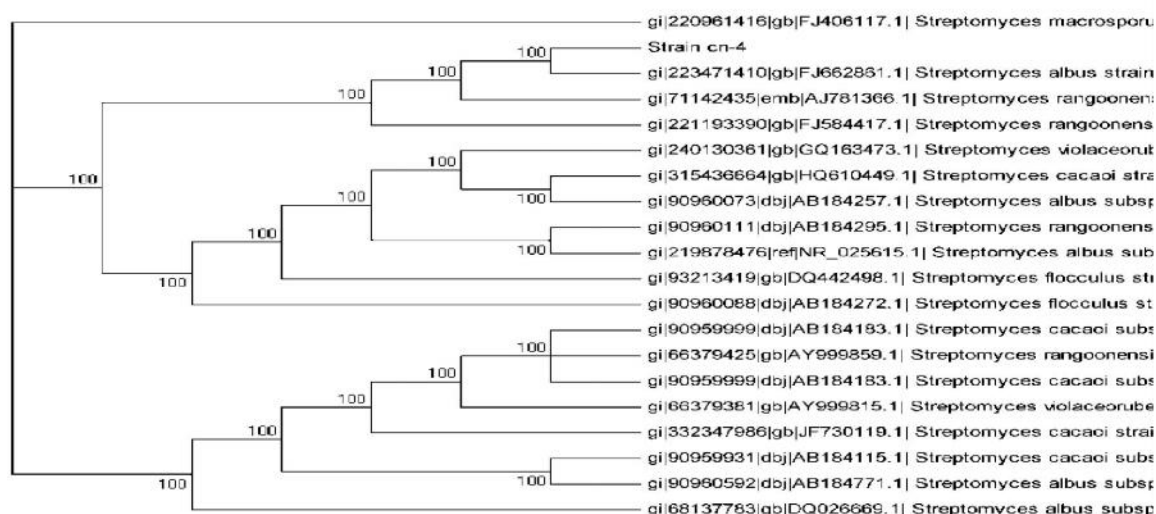

Fig 4 Phylogenetic tree derived from partial 16S rRNA gene sequence showing relationship between strain CN-4 and related members of the genus streptomyces spp, was constructed by using Maximum Likelihood method. Bootstrap values are expressed as percentages of 1000 replications.

phylogenetic tree was constructed based on Maximum likelyhood method using bootstrap analysis (Fig. 6). The strain was close to Streptomyces albus with a bootstrap value of 100. Based on the cultural, morphological, physiological and molecular analysis, the strain CN-4 was identified as Streptomyces albus CN-4.

CONCLUSION

The sporophore morphology of the strain was observed as spiral with rough surface and hence placed under the spira group of the family Streptomycetaceae .The phylogenetic tree was constructed based on Maximum likelyhood method using bootstrap analysis The strain was close to Streptomyces albus with a bootstrap value of 100. Based on the morphological, and molecular analysis, the strain CN-4 was identified as Streptomyces albus CN-4.

Reference

Adinarayana, G., Bapiraju, V.V.S.N.K., Sujatha, P., Premkumar, J., Saisha, V., Ellaiah, P. and Zeeck, A. (2005). 1-Hydroxy-1- norresistomycin, a new cytotoxic compound from a marine actinomycete, Streptomyces chibaensis AUBN-17. J. Antibiot., 58: 526-529.

Agate, A. D. and Bhat, J. V. (1963). A method for the preferential isolation of actinomycetes. Antonie Van Leeuwenhoek, 29: 297-304.

Al-Bari, M. A. A., Bhuiyan, M. S., Flores, M. E., Petrosyan, P., Garcia-Varela, M. and Islam, M. A. (2005). Streptomyces bangladeshiensis sp. nov., isolated from soil which produces bis-(2-ethylhexyl) phthalate. Int. J. Syst. Evol. Microbiol., 55: 1973-1977.

Alferova, I. V., Terekhova, L. P. and Prauzer, K. H. (1989) Selective medium with nalidixic acid for isolating antibiotic producing actinomycetes. Antibiot. Khimioter., 34: 344-348.

Anupama, M., Narayana, K. J. P. and Vijayalakshmi, M. (2007). Screening of Streptomyces purpeofuscus for antimicrobial metabolites. Res. J. Microbiol., 4: 1-3.

Arifuzzaman, M., Khatun, M. R. and Rahman, H. (2010). Isolation and screening of actinomycetes from Sundarbans soil for antibacterial activity. African J. Biotechnol., 9: 4615- 4619.

Baltz, R. H. (2005). Antibiotic discovery from actinomycetes: will a renaissance follow the decline and fall? SIM News, 55: 186-196.

Baltz, R. H. (2007). Antimicrobials from actinomycetes: Back to the future. Microbe, 2: 125-131.

Baskaran, R., Vijayakumar, R. and Mohan, P. M. (2011). Enrichment methods for the isolation of bioactive actinomycetes. Malaysian J. Microbiol., 7: 26-32.

Baumann, R., Ettlinger, L., Hutter, R. and Kocher, H. P. (1976).Control of melanin formation in Streptomyces glaucescens. In: Actinomycetes: the boundary microorganisms, (Ed. T. Arai) pp. 55-63, Toppan Co Ltd., Tokyo.

Bendict, R. G., Pridham, T. G., Leindenfelser, L. A., Hall, H. H. and Jackson, R. W. (1955). Further studies in the evaluation of carbohydrate utilization tests as aids in the differentiation of species of Streptomyces. Appl. Microbiol., 3: 1-6.

Berdy, J. (2005). Bioactive microbial metabolites. J. Antibiot., 58:1-26.

Bergy, M. E. (1968). Kalafungin, a new broad spectrum antibiotic, isolation and characterization. J. Antibiot., 21: 454-457.

Bhattacharya, B. K., Pal, S. C. and Sen, S. K. (1998). Antibiotic production by Streptomyces hygroscopicus D1.5: culture effect. Revista De Microbiologia, 29: 49-52.

Buchanan, R. E. and Gibbons, N. E. (1974). Bergey's Manual of Determinative Bacteriology, 8th Ed. The Williams & Wilkins Company, Baltimore, USA.

Cappuccino, J. G. and Sherman, N. (2004). Microbiology, a laboratory manual. Pearson Education, Inc., New Delhi, pp. 282-283

Chaiharn, M. and Lumyong, S. (2011). Screening and Optimization of Indole-3-Acetic Acid Production and Phosphate Solubilization from Rhizobacteria Aimed at Improving Plant Growth. Curr. Microbiol., 62: 173–181.

Chamberlain, K., and Crawford, D.L. (1999). In vitro and in vivo antagonism of pathogenic turf grass fungi by Streptomyces hygroscopicus strains YCED9 and WYE53. J. Ind. Microbiol. Biotechnol., 23 : 641-646.

Chandrasekhar, S. and Seshadri, R. (1964). Preliminary studies on the use of pulses and cereals in media for

antibiotic production by Streptomyces sp. Indian J. Microbiol., 5: 1- 8.

Chattopadhyay, D. and Sen, S. K. (1997). Optimization of cultural conditions for antifungal antibiotic accumulation by Streptomyces rochei G164. Hindustan Antibiot. Bull., 39: 64-71.

Chormonova, N. T. (1978). Isolation of Actinomadura. from soil samples on selective media with kanamycin and rifampicin. Antiblotiki, 23: 22-26.

Cross, T. (1968). Thermophilic actinomycetes. J. Appl. Bacteriol. 31: 36-53.

Davis, G. and Henrissat, B. (1995). Structural and mechanism of glycosyl hydrolases. Structure, 3: 853-859.

Demain, A.L. and Sánchez, S. (2009). Microbial drug discovery: 80 years of progress. J. Antibiot. 62: 5-16

Dietz, A. and Theyer, D. W. (1980). Actinomycete taxonomy. Society for Industrial Microbiology special publication, 6, pp. 26-31.

Ehmann, A. (1977). The van urk-Salkowski reagent- a sensitive and specific chromogenic reagent for silica gel thin-layer chromatographic detection and identification of indole derivatives. J. chromatogr., 132: 267-276.

Eiser, A. M. and McFarlane, W. D. (1948). Studies on the metabolism of Streptomyces griseus in relation to the production of streptomycin. Mycologia, 47: 420-422.

El-Enhancy, H. A., Farid, M. A. and El-Sayed, E. A. (2000). Influence of inoculums type and cultivation on natamycin production by Streptomyces natalensis. J. Basic Microbiol., 40: 333-342.

Ellaiah, P., Adinarayana, G., Saisha, V. and Vasu, P. (2005). An oligoglycosidic antibiotic from a newly isolated Streptomyces albovinaceus. Indian J. Microbiol., 45: 33-36.

El-Nakeeb, M. A. and Lechevalier, H. A. (1963). Selective isolation of aerobic actinomycetes. Appl. Microbiol., 11:75-77.

Glazebrook, M.A., Vining, L.C., White, R.L., Smith, K.C. and E.G.Chedrawy.(1993). Nutrient effects on growth and the production of 5 -hydroxy-4-oxonorvaline by Streptomyces akiyoshiensis. Can. J. Microbiol., 39: 536-542.

Glickmann, E. and Dessaux, Y. (1995). A critical examination of the specificity of the Salkowski reagent for indolic compounds produced by phytopathogenic bacteria. Appl Environ Microbiol 61: 793–796.

Gottlieb, D. (1961). An evaluation of criteria and procedures used in the description and characterization of Streptomyces, A cooperative study. Appl. Microbiol., 9: 55-60.

groundnut rust, Puccinia arachidis. World J. Microbiol. Biotechnol., 10: 342-345.

Gunaratna, K. R. and Balasubramanian, R. (1994). Partial purification and properties of extracellular chitinase produced by Acremonium obclavatum, an antagonist to the

Gupta, S. and Choudhary, K. C. (1971). Influence of various nitrogen sources on the production of antibiotic substances by Streptomyces nigrificans Waksman. Indian J. Mycol. Pl. Pathol., 1: 1-7.

Gurtler, A. and Stanisich, V. A. (1996). New approaches to typing and identification of bacteria using the 16S-23S rDNA spacer region. Microibolgy, 142: 3-16.

Hankin, L. and Anagnostakis, S. L. (1975). The use of solid media for the detection of enzyme production by fungi. Mycologia, 67: 597-607.

Hatano, K. (1997). Actinomycete population on mangrove rhizosphere. Res. Comm. Ins. Ferm., 18: 26-31.

Hayakawa, M. (2008). Studies on isolation and distribution of rare actinomycetes in soil. Actinomycetol., 22: 12-19.

Hayakawa, M. and Nonomura, H. (1987). Humic acid-vitamin agar,a new medium for the selective isolation of soil actinomycetes. J. Ferment. Technol., 65: 501-509.

Hayakawa, M. And Nonomura, H. (1989). A new method for the intensive isolation of actinomycetes from soil. Actinomycetol., 3: 95-104.

Hayakawa, M., Sadaka, T., Kajiura, T. and Nonomura, H. (1991). New methods for the highly selective isolation of Micromonospora and Microbispora. J. Ferment. Technol. 72: 320-326.

Haykawa, M., Isizawa, K. and Nonomura, H. (1988). Distribution of rare actinomycetes in Japanese soils. J. Ferm. Technol., 66: 367-373.

He, L., Li, W., Huang, Y., Wang, L., Liu, Z., Lanoot, B., Vancannevt, M. and Swings, J. (2005). Streptomyces jietaisiensis sp. nov., isolated from soil in nortern china. Int. J. Syst. Evol. Microbiol., 55: 1939-1944.

Heisey, R. M. and Putnam, A. R. (1990). Herbicidal activity of the antibiotics geldanamycin and nigericin. J. Plant Growth Regulators, 9: 19-25.

Henrissat, B. (1991). A classification of glycosyl hydrolases based on aminoacid sequence similarities. Biochem. J., 280: 309- 316.

Heydarian, S. M., Lily, M. D. and Ison, A. P. (1996). The effect of cultural conditions on the production of erythromycin by Saccharopolyspora erythraea in batch culture. Biotechnol. Letters, 18: 1181-1186.

Hirsch, C.F.T and Christensen, D.L. (1983). Novel method for selective isolation of actinomycetes.. Appl. Environ. Microbiol., 46: 925-929.

Ho, C., Lo, C., Lai, N., Cheah, H. and Wong, N. (2002).Actinomycetes isolated from soil samples from the cocker range sabah. ASEAN Rev. Biodiversity Environ. Conserv., 9: 1-7.

Holding, A. J. and Collee, J. G. (1971). Routine biochemical tests In: Methods in Microbiology, 6A. Academic Press, London, pp. 1-131.

Hoster, F., Schmitz, J. E. and Daniel, R. (2005). Enrichment of chitinolytic microorganisms: isolation and characterization of a chitinase exhibiting antifungal activity against phytopathogenic fungi from a novel Streptomyces strain. Appl. Microbiol. Biotechnol., 66: 434-442.

Hwang, B. K., Ahn, S. J. and Moon, S. S. (1994). Production, purification and antifungal activity of the antibiotic nucleoside, tubercidin produced by Streptomyces violaceusniger. Can. J. Botany, 72: 480-485.

Kadowaki, S., Saskiawan, I., Watanabe, J., Yamamoto, K., Bunno, M., Ichihara, Y. and Kumagai, H. (1997). Transglycosylation activity of N-acetylhexosaminidase from Penicillium oxalicum and its application to synthesis of a drug carrier. J. Ferment Bioeng., 83: 341-345.

Kamazawa, S., Asami, Y., Awane, K., Ohtani, H.,Fukuchi, C., Mikawa, T. and HAyase, T. (1994). Structural studies of new macrolide antibiotics, Shurimycins A and B. J. Antibiot., 47: 688-696.

Kampfer, P., Kroppenstedt, R.M. and Dott, W. (1991). A numerical classification of the genera Streptomyces and Streptoverticillium using miniaturized physiological tests. J. Gen. Microbiol., 137: 1831 – 1891.

Kaunat, H. (1969). Bisynthesis of indole derivatives by rhizosphere-specific bacteria and Actinomycetes. Zentralbl Bakteriol. Parasitenkd Infektionskr Hyg., 123: 501-515.

Kavitha, A. and Vijayalakshmi, M. (2009). Culturalparameters affecting the production of bioactive metabolites by Nocardia levis MK_VL 113. J. Appl. Sci. Res., 5: 2138-2147.

Kawase, T., Yokokawa, S., Saito, A., Fujji, T., Nikaidou, N.,Miyashita, K. and Watanabe, T. (2006). Comparison of enzymatic and antifungal propertiesbetween family 18 and 19 chitinase from S. Coelicolor A3 (2). Biosci. Biotechnol. Biochem., 70: 988-998.

Kerr, J. R. (1999). Bacterial inhibition of fungal growth and pathogenicity. Microbial Ecology in Health and disease, 11: 129-142.

Kim, B. S., Lee, J. Y. and Hwang, B. K. (1998). Diversity of actinomycetes antagonistic to plant pathogenic fungi in cave and sea-mud soils of Korea. Journal of Microbiology, 36: 86-92.

Kim, C. J., Lee, I. K. Yun, B. S. and Yoo, I. D. (1993).Concanamycin B, active substance against Phytophthora capsici produced by Streptomyces neyagawaensis 38D10 strain. Korean J. Microbiol. Biotechnol., 21: 322-328.

Kim, H. J., Lee, S. C. and Hwang, B. K. (2006). Streptomyces cheonanensis sp. nov., a novel streptomycete with antifungal activity. Int. J. Syst. Evol. Microbiol., 56: 471-475.

Kolbe, S., Fischer, S., Becinevic, A., Hinz, P. and Schrempf, H.(1998). The Streptomyces reticuli -chitin –binding protein CHB2 and its gene. Microbiol., 144: 1291-1297.

Kuminito, S., Lu, J., Esumi, H., Yamazak, Y., Kinoshita, N.,Honma, Y., Hamada, M., Ohsons, M., Ishizuka, M. and Takeuchi, T. (2003). Kigamicins, novel antitumor antibiotics. I. Taxonomy, isolation, physic-chemical properties and biological activities. J. Antibiot., 56: 1004-1011.

Kurup, V. P. and Fink, J. N. (1975). A scheme for identification of thermophilic actinomycetes associated with hypersensitivity pneumonitis. J. Clin. Microbiol., 2: 55-61.

Kuster, E. and Williams, S. T. (1964). Production of hydrogen sulphide by streptomycetes and method for its detection. Appl. Microbiol., 12: 46-52.

Kut-8. World J. Microbiol Biotechnol. (In press).

Kyung, M. Y. And Yong, K. P. (1996). A new method for the isolation of actinomycetes from soil. Biotechnol. Techn., 10: 541-546.

Lango, Z., Toth, L. G., Kovacs, G. and Cruz-Pizarro, L. (1999). Identification and biochemical characterization of Streptomyces galbus from the sediment of the high-mountain lake La Caldera (Sierra Nevada, Spain) Folia Microbiologica. 44: 651-656.

Lawrence, C. H. (1956). A method for isolating actinomycetes from scabby potato tissue and soil with minimal contamination. Can. J. Bot., 34: 44-47.

Lee, J. Y. and Hwang, B. K. (2002). Diversity of antifungal actinomycetes in various vegetable soils of korea. Can. J.Microbiol., 48: 407-417.

Leindenfelser, L. A., Shotwell, O. L., Bachler, M. J., Shannon, G.M. and Pridham, T. G. (1964). Antibotics against plant disease. Appl. Microbiol., 12: 508-512.

Li, Y. V., Terekhova, L. P. and Gapochka, M. G. (2002). Isolation of actinomycetes from soil using extremely high frequency radiation. Microbiology Moscow, 71: 105-108.

Li, Y., Wang, G. P. and Zhang, K. C. (2005). Preliminary identification and classification of soil actinomycetes LJ50 and MJ52. J. Human Agric. Univ., 31: 310-310

Li-Hui, S., Bo, J. and Zhi-Long, X. (2008). Aqueous two-phase extraction of 2, 3-butanediol from fermentation broths by isopropanol/ammonium sulfate system. Biotechnol. Lett. DOI 10.1007/s10529-008-9874-3.

Liu, X., Li, J., Ni, S., Wu, L., Wang, H., Lin, L., He, W. And Wang, Y. (2011). A pair of sulfur-containing geldanamycin analogs, 19-S-methylgeldanamycin and 4,5-dihydro- 19-S- methyl geldanamycin, from Streptomyces hygroscopicus 17997. J. Antibiot., 64: 519-522.

Locci, R. (1989). Streptomycetes and Related Genera. In:Bergey's Mannual of Systamatic Bacteriology, 4:2451-2469

Lounes, A., Lebrihi, A., Benslimane, C., Lefebvore, G. andGermain, P. (1996). Regulation of spiramycin biosynthesis in Streptomyces ambofaciens: effects of glucose and inorganic phosphate. Appl. Microbiol. Biotechnol., 45:204-211.

Mahadevan, B. and Crawford, D. L. (1997). Properties of antifungal biocontrol agent Streptomyces lydicus WYEC 108. Enzyme and Microbial Technol., 20: 489-493.

Malanicheva, I. A., Kozmian, L. I., Dudnik, I.U.V., Stromilova, L.I. and Novozhenov, M. I.U. (1992). Protoplast fusion in Streptomyces fradiae strains producing neomycin and tylosin. Antibiot. Khimioter., 37: 3-7.

Mandal, S. K., Mukhopadhyay, D. and Roy, D. K. (1981). Fermentative production of antibiotic by actinomycetes: Screening of organisms and production of oxytetracycline by Streptomyces rimosus. Ind. J. Microbiol., 21: 28-32.

Manulis, S., Shafrir, H., Epstein, E., Lichter and Barash, I. (1994). Biosynthesis of indole-3-acetic acid via indole-3-acetamide pathway in Streptomyces spp. Microbiol., 140: 1045-1050.

Marshmann, N. A. and Marshall, K. C. (1981). Bacterial growth on proteins in the presence of clay minerals. Soil Biology and Biochem., 13: 127-134.

Mellouli, M., Karray-Rebai, I., Sioud, S., Ben Ameur-Mehdi, R., Naili, B., Bejar, S. (2004). Efficient transformation procedure of a newly isolated Streptomyces sp. TN58 strain producing antibacterial activities. Curr microbial., 49: 400-406.

Merck, 1989, The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals (11th ed.), ISBN 091191028X

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugars. Anal. Chem., 31: 426-428.

Mitsutomi, M., Ohtakara, A., Fukamizo, T. and Goto, S. (1990). Action pattern of Aeromonas hydrophila chitinase on partially N-acetylated chitosan. Agriculture and Biological Chemistry, 54: 871-877.

Nanjo, F., Ishikawa, M., Katsumi, R. and Sakai, K. (1990). Purification, properties and transglycosylation reactions of -N-acetyl hexosaminidase from Nocardia orientalis. Agriculture and Biological Chemistry, 54: 899-906.

Narayana, K. J. P. and Vijayalakshmi, M. (2009). Chitinase production by Streptomyces sp. ANU 6277. Braz. J. Microbiol., 40: 725-733.

Narayana, K. J. P., Prabhakar, P., Vijayalakshmi, M., Venkateswarlu, Y. and Krishna, P.S.J. (2008). Study on Bioactive compounds from Streptomyces sp. ANU 6277. Pol. J. Microbiol., 57: 35-39.

Narayana, K.J.P., Ravikiran, D. and Vijayalakshmi, M. (2004). Production of antibiotics by Streptomyces species isolated from virgin soils. Indian J. Microbiol., 44: 147-148.

Nassar, A. H., El-Tarabily, K. A. and Sivasithamparam, K. (2003). Growth promotion of bean (Phaseolus vulgaris L.) by a polyamine producing isolate of Streptomyces griseoluteus. Plant Growth Regulation, 40: 97-106.

Nedialkova, D. and Naidenova, M. (2005). Screening the antimicrobial activity of actinomycetes strains isolated from Antarctica. Journal of Culture Collections. 4: 29-35. Newman, D.J. and Cragg, G.M. (2004). Curr. Med. Chem., 11: 1689-1709.

Nolan, R. D. and Cross, T. (1988). Isolation and screening of actinomycetes. In Actinomycetes in Biotechnology, Academic Press, London pp. 1-32.

Nolden, L., Beckers, G., Mockel, B., Pfefferle, W. Nampoothiri, K.M., Kramera R., Burkovskia, A. (2000). Urease of Corynebacterium glutamicum: organization of corresponding genes and investigation of activity. FEMS Microbiol Lett., 189: 305-310.

Nonomura, H. (1974). Key for classification and identification of 458 species of the Streptomycetes included in ISP. J. Ferment Technol., 52: 78-92.

Nonomura, H. (1988). Isolation, taxonomy and ecology of soil actinomycetes. Actinomycetol., 3: 45-54.

Oppenheim, A. B. and Chet, I. (1992). Cloned chitinase in fungal pathogen control strategies. Trends in Biotechnol., 10: 392-394.

Palleroni, N. J. (1980). A chemotactic method for the Isolation of Actinoplanaceae. Arch. Microbiol., 128: 53-55 Parag, S. S. and Rekha, S. S. (2007). Optimization of nutrional requirements and feeding strategies for Clavulanic acid

Perrine, F. M., Rolfe, B. G., Hynes, M. and Hocart, C. H. (2004). Gas chromatography-mass spectrometry analysis of indole- 3-acetic acid and tryptophan following aqueous chloroforamte derivatization of Rhizobium exudates. Plant Physiol. Biochem., 42: 723-729.

Pirouz, T., Karbasian, M. A. and Goofellow, M. (1999). Isolation of some aerobic actinomycete species from the soil of Zehaden county, South-East of Iran, Iranian J. Med. Sci., 24: 65-67.

Porter, J. N., Nilhelm, J. J. and Tresner, D. (1960). Method for preferential isolation of actinomycetes from soils. Appl. Microbiol. 8: 174-178.

Praveen, V. C. and Tripathi, K. M. (2008). Studies on optimum fermentation conditions for actinomycin-D production by two new strains of Streptomyces spp. Med. Chem. Res., 17: 114-122.

Pridham, T. G., Hesseltine, C. W. and Benedict, R. G. (1958). A guide for the classification of streptomycets according to selected group placement of strain in morphological sections. Appl. Microbiol., 6: 52-79.

Qin, J. Y., Xiao, Z.J., Ma, C. Q., Xie, N. Z., Liu, P. H. and Xu, P. (2006). Production of 2, 3-butanediol by Klebsiella pneumonia using glucose and ammonium phosphate. China J. Chem. Engg. 14: 132–136.

Rangaswamy, G., Oblisami, G. and Swaminathan, R. (1967). Antagonistic Actinomycetes in the soils of South India. Univ. Of Agric. Sci. Bangalore and U. S. Dept. Of Agric.

Rehn, D. (1983). Quantitative structure activity considerations on the spore-inhibiting activity of esters of 1, 3-dihydroxybutane. Methods Find Exp Clin Pharmacol., 5: 701-705.

Rehn, D., Siegert, W., Nolte, H. (1984). Antimicrobial activity of linear esters of 1, 3-dihydroxybutane. Pharm. Acta Helv. 59: 162-165.

Roy, R. N., Lasker, S. and Sen, S. K. (2006). Dibutyl phthalate, the bioactive compound produced by Streptomyces albidoflavus 321.2. Microbiol. Res., 161: 121-126.

Ruanpanun, P., Tangchitsomkid, N., Kevin, D. H. and Lumyong, S. (2010). Actinomycetes and fungi isolated from plantparasitic nematode infested soils: screening of the effective biocontrol potential, indole-3-acetic acid and siderophore production World J. Microbiol. Biotechnol., 26: 1569- 1578.

Saha, M. R., Ripa, F. A., Islam, M.A. and Khondkar, P. (2010) Optimization of Conditions and invitro Antibacterial activity of secondary metabolite isolated from Streptomyces sp. MNK7. Res. J. Appl. Sci., 6: 453-459.

Sahay, B. N. and Shrivastava, K. P. (1977). Study of growth, antibiotic and pigment production of six strains of Streptomyces on media with varying carbon and nitrogen sources. Indian J. Microbiol., 17: 141-143.

Savic, M., Bratic, I. and Vasilijevic, B. (2007). Streptomyces durmitorensis sp. nov., a producer of an FK506-like immunosuppressant. Int. J. Syst. Microbiol., 57: 2119-2124.

Sekhar, N., Bhattacharya, D., Kumar, D. and Gupta, R. K. (2006). Biocontrol of wood rotting fungi such as Streptomyces violaceusniger XL-2. Can. J. Microbiol., 52: 805-808.

Semedo, L.T.A.S., Linhares, A. A., Gomes, R. C., Manfio, G. P., Alviano, C. S., Linhares, L. F. and Coelho, R.R.R. (2001). Isolation and characterization of actinomycetes from Brazilian tropical soils. Microbiol. Res., 155: 291-299.

Semieniwiez, K. W. and Schrempf, H. (2007). Concerted responses between the chitin-binding protein secreting Streptomyces olivaceoviridis and Aspergillus proliferans. Microbiol., 153: 593-600.

Seong, C. N., Choi, J. H. and Baik, K. S. (2001). An inproved selective isolation of rare actinomycetes from forest soil. The J. Microbiol., 39: 17-23.

Sharma, D. D and Sinha, S. K. (1980). A survey of antagonistic actinomycetes from the soils of Agra region. Indian Phytopathol., 33: 581-585.

Sharma, H. and Parihar, L. (2010). Antifungal activity of extracts obtained from actinomycetes. Journal of Yeast and Fungal Research, 1: 197-200.

Shearer, M. C. (1987). Methods for the isolation of nonstreptomycete actinomycetes. Dev. Ind. Microbiol., 28: 91- 97.

Shilts, T., Erturek, U., Patel, N. J. and Chung, K. R. (2005). Physiological regulation of biosynthesis of phytohormone indole-3-acetic acid and other indole derivatives b the citrus fungal pathogen Colletotrichum acutatum. J. Biological Sciences, 5: 205-210.

Smith, J., Putnam, A. and Nair, M. (1990). In vitro control of diseases of Aspergillus officianalis L. with a Streptomyces or its polyenic antibiotic, Fariefungin. J. Agriculture and Food chemistry, 38: 1729-1733.

Solanki, R., Khamna, M. and Lal, R. (2008). Bioactive compounds from marine actinomycetes. Indian J. Microbiol., 48: 410- 431.

Stackebrandt, E., Rainey, F. A. and Rainey, N. L. W. (1997). Proposal for a New hierarchic classification system, Actinobacteria classis nov. Int. J. Syst. Bacteriol., 47: 479-491.

Sujata, P., Bapiraju, K.V.V.S.N. and Ramana, T.(2005). Studies on a new marine streptomycete BT-408 producing polyketide antibiotic SBR-22 effective against methicillin resistant Staphylococcus aureus. Microbiologial Res., 160: 119-126.

Sun, W., Huang, T., Zhang, Y. Q., Yang, Z. Y. and Liu, Z. H. (2007). Streptomyces emeiensis sp. nov., a novel streptomycetes from soil in china. Int. J. Syst. Evol. Microbiol., 57: 1635-1639.

Suzuki, K., Nagai, K., Shimizu, Y. and Suzuki, Y. (1994). Search for actinomycetes in screening for new bioactive compounds. Actinomycetol., 8: 122-127.

Swain, M. R. and Ray, R. C. (2008). Optimization of cultural conditions and statistical interpretation for production of indole-3-acetic acid by Bacillus subtilis CM5 using cassava fibrous residue. J. Sci. Ind. Res., 67: 622-628.

Syu, M. J. (2001). Biological production of 2, 3-butanediol. Appl. Microbiol. Biotechnol., 55: 10–18.

Taechowisan, T., Lu, C., Shen, Y. and Lumyong, S. (2005).Secondary metabolites from endophytic Streptomycesaureofaciens CMUAc 130 and their antifungal activity. Microbiol., 151: 1691-1695.

Takizawa, M., Colwell, R. R. and Hill, T. R. (1993) Isolation and Diversity of Actinomycetes in the Chesapeake Bay. Appl.Environ. Microbiol., 59: 997-1002.

Thakur, D., Bora, T.C, Bordoloi, G.N. and Mazumdar, S. (2009). Influence of nutrition and culturing conditions for optimum growth and antimicrobial metabolite production by Streptomyces sp. 201. J. Med. Mycol., 19: 161-167.

Thumar, J.T., Dhulia, K. and Singh, S.P. (2010). Isolation and partial purification of an antimicrobial agent from halotolerant alkaliphilic Streptomyces aburaviensis strain

Tresner, H. D., Hayes, J. A. and Backus, E. J. (1968). Differential tolerance of streptomycetes to sodium chloride as a taxanomic aid. Appl. Microbiol., 16: 1134-1136.

Turhan, G. and Grossmann, F. (1986). Investigation of a great number of actinomycete isolates on their antagonistic effects against soil-borne fungal plant pathogens by an improved method. Phytopathol., 116: 238-243.

Vaidya, R. J., Shah, I. M., Vyas, P. R. and Chhatpar, H. S. (2001). Production of chitinase and its optimization from a novel isolate Alkaligenes sylosoxydans: potential in antifungal biocontrol. World J. Microbiol. Biotechnol., 17: 691-696.

Van Loosdrecht, M. C. M., Lyklema, J., Norde, W. and Zehnder, A. J. B. (1990). Influence of interfaces on microbial activity. Microbiol. Rev., 54: 75-87.

Vasadeva, S. H., Thumar, J. T. and Singh, S. P. (2006). Secretion of a potent antibiotic by salt tolerant and alksliphilic actinomycete Streptomyces sannanensis strain RJT-1. Curr. Sci., 91: 1393-1397.

Vinogradova, K. A., Kirillova, N. P., Sokolova, P. A., Nikolau, P.A. and Shulgin, M. V. (1985). Regulation of heliomycin production by the carbon sources. Antibiot. Med. Bioteknol., 30: 264-271.

Vollmar, D., Thorn, A., Schuberth, I. and Grond, S. (2009). A comprehensive view on 4-methyl-2-quinazolinamine, a new microbial alkaloid from Streptomyces of TCM plant origin. J. Antibiot. 62: 439-444.

Watanabe, T., Kanai, R., Kawase, T., Tanabe, T.,Mitsutomi, M., Sakuda, S. and Miyashita, K. (1999). Family 19 chitinase of Streptomyces species: characterization and distribution. Microbiology, 145: 3353-3363.

Wenzel, S. C. and Bode, H. B. (2004). Novel polyene carboxylic acids from Streptomyces. J. Nat. Prod., 67: 1631-1633.

Williams, S. T. and Cross, T. (1971). Isolation, purification, cultivation and preservation of actinomycetes. Methods Microbiol., 4: 295-334.

Williams, S. T. and Davies, F. L. (1965). Use of antibiotics for selective isolation and enumeration of actinomycetes in soil. J. Gen. Microbiol., 72: 249-258.

Williams, S. T., Shameemullah, M., Watson, E. T. and Mayfield, C. I. (1972). Studies on the ecology of actinomycetes in soils VI. The influence of moisture on growth and survival. Soil Biol. Biochem., 3: 197-208.

Yamamura, H., Hayakawa, M. and Limura, Y. (2003). Application of sucrose gradient centrifugation for selective isolation of Nocardia spp. from soil. J. Appl. Microbiol., 95: 677-685.

Yeoh, H. H., Khew, E. and Lim, G. (1985). A simple method for screening cellulolytic fungi. Mycologia, 77: 161-162.

Yokoyama, K. and Mino, Y. (1990). Effect of heated bouvine powder on phases of soil microorganisms and growth of a soyabean. Res-Bull-Obihiro-Univ., 16: 225-230.

Yu, J., Liu, Q., Liu, Q., Liu, X., Sun, Q., Yan, J., Qi, X. and Fan, S. (2008). Effect of liquid culture requirements on antifungal antibiotic production by Streptomyces rimosus MY02. Bioresour. Technol., 99: 2087-2091

Zeng, A. P., Biebl, H. and Deckwer, W. D. (1991). Production of 2,3-butanediol in a membrane bioreactor with cell recycle.Appl. Microbiol. Biotechnol., 34: 463–468.

****