SCREENING OF DIFFERENT SPECIES OF BACILLUS AGAINST BROAD SPECTRUM ANTIBIOTICS

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Abstract :

Sewage is waste suspended or suspension in water that is intended to flow away from a community. Sewage effluent contains wide range of pathogenic microorganisms which may poses health hazards to human population when they are discharged into the environment. This study is carried out to systematically examine the pathogenic microorganisms in sewage samples isolated from Shekhawati region of Rajasthan. For this purpose survey was carried out at the Sewage Treatment plant of Mody Institute of Technology & Science, Laxmangarh, Sikar. In this survey we isolated 26 bacterial strains and identified them using biochemical and molecular methods. Different biochemical tests were performed during the study and in molecular identification by DNA isolation, PCR amplification using 16s rRNA primer and partial sequencing of purified product was done. The isolates were sequenced and 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. Growth pattern of these isolates was observed on different pH and Temperature. 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 have also did the antibiotic susceptibility of these isolates against various antibiotics (Ampicillin, Kanamycin, Chloramphenicol etc.) by measuring zone of inhibition in Mueller Hinton agar plate by Kirby Bauer method. Results of present studies indicates that the strains isolated from sewage water are resistant to most of the antibiotics available in market, it shows the necessity of new antibiotic to be developed to check the increasing ratio of bacillus infections through contaminated water.

Table 1: Morphological and Biochemical characteristics.

Bacterial Strains	Grams staining	Shape	Biochemical tests											
			Glucose test	Sucrose test	Lactose test	Nitrate reduction test	Indole Test	H2S test	MR Test	VP Test	Citrate Test	Urease Test	Catalase Test	Hugh 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

Table 2: Enzymatic activity.

Enzymatic Activity	Bacterial strains		
	Bacillus licheniformis	*Bacillus pumilis*	*Bacillus subtilis*
Protease Activity	+ve	_ve	_ve
Amylase Activity	+ve	_ve	_ve
Lipase Activity	+ve	+ve	+ve
Gelitinase Activity	+ve	+ve	_ve
Cellulase Activity	+ve	_ve	_ve

Table 3: Antibiotic susceptibility

Antibiotics	Bacterial strains		
	Bacillus licheniformis	*Bacillus pumilis*	*Bacillus subtilis*
	Zone diameter		
Ampicillin (10)	15 mm	20 mm	30 mm
Chloramphenicol (30)	35 mm	28 mm	90 mm
Kanamycin (30)	13 mm	17 mm	90 mm
Amoxicillin (30)	43 mm	35 mm	55 mm
Gentamycin (30)	30 mm	30 mm	35 mm
Amikacin (30)	12mm	28 mm	35 mm
Rifampicin (5)	30 mm	17 mm	45 mm
Amphotericin (20)	No zone	No zone	No zone

Partial Gene Sequences of *B. Subtilis* (603 bp)

AAAGCTCGTGTACACCTTCGGCGGCTGGCTCCTAAAAGGT
TACCTCACCGACTTCGGGTGTTACAAACTCTCGTGGTGTGA
CGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGG
CATGCTGATCCG.....
.....
.....
.CGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGA
GCTGACGACAACCATGCACCACCTGTCACTCTGCCCCCGA
AGGGGACGTCCTATCTCTAGGATTGTCAGAGGATGTCAAG
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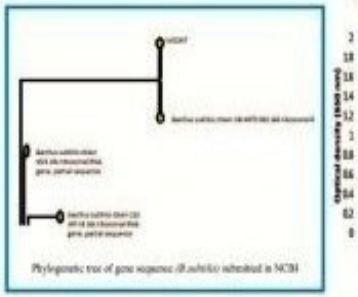
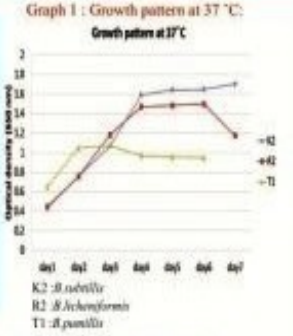
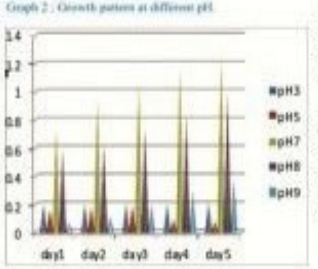


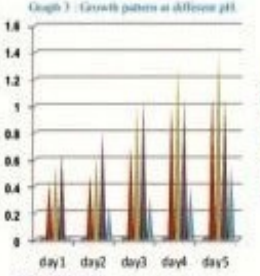
Graph 1 : Growth pattern at 37 °C:



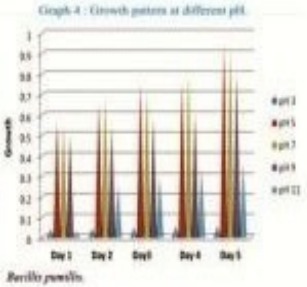
Graph 2 : Growth pattern at different pH.



Graph 3 : Growth pattern at different pH.



Graph 4 : Growth pattern at different pH.



Conclusion and Discussion

In present work the isolation of many bacterial cultures was done from STP sludge. Different strains of Bacillus were isolated from STP sludge and identified using Morphological and biochemical technique followed by Molecular characterization. Isolation, purification and identification of microorganism were done. Media used for isolation of microorganism from sample (STP sludge) was Nutrient Agar, Macconkey Agar etc. Raw sample were collected from Sewage Treatment Plant of MITS campus in 250 ml quantity and kept in conical flasks for further processing. These samples were under gone serial dilution to isolate the maximum number of different culture. After isolating these cultures on the basis of colony characteristics these bacterial cultures were under gone microscopical identification, for this gram staining technique was done. Once it was clear that the culture is gram positive or negative a table were formed and we differentiated the cultures and then designed other biochemical tests. Total 26 isolates were cultured. Out of these 26 samples only 13 samples were carried out for molecular characterization for the confirmation because maximum number of colonies and also due to lack of time and resources. Under molecular characterization isolation of genomic DNA was done, these DNA molecules under gone for PCR amplification and then for Sequencing, these sequences submitted in NCBI database for matching and identification. Some bacterial cultures which were confirmed after sequencing process are *E .coli, Bacillus licheniformis, Bacillus subtilis, Bacillus pumilus, Citrobacter freundii, Klebsiella pneumonia, Achromobacter etc.*

During the studies we observed increased resistance pattern in bacillus species against the various antibiotics available in market. This has been found that Bacillus licheniformis was resistant to antibiotics Kanamycin(30) , Amikacin(30) and Amphotericin(20) where as *Bacillus pumilis* was resistant against Amphotericin (20), its notable that *Bacillus subtilis* is highly susceptible to all the antibiotics used except Amphotericin(20) which shows no zone of inhibition against all the three bacterial cultures. **It confirms that Amphotericin** is a polyene antifungal drug, which often used intravenously for systemic fungal infections so that it is not effective against bacterial strains. At different pH the growth pattern of different species was surprising as all the three species shown maximum growth at pH 5 to 9 (Acidic to basic) which shows high resistance pattern of strains against pH, growth pattern of bacillus strains at 37°C reveals that bacillus subtilis has increasing growth on 7th day whereas both other strains has entered in decline phase. It also predicts the most resistance strain is *bacillus subtilis*. Microbiological analysis of the samples showed that the sewage water contains high number of micro organisms which may be harmful as well as beneficial for environment. As beneficial micro organisms they work as a decomposer of pollutants but as a harmful creature they can affect on human health and also agriculture. Keeping this view in mind this work is important for Environment, Agriculture and also for human and animal life.

Reference :

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