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COMPARATIVE PRELIMINARY SCREENING OF HEAVY METAL TOLERANT RHIZOBACTERIA ISOLATED FROM THE TWO FERNS *TECTARIA COADUNATA* (J. SM.) C. CHR. AND *PHYMATOSORUS CUSPIDATUS* (D. DON) PIC. SERM. OF DARJEELING DISTRICT

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ABSTRACT

Environment pollution with heavy metal toxicity is now a worldwide issue due to different anthropogenic activities viz. mining, industrial as well as agricultural activities. Heavy metals are not easily degradable in nature and they can accumulate in body of any organisms from primitive microbes to plants as well as animals and effected their biological system. Using microbes as bioremediant is novel approach and it is cost-effective as well as highly efficient. In this present study bacteria isolated from the fern *Tectaria coadunata* (J. Sm.) C. Chr. and *Phymatosorus cuspidatus* (D. Don) Pic. Serm. and screened against one heavy metal nickel in different conc. The heavy metal was used in 0.5 mg/ml, 1 mg/ml, 1.5 mg/ml and 2 mg/ml conc. The twelve strains are isolated from two fern roots which were effective against in all the conc. except at 2.0. mg/ml conc. after 48 hours incubation period.

Keywords : Phytoremediation, Nickel, Heavy metal degrading Bacteria.

INTRODUCTION

In past few decades environmental pollution level has increased and it became harmful to humans as well as other livestock. Now-a-days heavy metal contamination in soil becoming a worldwide problem due to mining, industrial, domestic, medical and agricultural waste activities. A major factor towards this pollution is lack of awareness and appropriate knowledge among the people behind improper disposal and management (Bajpai and Gauba, 2018). Heavy metals are those elements which have metallic properties and an atomic number greater than 20. Cd, Cr, Cu, Hg, Pb and Zn are the most common heavy metals contaminants (Tangahu *et al.*, 2011). Among them some are essential for plant growth and development such as Ni, Pb, Fe, Mn, Cu and Zn (Kumar *et al.*, 1995). There are also non-essential heavy metals such as Cd, Hg, Pb, Cr and As present in soils which have no known biological or physiological role in plant system (Kumar *et al.*, 1995). Both essential and non-essential heavy metals are toxic at higher concentration because they cause oxidative stress by forming free radicles or they replaced essential metals in pigment or enzymes and causes improper function along with inhibit growth and development with multiple adverse health effects (Sajeev *et al.*, 2012, Bajpai and Gauba, 2018).

Several methods are applied to remediate heavy metals from environment but most of them are costly and far away from their optimum performance. The chemical

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technologies generate large volumetric sludge and increase the costs (Rakhshaee *et al.*, 2009). The synthetic techniques not only affect soil microbiota but also make soil infertile and do not remediate completely (Bajpai and Gauba, 2018). The disadvantages of these conventional methods are only applicable when heavy metals were below 100 mg/L (Tomko *et al.*, 2006). Presently, phytoremediation become an effective and affordable technology which remediate heavy metals from contaminated soil. Phytoremediation is the technique where green plants (green grasses, ferns and woody species) are used to remediate contaminated soil, sediments and water. This process takes advantage of the unique and selective uptake capabilities of plant root systems, together with the translocation, bioaccumulation and contamination degradation abilities of the entire plant body (Sajeev *et al.*, 2012). Among plants Pteridophytes are mostly used in phytoremediation because they are herbaceous, shrubby and non-consumables (mostly) (Praveen and Pandey 2020). As they have no role in food chain, they received much more attention than Angiosperms (Prabhu *et al.*, 2016). But more exploration is needed in Pteridophytes because only some pteridophytes are used in phytoremediation.

Though Pteridophytes are used in phytoremediation due to their short life cycle but heavy metals also effect soil microbiota by reducing their total biomass (Brookes and McGrath, 1984; Fliessbach *et al.*, 1994); decreasing their specific populations (Chaudri *et al.*, 1993; Koomen *et al.*, 1990); shifting their microbial community structure (Frostegård *et al.*, 1993; Gray and Smith, 2005). Due to sensitivity and sequestration ability microbes are used for bioremediation (Hallberg and Johnson, 2005; Kao *et al.*, 2006; Umrania, 2006).

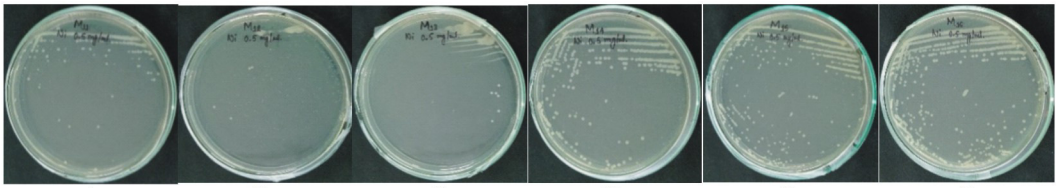
MATERIALS AND METHODS

Plant roots were collected from Rongtong (Latitude → 26° 40' N – 27° 00' N; Longitude → 87° 55' E – 88° 10' E), Darjeeling district. After collecting plant roots they were washed with tap water, cut into small pieces and placed in NA plate at 37°C for 24 hrs. Next day the bacteria which were grown around the small pieces were streaked several times to get the pure culture. Here we got twelve morphologically different isolates from the two ferns *T. coadunata* and *P. cuspidatus*. The isolates were named as M11, M12, M13, M14, M15 and M16 which were isolated from *T. coadunata* and M21, M22, M23, M24, M25 and M26 from *P. cuspidatus*. The isolates were grown on different concentration of heavy metal containing medium like nickel in 0.5 mg/ml; 1.0 mg/ml; 1.5 mg/ml and 2.0 mg/ml concentrations and incubate for 48 hrs at 37°C temperature.

OBSERVATIONS

***Tectaria coadunata*:** Among the six isolates, no isolates were grown on 1.0 mg/ml; 1.5 mg/ml and 2.0 mg/ml conc. of nickel heavy metal containing medium. Only in 0.5 mg/ml conc. isolates were grown. After 24 hrs. M11 showed low growth but M12 and M13 showed very low growth whereas M14 and M15 showed moderate growth in 24 hrs. But

PLATE I



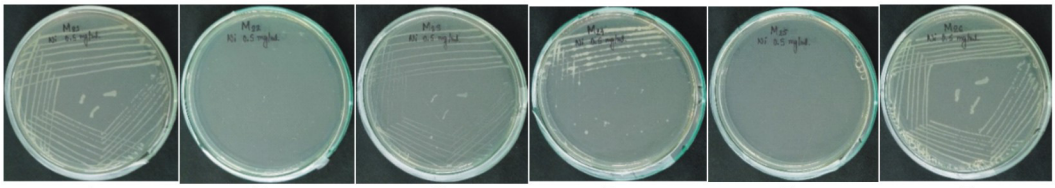
A B C D E F

Fig. 1 : Isolates are against 0.5 mg/ml nickel conc. after 24 hrs. of *Tectaria coadunata*



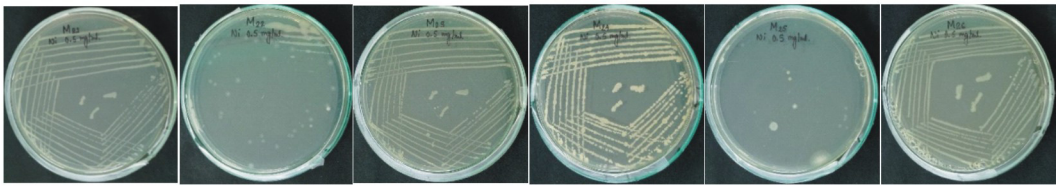
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Fig. 2 : Isolates are against 0.5 mg/ml nickel conc. after 48 hrs of *Tectaria coadunata*



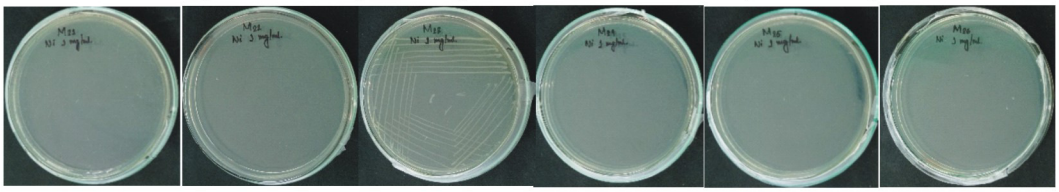
A B C D E F

Fig. 3 : Isolates are against 0.5 mg/ml nickel conc. after 24 hrs of *Phymatosorus cuspidatus*



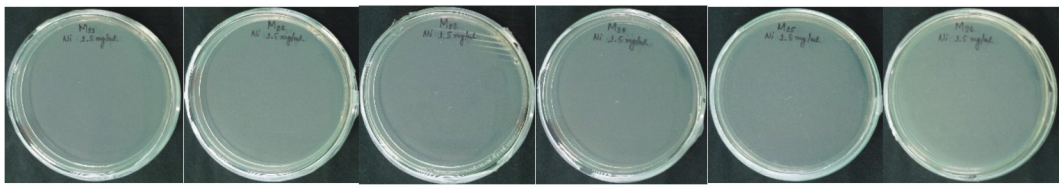
A B C D E F

Fig. 4 : Isolates are against 0.5 mg/ml nickel conc. after 48 hrs of *Phymatosorus cuspidatus*



A B C D E F

Fig. 5 : Isolates are against 1.0 mg/ml nickel conc. after 48 hrs of *Phymatosorus cuspidatus*



A B C D E F

Fig. 6 : Isolates are against 1.5 mg/ml nickel conc. after 48 hrs of *Phymatosorus cuspidatus*

M16 showed high growth within 24 hrs. When after 48 hrs (actual observation period) it was observed then it was found that M11, M14 and M15 showed highly growth whereas M12 and M13 showed moderate growth but M16 showed very high growth.

***Phymatosorus cuspidatus*:** Among the six isolates, no isolates were grown on 2.0 mg/ml conc. of nickel heavy metal containing medium. Only M23 isolate showed low growth in 1.5 mg/ml conc. and this same isolate showed very high growth in 1.0 mg/ml conc. medium. Whereas, in 0.5 mg/ml conc. after 24 hrs M21, M23 and M26 showed very high growth but M22 and M25 showed no growth. Whereas M24 showed moderate growth in these hrs. After 48 hrs (actual observation period) it was observed that M21, M23, M24, M25 and M26 showed very high growth whereas M22 showed low growth.

RESULTS AND DISCUSSION

The isolates of *Tectaria coadunata* only effective at 0.5 mg/ml conc. (500 ppm) (PLATE I, Fig. 1 and 2). But when conc. was increased, they are not effective against nickel. So, at low conc. bacteria are used as bioremediant as they are unable to grow at higher conc.

Whereas, the isolates of *Phymatosorus cuspidatus* they are highly effective at 0.5 mg/ml conc. (PLATE I, Fig. 3 and 4) but there is only one bacterium which is effective against 1.0 mg/ml (PLATE I, Fig. 5) and 1.5 mg/ml conc. (PLATE I, Fig. 6). They are also ineffective at 2.0 mg/ml conc.

CONCLUSION

In case of rhizobacteria obtained from *T. coadunata* can grow i.e. utilized Ni in low conc. But the rhizobacteria obtained from *P. cuspidatus* can grow in lowest conc. of Ni easily. These type of rhizobacteria can remove complex contaminants as well as they increase Ni uptake from soil by changing their phases. So, these rhizobacteria should be utilized as a potent bacteria for phytoremediation. These strains should be identified and can be cultured artificially for the use as a potent agent for phytoremediation in polluted area.

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