Cellulose can be obtained from four major sources. Plants provide the bulk of celluloses. Another source of cellulose comes from the biosynthesis of it by various microorganisms like bacteria, algae, fungi. The remaining two sources are less common.

Cellulose present in the domestic waste is degraded by cellulolytic microorganisms into other useful products. For everyday use of man they play a very important role in the biosphere by breakdown of cellulose into various economically important products such as compost, antibiotics, monomeric sugars, microbial biomass protein, etc. The extracellular enzymes produced by microorganisms helps in the biodegradation of organic contaminants. For microbes these contaminants are considered as the microbial food source or substrates (Maier et al., 2000).

Cellulosic activities have been reported in many microorganisms including fungal and bacterial strains both anaerobic and aerobic. Cellulolytic bacterial species include *Butyrivibrio fibrisolvens, Ruminococcus albus, Methanobrevibacterium ruminatium, Trichonympha* and *Bacteroides succinogenes* (Milala et al., 2005 and Schwarzz , 2001).

High growth rate bacteria have good potential to be used in cellulase production as compared to fungi (Sethi et al., 2013). Commercially production of enzymes are done on refined substrates and usually on patented organisms hence they are expensive. So it is necessary to use cheaper substrates from local sources for the production of enzymes.

It is always challenging to prevent plants from phytopathogens. Different approaches have been evaluated for more than a century to control phytopathogens. However, among the different methods used chemical and synthetic strategies have gained the most limelight. The main reason behind this is the fast action of synthetic chemical against pests. This led to over consumption of chemical pesticides in many countries (Zhang et al., 2011). The use of biological control agents (BCA) to eliminate the actions of phytopathogen has proven to be more useful than chemical approach.

Amongst all known mechanisms of biocontrol, secretion of the lytic enzymes like cellulase is recognized as an effective way to deter phytopathogens inhabiting the vicinity of the rhizosphere. These biocontrol enzymes utilize various mechanisms involved in the removal of phytopathogens and indirectly help in plant's growth and survival.

The application of cellulases in agriculture is reported in improvement in crop growth and control of plant diseases. For this purpose, the combinations of cellulases, hemicellulases, and pectinases are mostly used. It is reported that these bacteria play an important role in minimizing application of chemical fertilizers thereby improving plant development, controlling potential plant pathogens and protecting plants from diseases.

There are various details of application of bacteria such as plant growth-promoting rhizobacteria (PGPR) to improve plant performance. Rhizosphere microbes are well known for their ability to promote plant growth and in the control of phytopathogens that are capable of causing disease in plants. Rhizospheric microbes provide plants with tolerance towards phytopathogens by various means. It is reported that these bacteria have a major role in reducing use of chemical fertilizers to improve plant development and protecting it from plant pathogens and diseases. Some studies are about possible interactions between bacterial cellulase production and bacterial antibiotic production against phytopathogenic fungi.

Materials and Methods:

Sampling : Sampling of cellulase producing microorganisms was done from two different sites, A- garden soil and B- a playground of Patna, Bihar. Rhizospheric soil samples from a depth of about

10cm were collected in sterile polythene bags and brought to the laboratory, Dept. of Microbiology, Patna Women's College under aseptic conditions for further processing. Soil sample A had a pH of 6.8 while B had a pH of 7.0.

Isolation of bacteria : Isolation of bacteria was done on Carboxy Methyl Cellulose Agar (CMC) plates through serial dilution (10^{-1} to 10^{-7}) and spread-plate technique (Aneja, 2015). The inoculated CMC plates were incubated at 37ºC for 3-5 days under regular observation. Morphologically different bacterial colonies were repeatedly sub cultured to get pure isolates. Each pure isolate was then maintained on NA slants at 4ºC.

Screening of cellulase producing bacteria : Purified bacterial isolates were streaked on the CMC plate .The inoculated plates were incubated at 37°C for 1-2 days. After growth was observed, the plate was flooded with Gram's Iodine solution. Formation of halo zone (clear zone) around the colony confirms cellulase producing bacteria. This was followed in accordance with the works of Philip et al, (2016).

Production of cellulase crude enzyme : Cellulase enzyme was produced using growth medium. The screened isolates were inoculated in culture broth in a shaker incubator at 200 rpm at 37˚C for 48 h. Then the cell free extract was obtained by centrifugation at 6000 rpm for 15 mins in a cooling centrifuge. The supernatant (crude enzyme) obtained after centrifugation was stored for further estimation. The enzymes produced were named on the basis of bacteria isolated from different soil samples as A1, A2, A3, B1 and B3 bacterial enzymes.

Phytopathogenic fungal culture: The phytopathogenic fungal culture was procured from the Department of Microbiology- Patna Women's College. Pure cultures of three organisms were taken namely- Trichoderma, Aspergillus and Penicillium.

Effect of enzyme on phytopathogenic fungi: The fungal cultures were spread using sterile cotton swabs dipped in peptone water on MHA (Mueller Hinton Agar) medium. Disc diffusion assay was performed for bacterial crude enzymes and the plates were incubated at 26˚ C for 24 hours.

Results and Discussion :

Sampling : Two soil samples were collected each from a different sampling site. The physio-chemical study was done for both the samples (Table 1).

Isolation and screening of bacteria : A total of 17 morphologically different bacterial colonies appeared on CMC plates at 37˚C (Table 1). 10 were isolated from soil sample A and were designated A1, A2, A3, A4, A5, A6, A7, A8, A9, A10, while from sample B they were 7 namely, B1, B2, B3, B4, B5, B6, B7.

Colony forming unit (CFU) was high for sample A as compared to sample B. Among 17 isolates, a total of 06 bacterial isolates produced a halo zone around colonies when treated with gram's iodine on CMC plates namely, A1, A2, A3, B1and B3 which were estimated as cellulase producers (Table 1).

The mechanism lying behind the formation of halo zone around cellulase producing bacterial colonies is that those bacterial colonies were capable of solubilizing cellulose.

Table 1. Sampling, isolation and screening of cellulase producing bacteria

Sampling Sites	pH	Characteristics	Total number of bacterial isolates	CFU/ml	Total number of cellulase producing positive isolates
A	6.8	Brownish, dry	10	2.5x 106	03 (A1,A2,A3)
B	7.0	Deep brownish,wet	07	1.4 x 106	02 (B1,B3)
Total			17		05

Phenotypic Characterization

Morphological study

Morphological study of 06 best cellulase producing positive isolates showed quite variation (Table 2). A1 was oval in shape with irregular margin, off white in colour, small, slightly elevated colony, A2 was circular in shape with regular margin, white in colour, large colony elevated at center, A3 was circular in shape with regular margin, white in colour, large colony having a bulge in center, B1 was oval in shape with irregular margin translucent small elevated colony, B3 was circular in shape with regular margin light pink in colour small flat colony.

Table 2. Morphological characterization of cellulase producing bacteria

Characteristics	A1	A2	A3	B1	B3
Margin	irregular	regular	regular	irregular	regular
Elevation	Slight elevated	elevated (center)	Bulge in center	elevated	flat
Colour	off white	white	white	translucent	light pink
Size	small	large	large	small	small
Shape	oval	circular	circular	oval	circular
Gram character	+ve (diplo *Bacillus)*	+ve (mono *Bacillus)*	-ve (mono *Bacillus)*	+ve (mono *Bacillus)*	+ve (mono *Bacillus)*

Effect of bacterial cellulase on phytopathogenic fungi

The effect of bacterial cellulase on phytopathogenic fungi was based on the zone of inhibition observed after performing disc diffusion assay of bacterial enzymes at 26˚C for 24 hours (Table 3).

Bacterial enzyme A1 was slight, negligible and highly effective against *Trichoderma, Aspergillus* and *Penicillium* fungal cultures respectively. Enzyme A2 was slightly effective against *Trichoderma* and *Penicillium* while very minutely effective against *Aspergillus.* Enzyme A3 was moderately effective against *Trichoderma* and *Penicillium* while slightly effective against *Aspergillus.* Enzyme B3 was slightly effective against *Trichoderma* and moderately effective against *Aspergillus* and *Penicillium* cultures.

Table 3. Effect of bacterial cellulase on phytopathogenic fungi

Bacterial enzyme	Effects on phytopathogenic fungus		
	Trichoderma	Aspergillus	Penicillium
A1	slightly effective	negligible effect	highly effective
A2	slight effective	minutely effective	slightly effective
A3	moderate	slight effective	moderate
B1	moderate	effective	moderate
B3	slight effective	moderate	moderate

Conclusion:

Among 17 bacterial isolates, 05 isolates produced halo zone (clear zone) around bacterial colony which were estimated to be cellulase producers. From sampling site A, total isolates were 10 out of which 03 were positive. Thus, approximately 30% of them showed positive activity. Similarly, from sample B, 02 out of 07 isolate approximately 29% positive activity.

Bacterial crude enzyme B1 showed the best result for inhibition of phytopathogenic fungi that has moderate and effective results on all the three organisms under test namely, Trichoderma, Aspergillus and Penicillium. While crude enzyme A1 showed least inhibition on fungal cultures.

Phenotypic characterization of best isolates gave a preliminary idea about generic categories though for authentication detailed biochemical analysis as well as molecular characterization through 16S rRNA gene sequencing and phylogenetic analysis is required for identification up to generic and species level, respectively.

Owning to the initial levels of the effect of bacterial cellulase on phytopathogenic fungi it should be considered as a very important method to control plant diseases Bacterial cellulase can be easily produced under inexpensive means at a high rate that can bring notable change in the control of phytopathogenic fungi to reduce plant diseases.

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