Asian J. of Bio Sci. (April, 2008) Vol. 3 No. 1 : (133-136)

Streptomyces from heavy metal contaminated soils of mines of Orissa, India

J. SABAT AND N. GUPTA*
Microbilogy laboratory, Division of Biotechnology, Regional Plant Resource Centre, BHUBANESWAR (ORISSA) INDIA

(Accepted : February, 2008)

Sixty isolates of *Streptomyces* were isolated from heavy metal contaminated soils of different mines of Orissa.. These isolates were studied for their gram staining properties , extra cellular and fermentative activity . All cultures were branching and rods, gram positive. They showed varied performance during the biochemical and enzymatic tests and analysis. The importance of these isolates is discussed with respect to their distribution in such a extreme habitat of heavy metal contaminated soils of mines of Orissa.

Key words : Streptomyces, Mines, Matal contaminated soil.

INTRODUCTION

Streptomyces are a large and heterogeneous group of prokaryotic organisms. Knowledge of *Streptomyces* biology is rapidly increasing because these organisms have attracted , and continuing to attract attention from the stand point of antibiotic production, mineralization of matter , and R DNA technology (Joo, 2005;. Clark *et al*., 2004; Zhang *et al*., 2004; Hashimoto *et al.,* 2004). The diversity of habitats , ranging from mesothermal to extremely thermal , normal to saline conditions adapted for growth and survival is yet another important feature of this group of organisms (Xiong *et al*., 2005;.Basil *et al*., 2004). Since very rare reports and / or no proper reports are available on *Streptomyces* of mines of heavy metals , it was thought of to explore the possibility of obtaining this group of bacteria from soils of different mine sites of Orissa.

MATERIALS AND METHODS

Study sites and sample collection

Chromite mine Sukinda (Cuttack Dist) :

The Sukinda is the largest single chromiferous fields mass in orissa which is very close to the trifurcation of Cuttack, Dhenkanal and Keonjahr districts of Orissa,. It lies in a watery sloping valley between Mahagiri and Daitari hill ranges between $21^0$00' and $21^0$04' N latitudes and $85^0$40' and $85^0$50' E longitudes in the district of cuttack. The climate is subtropical and wet abundant seasonal rainfall during monsoon months from June to September. Chromite mine soil was loamy sandy with pH of 6.2 This mine soil was endowed with high concentration of Chromium , Iron and aluminum .

Sample collection :

The total mine area was divided into different sectors according to the type of soil and mining processes.

1. Sample collected from the plantation sites (done by RPRC) on the over burden soil
2. Sample collected from the only overburdened soil
3. Samples collected from the mining sides of different top layers.
4. Samples collected from the pure ore of different kinds.

In total 35 different types of samples were collected and brought to the laboratory for isolation.

Manmora manganese mine, Joda (Keonjhar district):

The Manmora manganese mine is laocated at Joda in the district of Keonjhar which is 280 km away from Bhubaneswar, the capital of Orissa. The mining area lies at $22^0$05' N latitude and $88^0$17 E' longitude. The maximum temprarature in the locality ranges from 30^0C to 47^0C and minimum from 5^0C to 2^0C. in different seasons of the year. Annual rainfall varies between 3.9 mm to 320 mm. Soil analysis revealed that the soil was sandy loam in texture and acidic in nature. Organic, carbon, nitrogen, phosphorus, potassium contents are generally low. Due to porous soil, water holding capacity is also very less. This mine soil was endowed with high concentration of Manganese, Iron and nickel .

Sample collection :

The total mine area was divided into different sectors according to the type of soil and mining processe111.

* Author for Correspondence

1. sample collected from open cast mine
2. sample collected from approach to the Quarry
3. samples collected from upper layer dumping area
4. samples collected from loading point
5. samples collected from old plantations (10-15 years)

In total 67 different types of samples were collected and brought to the laboratory for isolation.

South Bolunda colliery. Talcher (Dhenkanal District): The south Bolunda colliery, Talcher is situated in the district of Dhenkanal at a distance of about 88 kms north west of Cuttack in the valley of Bhrahmani river at 564 m . It lies between $20^0$29' N and $21^0$42' N latitudes and $84^0$16' E and $86^0$20' E longitudes. Talcher has the largest reserve of coal in Orissa. The mineral wastes produced from these mines during the 30 years, have given rise to large number of artificial hills and hillocks stretching over several hectares of land. The texture ranges from loamy sand to loamy in texture and acidic. Unlike other mine spoils, organic carbon content was quite high. However, nitrogen, phosphorus, potassium content and water retaining capacity is very low. The soil is characterized by high concentration of Fe,Ca,Al.

Sample collection :

The total mine area was divided into different sectors according to the type of soil and mining processes.

1. Sample collected from plantation sites(done by RPRC) Quarry no. 1.
2. Sample collected from the Quarry no. 2 dumping yard.
3. Samples collected from East wing plantation
4. Samples collected from west wing sites at plantation over burden area.

In total 48 different types of samples were collected and brought to the laboratory for the isolation of various bacteria and fungi by following the different methods of their isolations.

The Streptomyces were isolated from the soils samples obtained from mines sites. Dilution plate technique was followed for the isolation of Streptomyces on Starch casein agar medium of pH 7.2. Gram staining and morphological studies were done through slide preparations and microscopic observations. All tests viz. MRVP, fermentation, amylase and protease production, citrate utilization, catalase and H2S production tests were performed according to Aneja, 1993.

Results and Discussion

The present study was carried out to isolate the specific group of Bacteria i.e *Streptomyces* sp. from heavy metal contaminated soil and plants of different mine sites of Orissa. In results, total 60 different isolates of *Streptomyces* were obtained from 150 samples collected from 15 different sites belonging to these mine area viz. (i) Sukinda (35 samples – 5 sites), Joda (73 samples – 20 from plants and 53 soils), Talcher (42 samples – 35 soil, 5 plants and 2 water samples) (Fig. 1,2,3). The detailed morphological study of these isolates have shown that all isolates were gram positive, rod/ branching, amorphous, whitish and grayish pock forming colonies.

Characterization of *Streptomyces* isolates obtained from different sites of Sukinda, Joda and Talcher have shown that out of 7 isolates of top layer site –1 obtained from 5 different sites of Sukinda mine area, all were amylase +, MR+ , protease –ve , citrate –ve , catalase – ve and H_2S –ve except S3 and S4 that are fermentative for all three sugars tested. Only 2 isolates of *Streptomyces* were obtained from Nickeliferous ore and these were MR +ve , fermentative , amylase producers and H_2S positive. So as to S10 of site no. 3 also MR+, Non fermentative and amylase producers. *Streptomyces* of serpentine soil i.e. S11 and S12 have not shown any extracellular activity except acidic and alkaline product producers: Hence , S11 was VP+ and S12 was MR–ve . *Streptomyces* isolates from different plant rhizosphere of RPRC plantation were fermentative and amylase producers except S35 that was nonproducer of both amylase and protease. A varied results have been obtained in case of all isolates of this sites for the positive and negative activity towards catalase, citrate, H_2S production tests.

Total 14 isolates of *Streptomyces* were obtained from Joda mines of Orissa. All were MR (+) , VP (-) and Fermentative except S 41- 43. All were amylase and catalase producers except S41 . Similarity , in site two, all isolates were amylase + ve , MR +, fermentative exceptS 47 and S48. These strains were protease producers. In site 3 also all were MR+ but nonfermentative except S51 that was Lactose +. All are citrate negative , protease and H_2S positive. Similarly S52 and S53 was found to be MR+, VP–, fermentative, amylase producer but neither producer of protease nor citrate utiliser .

Third mine area Talcher was also studied for this purpose. All 7 isolates were MR (+) VP (-), Fermentative, amylase (+),citrate (-) nonproducers of protease except S58. In case of catalase and H_2S varied results have been observed.

The occurrence of *Streptomyces* has been reported well (Kang *et al.*,2004; Selvin *et al.*,2004). But it is important to note that occurrence of such a good no. of *Streptomyces* indicates the rich diversity of microbes

extreme habitat that is contaminated with heavy metal such as chromium , nickel, cobalt iron and manganese. Due to mining activity, considerable amount of heavy metals are also released into the atmosphere. Although, sometimes these metals are essential for synthesis of a number of enzymes and electron transfer proteins, high levels of it are toxic to microbes (Saxena and Srivastava, 1999).

But the present study reveals the high resistance of *Streptomyces* towards such mechanized activity of mining so as to the heavy metals for which very rare group of organisms adopt a resistance phenomenon. Our isolates may have such properties very well for that these would be categorized and recognized in future.

Steptomyces it self is named as the factory of antibiotic (Kiviharju *et al*., 2004; Wang and lazarovits *et al.*, 2004).The present isolates / strain may be new to the taxa and/or may be endowed with such type of extracellular production. *Streptomyces* are remarkably versatile chemists. The secondary metabolites produced by fermentation of these organisms include simple amino acid analogs as well as complex macrocyclic compounds. Since our isolates were fermentative of nature as some of the them were active for all these sugar type may be useful in this direction (Gunnarsson *et al*., 2004).

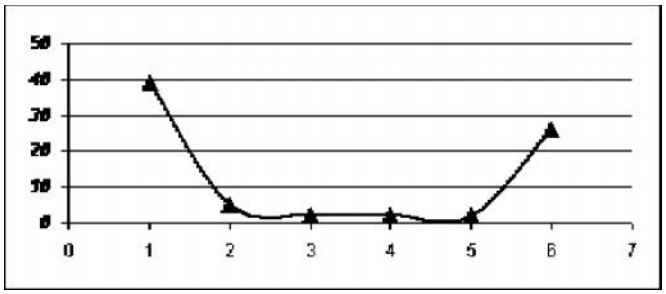

Fig. 1 No. of Streptomyces isolates found in various sites of Sukinda mines of Orissa 1= Total no. f isolates, 2= Top layer ,3= Nikeliferus, 4 = Chromite pure, 5 = Serpentine soil, 6= RPRC plantations

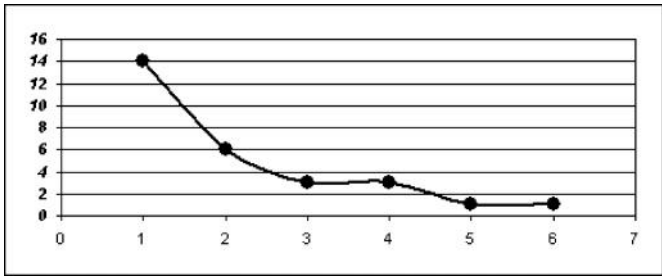

Fig. 2 No. of Streptomyces isolates found in various sites of Joda mines of Orissa 1= Total no. of isolates, 2= approach to quarry,3= Loading point 4 = old plantation, 5 = dumping area , 6= quarry barren land

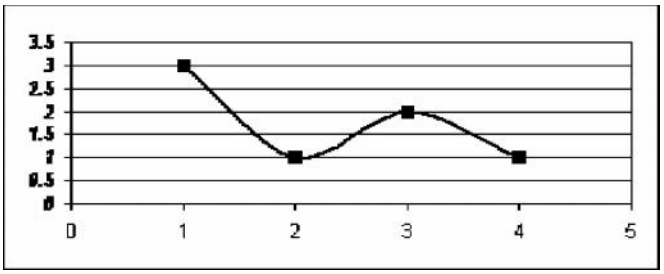

Fig.3 No. of Streptomyces isolates found in various sites of Talcher mines of Orissa 1= Quarry no.1, 2= Quarry no.2, 3=east wing 4 = west wing

REFERENCES

Aneja, K.R. (1993). *Experiments in Microbiology, Plant Pathology and Tissue culture, Wishwy Prakashan , Willey Eastern Ltd.*

Basil, A.J., Strap, J.L., KnotekSmith, H.M. and Crawford, D.L. (2004). Studies on the microbial population of the rhizosphere of big sage brush (*Artemisia tridentata*). *Journal of Industrial Microbiology and Biotechnology.*, **31**(6) : 278-288.

Clark,B., Capon, R.J., Stewart, M., Lacey, E., Tennant, S. and Gill, J.H.(2004). Blanchaquinone : A new anthraquinone from an Austalian *Streptomyces sp*., *Journal of Natural Products*., **67**(10): 1729-1731.

Gunnarsson, N., Bruheim, P. and Nielsen, J. (2004). Glucose metabolism in the antibiotic producing Actinomycetes *Nonomuraea sp*. ATCC39727. *Biotechnology and Bioengineering*., **88**(5): 652-663.

Hashimoto, Y., Herai, S., Higashibata, H. and Kobayashi, M. (2004). Analysis and application of the gene cluster involved in nitrile degradation of industrial Rhodococcus. *Journal of the Japan Society for Bioscience Biotechnology and Agrochemistry,* **78**(11): 1073-1075.

Joo, G.J. (2005). Production of an antifungal substance for biological control of *Phytophthora capsici causing* Phytophthora blight in red peppers by *Streptomyces hastedii, Biotechnology Letters,* **27**(3): 201-205.

Kang, J.H., Ri, N. and Kondo, F. (2004). *Streptomyces sp*. strain isolated from river water has high bisphenol A degradability : *Letters in Applied Microbiology,* **39**(2): 178-180.

Kiviharju, K., Leisola, M. and Eerikainen, T. (2004). Optimisation of *Streptomyces peucetius var. caecius N47* cultivation and epsilonrhodomycinone production using experimental design and response surface methods. *Journal of Industrial Microbiology and Biotechnology,* **31**(10): 475-481.

Saxena, Deepa. and Srivastav, Sheela. (1999). Copper resistance in *Candida guilliermondii* strain DS31: *Current Science,* **76**(2)**.**

Selvin, J., Joseph, S., Asha, KRT., Manjusha, W.A., Sangeetha, V.S., Jayaseema, D.M., Antony, M.C. and Vinitha, A.J.D. (2004). Antibacterial potential of antagonistic *Streptomyces sp*. Isolated from marine sponge *Dendrilla nigra*. *FEMS Microbiology Ecology.*, **50**(2): 117-122.

Wang, A.X. and Lazarovits, G. (2004). Enumeration of plant pathogenic streptomyces on postharvest potato tubers under storage conditions , *Candian Jounal of Plant Pathology-Revue canadienne de phytopathologie,* **26**(4): 563-572.

Xiong, L.X., Li, J.Z. and Wang, H.L. (2005). *Streptomyces avermitilis* from marine. *Journal of Environmental Sciences* – China, **17**(1): 123-125.

Zhang, S.J., Yu, H.Q. and Wu, L.X. (2004). Degradation of calcium lignosulfonate using gamma-ray irradiation, *Chemosphere,* **57**(9): 1181-1187.