ANALYSIS OF ACCEPTANCE OF BACILLUS TO VARIOUS HEAVY METALS AND ANTIBIOTICS

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ABSTRACT

Present study is carried out to systematically examine the pathogenic microorganisms in sewage samples isolated from Shekhawati region of Rajasthan. In this survey 26 bacterial strains were isolated and identified using biochemical and molecular methods. Molecular identification of isolates was done by DNA isolation, PCR amplification using 16S rDNA primer and partial sequencing of purified product after sequencing, out of 26, 10 sequences were submitted in NCBI which designated accession no. Out of these 10 isolates *Bacillus pumilis*, *Bacillus subtilis* and *Bacillus licheniformis* were taken for further studies. There is growing awareness of the need for development of new antimicrobial agents for the treatment of human, animal and plant diseases. Keeping this fact in view, we did the antibiotic susceptibility of these isolates against various antibiotics (Ampicillin, Erythromycin, Chloramphenicol etc.) by measuring zone of inhibition in Mueller Hinton agar plate by Kirby Bauer method comparing with growth pattern at different pH, temperature and various other environmental factors. Results of present studies indicates that the strains isolated from sewage water are resistant to most of the antibiotics and susceptible to Chromium, Cadmium, Mercury and some other heavy metals whereas Zinc and Iron are supporting the growth of bacillus at various parameters. Till date *B.licheniformis* has been checked for its resistance pattern to eight heavy metals. This study can be used for bioremediation of the environment hazards recombinants will also be examine for the same against positive strains.

MORPHOLOGICAL & BIOCHEMICAL CHARACTERISTICS

Bacterial Strains	Grams staining	Shape	Glucose test	Sucrose test	Lactose test	Nitrate reducton test	Indol e Test	H ₂ S Test	M/R Test	VP Test	Citrate Test	Urease Test	Catalase Test	Hugh Lefson Test
Bacillus licheniformis	+ve	Rod	Acid+	Acid+	-ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve
Bacillus pumilis	+ve	Rod	Acid+	Acid+	-ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve
Bacillus subtilis	-ve	Rod	Acid gas +	Acid gas +	-ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve

Enzymatic Activity	Bacterial strains	Amicillin 10	Chloramphenicol 120	Kanamycin 30	Amoxacillin 30	Tetracycline 30	Amikacin 30	Rifampicin 5	Amphotericin 20
Bacillus licheniformis	Bacillus pumilis	Bacillus subtilis	19 mm	35 mm	13 mm	43 mm	38 mm	12mm	No zone
Protease	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Amylase	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Lipase	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Gelatinase	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Cellulase	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Bacillus licheniformis	Bacillus pumilis	Bacillus subtilis	20 mm	28 mm	37 mm	35 mm	30 mm	17 mm	No zone
Protease	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Amylase	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Lipase	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Gelatinase	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Cellulase	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve

PARTIAL GENE SEQUENCES OF B. SUBTILIS USING 16SRDNA PRIMER

AAAGCTCGTGACACCTTCGGCGGCTGGCTCCTAA
AGGTTACCTCACCAGACTTCGGGTGTTACAACTCTC
GTGGTGTGACGGGCGGTGTGTACAAGCCCGGGA
ACGTATTCACCGCGGCATGCTGATCCG.....
.....CGCTCGTTGCGGGACTTAACCCAACATCTCAC
GACACGAGCTGACGACAACCATGCACCACCTGTCA
CTCTGCCCGGCAAGGGGACGTCCTATCTCTAGGATT
GTCAGAGGATGTCAAGACCTGGTAAGGTTCTTCGTG
TTGCTTCAAATTAACCAATGCTCTACCGCCTGTG
CGTGCCCTACCCCAATTCCTCCGAGTCTCAGTCTTG
CCCACCGCACTCCCC

PHYLOGENETIC TREE

GROWTH OF BACTERIA AT 37°C

GROWTH PATTERN AT DIFFERENT pH.

Bacillus subtilis

Bacillus licheniformis

Bacillus pumilis.

INTERACTION OF BACILLUS LICHENIFORMIS (R2) WITH VARIOUS METALS :

CONCLUSION

Present study reveals that the growth of *B. licheniformis* at 37°C shows a uniform pattern till day 6 but decreases at 7th day in rapid manner, as far as pH is concern maximum growth of *B. licheniformis* occurs at pH 7 which is known as neutral pH slight variation in growth has been observed on pH 5 and 9 respectively. Enzymatic analysis of bacillus also taken during study which shows positive result of *B. licheniformis* for Protease, Amylase, Lipase, Gelatinase and Cellulase. Present investigation is important due to heavy metal tolerance studies which showing remarkable variation among Cu, Zn, Fe and Cd.

REFERENCE

1. Zach, A., Latif, M., Binner, E., and Lechner, P. (1999) Influence of mechanical-biological pretreatment on the toxicity of municipal solid waste. *Compost Sci. Util.* 7(4), 25–3
2. Ali S, Roberto F, La fuente, Dona AC (1998). Meta pathway degradation of phenolics by thermophilic Bacilli. *Enzyme Microbial Technol.* 23: 462-468.
3. Alföldi L (1988) Groundwater microbiology: problems and biological treatment-state-of-the-art report. *Water Sci Technol* 20: 1–31.
4. B. Cao, K. Nagarajan, and K. C. Loh, "Biodegradation of aromatic compounds: current status and opportunities for biomolecular approaches," *Applied Microbiology and Biotechnology*, vol. 85, no. 2, pp. 207–228, 2009.