



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	Indole Test	H2S Test	M/R Test	VP Test	Citrate Test	Urease Test	Catalase Test	High Leifson 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 +	Acid gas +	+ve	_ve	_ve	+ve	_ve	+ve	+ve	+ve	+ve

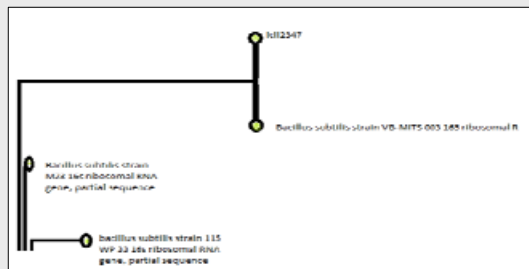
Enzymatic Activity	Bacterial strains			Antibiotics		
	Bacillus licheniformis	Bacillus pumilis	Bacillus subtilis	Ampicillin 10	Chloramphenicol 120	Kanamycin 30
Protease	+ve	_ve	_ve	19 mm	35 mm	13 mm
Amylase	+ve	_ve	_ve	20 mm	28 mm	43 mm
Lipase	+ve	+ve	+ve	30 mm	30 mm	35 mm
Gelatinase	+ve	+ve	_ve	30 mm	30 mm	38 mm
Cellulase	+ve	_ve	_ve	30 mm	30 mm	12mm
				30 mm	17 mm	45 mm
				No zone	No zone	No zone

PARTIAL GENE SEQUENCES OF B. SUBTILIS USING 16SRDNA PRIMER

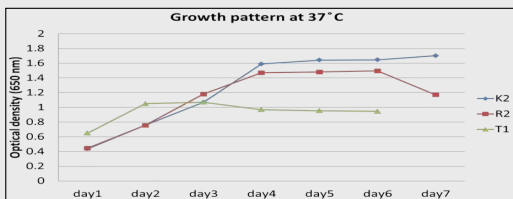
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.....CGCTCGTTGCGGGACTTAACCCAACATCTCAC
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GTCAGAGGATGTCAGACCTGTAAGGTTCTTCGTG
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PHYLOGENETIC TREE

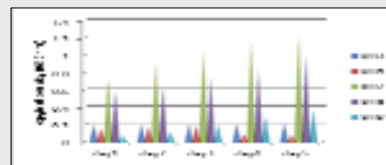


GROWTH OF BACTERIA AT 37°C

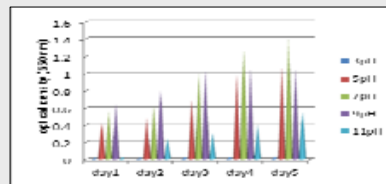


K2 : *B.subtilis*, R2 : *B.licheniformis*, T1 : *B.pumilis*

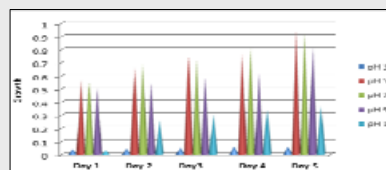
GROWTH PATTERN AT DIFFERENT pH.



Bacillus subtilis

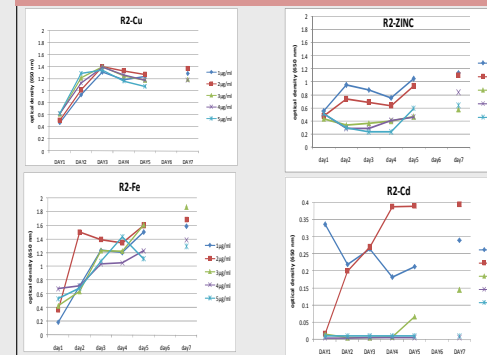


Bacillus licheniformis



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.

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