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RESEARCH **P**APER

Isolation and screening of cellulase producing bacterial strains from Cafeteria waste

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Cellulases are a group of enzymes that are involved in the breakdown of cellulose into simpler sugars. These enzymes have tremendous application in the production various products like pulp and paper, textiles etc. Plants, animals and various microorganisms like protozoans, bacteria and fungi naturally produce these enzymes. Since most industrial applications utilize fungi and bacteria for fermentation, this study was also designed to isolate cellulose producing bacterial strains. In this study, food waste was collected from cafeteria and used for isolation of cellulase producing bacterial strains. The waste was pulverized using mortar and pestle subsequently used for making serial dilution, followed by the spreading of diluents on nutrient agar plates. Morphologically distinct colonies were selected and streaked to obtain single colonies. Cellulase production potential of the selected 8 isolates was tested by streaking each strain on agar plates enriched with different concentration of carboxymethylcellulose (as the sole carbon source). The plates were incubated for 72-120 hours followed by staining with 1% (v/v) Congo-red dye. Further the plates were decolourised by 1M Sodium chloride solution. A zone of clearance was observed around the bacterial strains capable of cellulase production because of their ability to hydrolyze cellulose. Further, the ability of the isolates to utilize varying concentration of CMC was tested in order to ascertain. The study resulted in isolation of 3 cellulase producing bacterial strains from cafeteria waste which can be utilized in cellulase production in the industries and also in agricultural waste management.

Key words : Cellulase, Cellulose hydrolysis, Waste, Paper, Pulp industry, Microbial product

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INTRODUCTION

Cellulose is a complex carbohydrate polymer composed of Glucose as the basic unit. It is a polysaccharide, which is an important molecule of plant cell wall. As India is an agriculture-based economy, the cellulose waste generated after crop harvesting is in huge amount. World over scientists are working on devising new methods to utilize the major Carbon content of cellulose. Biotechnological transformation of cellulosic biomass to respective simpler sugar forms is potentially a sustainable approach for development of novel bioprocesses and products. Cellulases are a class of enzymes that hydrolyze β-1, 4 linkages in a cellulose chain essentially converting them into simpler sugars (Henrissat, 1991). High costs of Cellulases are one of the largest obstacles for commercialization of biomass bio-refineries because a large amount of cellulase is consumed for biomass saccharification (Zhang and Zhang, 2013 and Zhu *et al.*, 2011).

Microbial Cellulases have irrevocably become the principle biocatalysts due to their complex nature and

extensive industrial application. These enzymes are synthesized by a large assortment of micro-organisms including both fungi and bacteria (Kubicek *et al.*, 1993) and purified for the use in industries like pulp and paper, textile, laundry, biofuel production, food, agriculture and breweries (Dienes *et al.*, 2004). Since cellulase converts cellulose into simpler sugar forms, which can further be used for fermentation, they are also employed for bioethanol production and biofuels where materials like rice straws primarily comprising of cellulose are used (Chandra *et al.*, 2007)

Acinetobacter, *Bacillus* and *Pseudomonas* sp. are quite commonly used for bacterial production of cellulase at the industrial scale due to their ability to produce and secrete large quantities of extracellular enzymes or on the surface (Mawadza *et al.*, 1996).

Saccharification and fermentation (SSF) processes are combined simultaneously for enzymatic hydrolysis of cellulose with subsequent fermentation of reducing sugar (glucose) which can ultimately be converted to ethanol. SSF studies from lignocellulosic biomass such as wheat and rice straw, corn stalk, corn cobs, and forestry wastes using cellulase from natural sources (Howard *et al.*, 2003) have already been reported.

Thus, due to many such salient applications of this enzyme it is essential to isolate and identify high-cellulase producing bacterial strains. In this study, we have collected a nutritious waste from a university cafeteria and aimed to isolate high-cellulase producing bacterial strains from the same.

Research Methodology

Sample collection :

Food waste was acquired from the Cafeteria situated near Amity University Uttar Pradesh (Noida) in a sterilized container.

Isolation of bacterial strain :

For the purpose of isolation of a novel bacterial strain, nutrient agar medium (0.5% peptone, 0.3% beef extract, 0.5% sodium chloride and 2% agar) was prepared and autoclaved at 121°C, 15 psi for 20 minutes. After cooling, the medium was poured into sterilized plates and left to solidify. These plates were then further used for isolation of bacterial strains by serial dilution.

Collected waste was weighed and 5g of waste was mashed with the help of mortar and pestle. This pulverized pulp was then mixed with Distilled sterilized water and the left to settle down for some time. The upper clear layer of water was used for making serial dilution from 10^{1}-10^{8}. Different dilutions of the waste 10^{-6}, 10^{-7}, 10^{-8} were spread on the nutrient agar plates. These plates were then incubated at 37°C for 24-48 hours. Colonies that were visually distinguishable were selected and streaked onto a fresh nutrient agar plate, to isolate single colonies, thus giving us a pure culture of the desired bacterial strain. Isolated strains were stored at 4°C.

Screening of cellulase producing bacterial strains:

Although a large number of micro-organisms are capable of degrading cellulose, only a handful of these micro-organisms produce significant quantities of cellulase enzymes capable of completely hydrolyzing cellulose. The isolated colonies of bacteria were subsequently picked and inoculated onto agar plates containing different concentration of carboxymethyl cellulose (0.2%-1%), Potassium dihydrogen phosphate 0.5g/L, Magnesium Sulphate 0.25g/L and the pH was set to 6.8-7.2. These plates were then incubated at 37°C for 72-120 hours. Post-incubation the plates were flooded with 1% (v/v) Congo-red for staining and de-colorized with 1M sodium chloride solution. The degradation zones were clearly visible around the bacteria, showing that the strains could hydrolyze varied concentration of CMC.

Research Findings and Analysis

Waste from cafeteria was collected which was a mixture of fruit and vegetable peels, fruit pulp and waste food. The waste was pulverized in a sterile mortar pestle with the help of autoclaved double distilled water, making sure that the bacterial strains were isolated from the waste itself. By serial dilution and spreading, 8 visually distinct colonies were isolated and purified by streaking (Fig. 1).

The purified colonies were tested for their cellulose hydrolysis potential. The bacterial strains to be tested were streaked across the cellulose agar plate by incorporating cellulose (as an only carbon source) into the medium. The growth of a bacterium on this medium suggested the possibility of Cellulase production by the bacterium to utilize the cellulose present in the medium, as there is no other carbon source available. Cellulase production was confirmed by flooding the plate with Congo-red dye and de-colourised with 1M sodium chloride solution. A clear zone is visible around the

cellulase producing bacterial strains after decolourization. These zones are a result of hydrolysis of cellulose by cellulose, while no clear zones was found around cellulase negative bacterial strain. It was observed that a total of 3 isolates had the ability to produce the cellulase enzyme. The ability of these isolates to degrade different concentration of cellulose was also tested and it was observed that that the most effective degradation was done at 0.2% of CMC (Fig. 2) by strain number ART_PS3 and ART_PC2.

Conclusion :

Cellulases are cellulose-degrading enzymes, which are diversely applied in various industries. Bacterial Cellulases are quite useful in the agricultural waste management. These enzymes can be synthesized by optimizing the cultural conditions for Cellulase producing bacteria. In the current study, we were able to isolate 8 distinct bacterial strains from cafeteria waste. Three strains were found to produce extracellular Cellulase out of which ART_PS3 and ART_PC2 showed highest

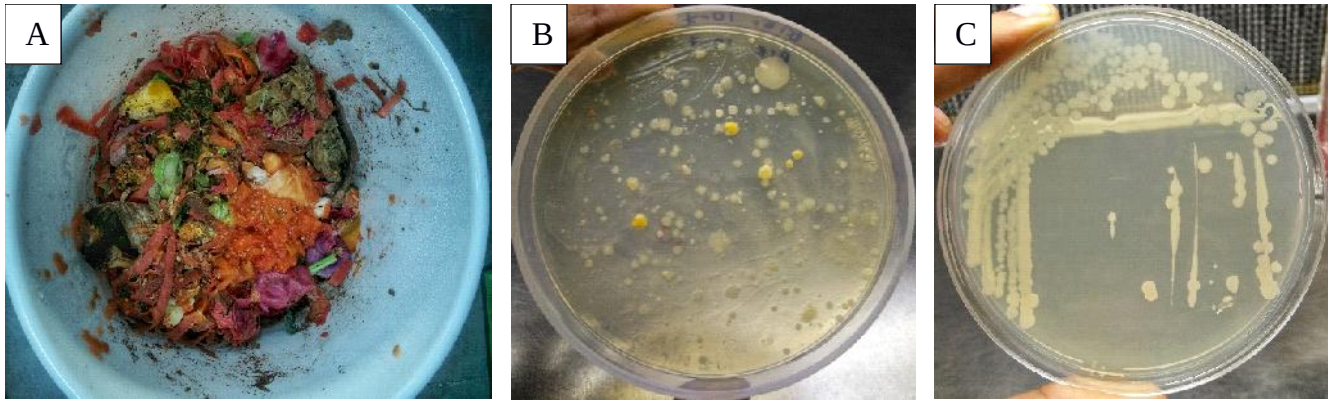

Fig. 1 : A) Waste collected from Cafeteria B) Spreading of diluents after serial dilution C) Streaking of isolated colonies

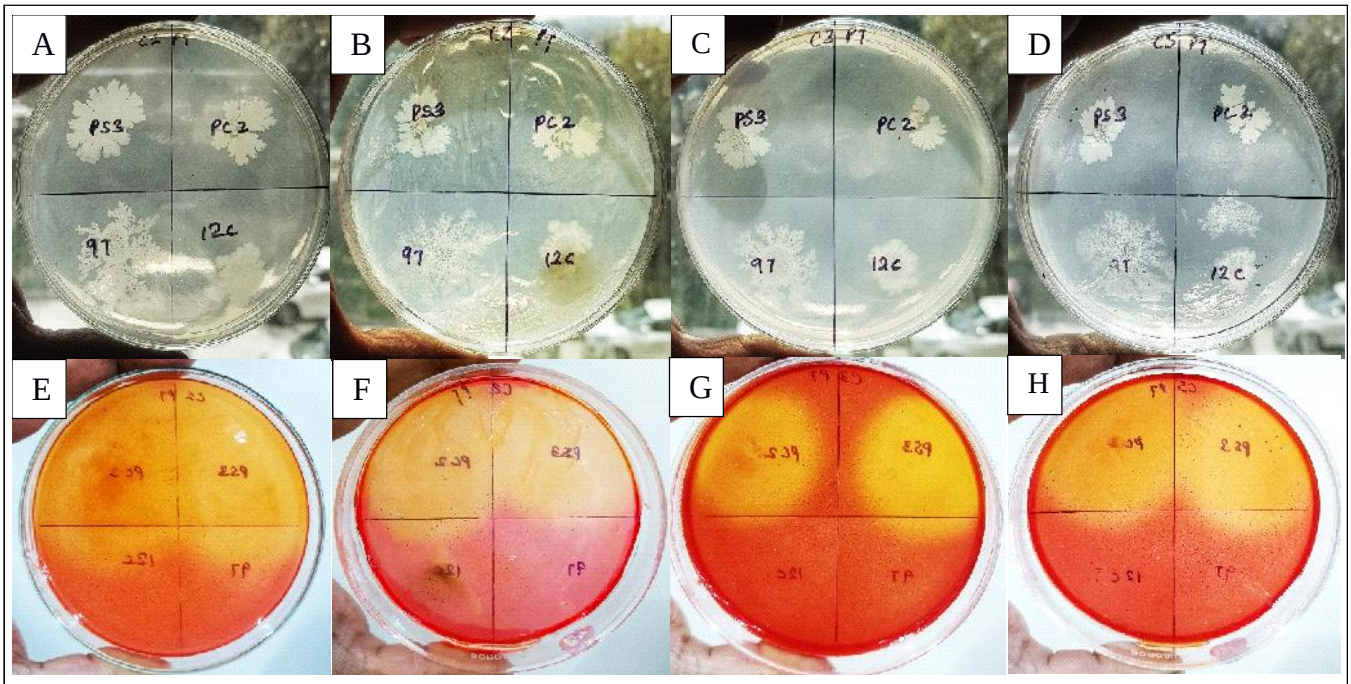

Fig. 2 :(A-D) Growth of Isolated bacterial strain on varying concentration of CMC (0.2%- 1%). (E-H) Production of cellulase on different concentration of CMC as depicted by the distinct zone of clearance

cellulase activity. Further studies are being undertaken to identify the optimum conditions for maximizing the cellulase production by these bacterial strains.

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