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ISOLATION OF CHROMIUM TOLERANT NATIVE FUNGI COLLECTED FROM SUKINDA MINING AREA

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Abstract– Elevated chromium contamination is a prime concern in the Sukinda Valley of Odisha, India. As a chromite hub of our country, the anthropogenic activity is at its peak. Due to over-exploitation through mining, the Sukinda atmosphere is now polluted with hexavalent chromium concentration far beyond its permissible limits. Certain physicochemical technologies for these problems are either high-cost demanding or produce secondary toxins. So mycoremediation can be an effective alternative for degrading hexavalent chromium as it is a well-known decomposer of the environment. This experiment aim to isolate chromium-tolerating potential fungal species from the Sukinda Valley area. Five different soil samples were collected from different areas of the Sukinda region and tested for physic-chemical parameters. The isolation and screening was done in a Potato dextrose agar medium. The results confirm three different species having a potential to tolerate up to a concentration of 5000 ppm whereas the highest tolerance potential was 9000 ppm found in species of *Aspergillus niger*. These remarkable tolerance abilities confirm that these species can effectively apply as bioremediation agents for removing the hexavalent chromium concentration in Sukinda mining areas.

INTRODUCTION

Chromium is one of the most widely used metals all over the world (Zyaed *et al*., 2003). Due to its significant properties like resistance to corrosion, hardness, high melting point and high oxidation ability it has been used in industries like leather, steel, chemicals, steel and refectories (Mishra *et al.*, 2013). Sukinda Valley of Odisha, India is a well-known chromite hub contributing almost 98% of the total chromium production of our country (Suman *et al*., 2020). The exceeding anthropogenic activity especially mining leads to Sukinda Valley contamination of the soil, air and water with high saturation of chromium, mostly hexavalent chromium causing severe damage to the biotic community both directly and indirectly of that locality (Sahoo *et al.,* 2017). So the chromium pollution of Sukinda Valley needs great concern.

Chromium is an essential element in the food chain as it is considered as a microelement in plants but depending on its oxidation it causes toxicity (Sharma *et al*., 2020). Chromium has different oxidation states but only trivalent and hexavalent forms are found to be stable. Among these two forms, the trivalent form is found to be less toxic because of its bigger size, impermeable through biological membranes again it is directly excreted by the excretory system of animals (Chatterjee *et al.*, 2015). Whereas hexavalent chromium due to its smaller size and similarity with sulphate ions, easily gets absorbed by the sulphate channel into the cell and its redox capacity produces ROS complex and results in DNA damage. Considering the lethal effect of chromium concentration WHO announced a limit to the concentration of chromium beyond which it will be considered as toxic. However, according to the USEPA report (U.S. Environmental Protection Agency, 2004h), the chromium level in the Sukinda area is much more exceeded than normal. Hence proper protocols with immediate action to lower down the toxicity level were essential.

Among several remediation protocols, microbial remediation was the most popular, effective and affordable procedure to be applied on a large scale (Khalid *et al*., 2017). Bioremediation is the application of living beings to remediate heavy metal toxicity. In this versatile microbial world, fungi can be a better option to be applied for chromium bioremediation as they are related to metals in various metabolic aspects (Kumar *et al.*, 2011) Again fungi are known to be excellent decomposers (Jacob *et al*., 2018) and there are evidence of having the ability to grow in metals contaminated area. So our experiment aims to study and establish a relationship between chromium and fungi of Sukinda area and determine the maximum chromium tolerance potential of fungi to survive in high chromium concentrated area

MATERIALS AND METHODS

Collection of soil sample

Chromium contaminated mine soil samples were collected from 3 different mining sites near areas of Sukinda, Odisha namely IMFA, Jindal and Kamardha allocated between latitudes 20°53′ and 21°05′ and longitudes 85°40′. The soil samples are collected from 10 cm. depth under the soil surface and preserved in clear plastic bags. The samples were then transported to the laboratory for further investigations.

Physiochemical parameters

The soil samples' physio-chemical parameters were then analyzed which include pH, Temperature, Electrical conductivity total chromium and hexavalent chromium. The total and hexavalent chromium were determined by the APHA method using AAS.

Isolation and primary identification of fungi

To isolate the fungal species serial dilution followed by a spread plate was done in a Potato Dextrose Agar medium (PDB) with an incubation for five days at 30 ^{o}C. Streptomycin (35 mg/ml) was added to avoid bacterial contamination. After the incubation period, the different mycelia were separated and subcultured till pure form was achieved. The fungal samples were primarily identified through phenotypical (shape, texture, diameter and colony appearance) and microscopic observation (septation of mycelium, texture of conidia/spore and shape of spore etc.). The characteristic features were then compared with those described using LPCB staining.

Screening for tolerant species

The chromium tolerance activity of fungal isolates can be identified by introducing a rising concentration of Potassium dichromate (K2Cr2O7) as a chromium source to the growth medium, i.e. Saboured Dextrose Agar. Each fungal isolate is inoculated to different concentrations of chromium (0, 100, 500, 1000, 2000 … up to 10000 ppm) contained in Petri plates and incubated at 30 ^{o}C for a week (Iram *et al.*, 2013). The growth was monitored and the diameter of mycelia growth was calculated from the point of inoculation. Tolerance potential fungi were identified by calculating the tolerance index.

Identification of tolerant species

For identification isolation of genomic DNA is necessary. The gemomic DNA was derived from fresh mycelia using SDS (Sodium Dodecyl sulphate) method (Swain *et al.*, 2018). The molecular characterizations of all fungal isolates were based on internal transcribed spacer (ITS) regions. Translation elongation factor 1(TEFI) and RNA polymerase B-larger subunit-II (RPB-II) regions per standard methods (Tedersoo *et al*., 2018).

RESULTS AND DISCUSSION

Soil sample Physio-chemical parameters analysis

The result of physico-chemical parameter described in Table 1.

In total four soil samples were collected from different sites of Sukinda area. During the collection of samples the environmental of soil sample temperatures range between 35 ^{o}C-39 ^{o}C. The physico-chemical parameter analysis (Table 1) revels that the pH of Sukinda region varies from 7.1 to 7.9 indicating alkalinity nature of soil. The alkalinity may be due to the elevated concentration of chromium (Mishra *et al*., 2010). As per Alam *et al*., 2011 the chromium dominated sites usually shows alkalinity varying a pH from 6.5 to 9.0. Moreover the electrical conductivity the collected samples fall

Table 1. Identification and sequencing of microbilal species in the soil sample

Strain designation	Sources of collection	Place of collection	Species identified	NCBI Accession number
LSS1	Soil Sample	Sukinda mining area	*Aspergillus niger*	OM108171

between 58 to 67 μS/cm.

The total chromium concentration of the agricultural land, overburden, sediments, and ore soil were found to be 2900 mg/kg, 6700 mg/kg, 3300 mg/kg, and 7800 mg/kg respectively whereas the same experiment by Long *et al.*, 2013 showed near about 20,000 mg/kg and regarding the hexavalent chromium the highest concentration is found to be 989 mg/kg, i.e. in ore soil but in his experiment, it was found to be 242 mg/kg the lowest concentration was observed in agricultural land, i.e. 233 mg/kg which is also far from the prescribed limits of EPA (Das *et al*., 2013). This may be due to exceeded open-cast mining and low-grade treatment of discharged wastewater and soil s (Ahemad, 2015). Table 1 Physico-chemical parameter analysis.

Isolation of fungal isolates

Through serial dilution and spread plating the maximum CFU/ml was found to be in agricultural field i.e. 6.0 x 10^2 and the lowest was found to be sediment with a CFU/ml of 1.0 x 10^2. Among those fungal isolates (twenty two) 6 showed resistance towards a concentration of more than 500ppm (Table 2), they were named as OBS1 , OBS2 ASF1, LSS1, LSS2 and LSS3, from which three of them (LSS1, LSS2 and LSS3) are able to tolerate up to 5000ppm and only one showed tolerance to 9000ppm (LSS1).

Identification of fungal isolate

Out of three isolates only the highest tolerant species was designated for identification. Based on the morphological characteristics and molecular identification, isolate was identifies as were identified as *Aspergillus nige*r (LSS1). The ITS sequence data deposited with GenBank viz. Accession number. OM108171. The species were identified by BLASTN search on the NCBI site and identification was confirmed by comparing the sequences with authentic sequences from gene Bank, a phylogenetic tree constructed on (Figure 1).

It was interesting to note that out of 6 isolates 3

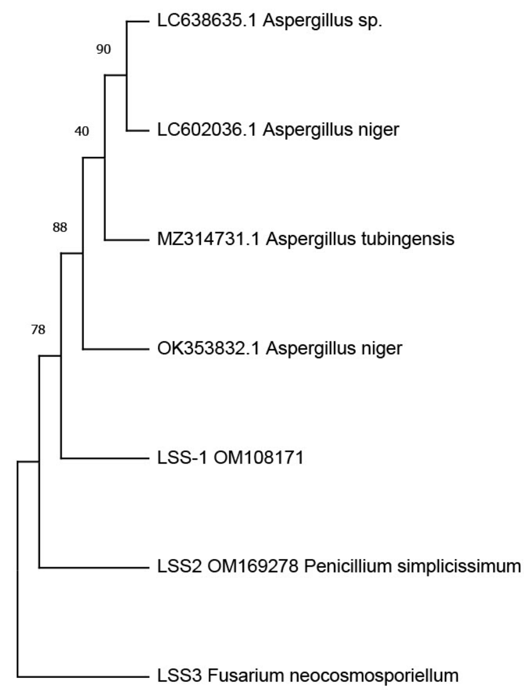

Fig. 1. Phylogenetic tree of LSS1

Table 2. Physico-chemical parameters of the soil samples

Samples	Temp. (°C)	pH	EC (μS/cm)	Total Crmg/kg	Cr(VI)mg/kg
Agricultural land	37	7.1	67	2900	233
Over-burden	37	7.62	61	6700	701
Sediments	38	7.8	58	3300	313
Ore Soil	38	7.69	63	7800	989

Table 3. Screening of Chromium tolerant species in the soil samples

Fungal isolates	Concentration in ppm									
	500	1000	3000	4000	5000	6000	7000	8000	9000	10000
LSS1 *Aspergillus sp.*	++++	++++	++++	++++	++++	+++	++	++	+	—
LSS2 *Peniciliumsp*	++++	+++	+++	+++	+++	++	+	+	—	—
OBF1 *Aspergillus sp.*	++++	++	—	—	—	—	—	—	—	—
OBF2 *Penicillium sp.*	++++	++	—	—	—	—	—	—	—	—
LSS3 *Fusarium sp.*	++++	+++	+++	+++	+++	++	++	++	—	—
ASF2 *Aspergillus sp.*	++++	++	—	—	—	—	—	—	—	—

assumed to be *Aspergillus sp.* which indicates probable abundance of the species in the studied area. However, it needs detailed investigation using more numbers of isolates.

Summary and conclusion

In total twenty-two native fungi were isolated from different contaminated sites of Sukinda Valley. Six species among them show a remarkable tolerance capacity. LSS1, LSS2 and LSS3 are the potential varieties tolerating up to 5000 ppm of Cr (VI) concentration. The most tolerant variety was identified as LSS1 (*Aspergillus nige*r) having a tolerance potential of 9000 ppm.

This high tolerance capacity of the native fungi signifies that these species must possess a proper mechanism for utilization of the Cr (VI) inside or outside of their body. Hence proper and deep investigation of these native species is essential to make an efficient bioremediation agent that can eradicate chromium pollution in Sukinda areas.

REFERENCES

Ahemad, M. 2015. Enhancing phytoremediation of chromium-stressed soils through plant- growth-promoting bacteria. *Journal of Genetic Engineering and Biotechnology.* DOI: 10.1016/j.jgeb.2015.02.001.

Alam, M.Z., Ahmad, S. and Malik, A. 2011. Prevalence of heavy metal resistance in bacteria isolated from tannery effluents and affected soil, *Environmental Monitoring Assessment*. 178: 281–291.

Chatterjee, Sreemoyee, 2015. Chromium Toxicity and its Health Hazards. *International Journal of Advanced Research*. 3: 167-172.

Das, S., Patnaik, S.C., Sahu, H.K., Chakraborty, A., Sudarshan, M. and Thatoi, H.N. 2013. heavy metal contamination, physico-chemical and microbial evaluation of water samples collected from chromite mine environment of Sukinda, India. *Transactions of Nonferrous Metals Society of China.* 23: 484-549.

EPA (U.S. Environmental Protection Agency), 2004h. Nationwide Identification of Hardrock Mining Sites. Evaluation report. Report No. 2004-P-00005. Office of Inspector General, U.S. Environmental Protection Agency, Washington, DC. March 31, 2004 [online]. Available: http://www.epa.gov/oig/reports/2004/20040331-2004-p-00005.pdf [accessed Dec. 5, 2006].

Iram, S., Zaman, A., Iqbal, Z. and Shabbir, R. 2013. Heavy metal tolerance of fungus isolated from soil contaminated with sewage and industrial wastewater. *Polish Journal of Environmental Studies.* 22(3).

Jacob, J.M., Karthik, C., Saratale, R.G., Kumar, S.S., Prabakar, D., Kadirvelu, K. and Pugazhendhi, A. 2018. Biological approaches to tackle heavy metal pollution: a survey of literature. *Journal of Environmental Management.* 217: 56-70.

Khalid, S., Shahid, M., Niazi, N.K., Murtaza, B., Bibi, I. and Dumat, C. 2017. A comparison of technologies for remediation of heavy metal contaminated soils. *Journal of Geochemical Exploration.* 182: 247-268.

Kumar, A., Bisht, B.S., Joshi, V.D. and Dhewa, T. 2011. Review on bioremediation of polluted environment: a management tool. *International Journal of Environmental Sciences*. 1(6): 1079-1093.

Long, D., Tang, X., Caiz, K., Chen, G., Shen, C., Shi, J., Chen, L. and Chen, Y. 2013. Cr(VI) resistance and removal by indigenous bacteria isolated from chromium- contaminated soil. *Journal Microbiology Biotechnology*. 23: 1123–1132.

Mishra, Haripriya and Sahu, Himanshu. 2013. Environmental Scenario of Chromite Mining at Sukinda Valley - A Review. 287-292.

Mishra, V., Samantaray, D.P., Dash, S.K., Mishra, B.B. and Swain, R.K. 2010. Study on hexavalent chromium reduction by chromium resistant bacterial isolates of Sukinda mining area. *Our Nature*. 8(1): 63-71.

Sahoo, Himadri, 2017. Geology, Mining and Environment with an Overview of Sukinda Chromite Mines. 10. 29011/*JEES*-125.

Sharma, A. and Kapoor, D. Wang J. 2020. Chromium Bioaccumulation and Its Impacts on Plants: An Overview. *Plants (Basel).* 9(1):100. doi:10.3390/plants9010100

Suman Nayak, Paresh Kale and Balasubramanian, P. 2020. Inhibition assays of horseradish peroxidase by hexavalent chromium and other heavy metals. International *Journal of Environmental Analytical Chemistry*. 1-13.

Swain, E., Gadekar, A.A., Mane, S.S. and Meher, J. 2018. Determination of Genetic Diversity among *Sclerotium rolfsii* Isolates Causing Collar Rot of Chickpea using Simple Sequence Repeat (SSR) Markers. *Int. J. Curr. Microbiol. App. Sci.* 7(6): 1190-1197.

Tedersoo, L., Sánchez-Ramírez, S., Koljalg, U., Bahram, M., Döring, M., Schigel, D. and Abarenkov, K. 2018. High-level classification of the Fungi and a tool for evolutionary ecological analyses. *Fungal Diversity.* 90: 135-159.

Zayed, A.M. and Terry, N. 2003. Chromium in the environment: factors affecting biological remediation. *Plant and Soil.* 249(1): 139-156.