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Isolation and Screening of Cellulase Producing Bacteria from Soil

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A B S T R A C T

The aim of this study is to display the potential of various isolates screened from soil have cellulase producing ability. For the selective isolation of cellulase producing isolate cellulose was added in the nutrient agar media (NAM). After that screening was performed with congo red to check the zone of hydrolysis on NAM plates containing cellulose. This process is repeated several times to obtain a clear transparent zone of hydrolysis on plates. The diameter of zones was measured for the seletected isolates. Total 10 bacterial isolates were obtained from soil. Out of 10, 5 were selected for screening. Highest zone diameter was 9±1, found in isolate SHI III. This paper demonstrating the isolation of cellulolytic bacteria from soil samples collected from local area of ishanpur, bulandshahar. So, it is a brief representation of isolation and screening of cellulase producing bacterial strain.

Keywords: Cellulase, Isolation, Screening, Bacteria, Congo red dye

1 INTRODUCTION

Cellulose is the major renewable, abundant and cheaper source of biopolymer on earth. (Lynd et al., 2008). Lignocellulose composed of consists of cellulose, hemicellulose and lignin. The breakdown of these lignocelluloses to their respective monomers for the generation of useful products is of great concern and the whole process using chemicals is so expensive that is seems to be unattractive for the industries to be used (Putro et al., 2016). Conventional methods also polluting in nature, so they can be replaced by enzymatic methods. Microbial sources of enzyme are highly efficient and cause less pollution (Desai and Iyer, 2016; Kaur et al., 2016). Cellulose is the structural component of the primary cell wall of green plants, many forms of algae and the oomycetes. Cellulase degradation and its subsequent utilization is important for global carbon sources. More research should be aimed in obtaining new microorganisms producing cellulase enzymes with higher specific activities and greater efficiency (Subramaniyan and Prema, 2000). Over the years, a number of microorganisms, in particular fungi, possessing cellulose-degrading enzymes have been isolated and studied extensively (Bhat et al., 1997). Cellulolytic enzymes play an important role in natural biodegradation processes in which plant lignocellulosic materials are efficiently degraded by cellulolytic fungi, bacteria, actinomycetes and protozoa. In industry, these enzymes have found novel applications in the production of fermentable sugars and ethanol, organic acids, detergents and other chemicals. Cellulases provide a key opportunity for achieving tremendous benefits of biomass utilization (Wen et al., 2005). Cellulolytic enzymes are synthesized by a number of microorganisms. Fungi and bacteria are the main natural sources of cellulose degradation (Lederberg, 1992). Biotechnology of cellulases and hemicellulases began in early 1980s, first in animal feed followed by food applications (Voragen, 1992; Thomke et al., 1999). Several fungal sources of cellulase have been reported in literatures with limited bacterial

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sources. As bacterial cellulase have high growth rate in comparison to fungal microbes. So, therefore more research has to be done on bacterial isolates, which have capability to produce high cellulase enzyme titre (Singh et al., 2020). The major industrial applications of cellulases are in textile industry for bio-polishing of fabrics and producing stonewashed look of denims, as well as in household laundry detergents for improving fabric softness and brightness. Besides, they are used in animal feeds for improving the nutritional quality and digestibility, in processing of fruit juices, in baking etc. Utilization in deinking of paper is yet another emerging application. The cellulases that are used so far for the above-mentioned industrial applications are those from fungal sources (Tolan and Foody, 1999). So, it is prerequisite to check the potential of bacterial microbes to produce cellulase enzyme. Efforts has been concentrated on the isolation and screening of different microbes (bacteria) for cellulase production.

2 MATERIALS AND METHODS

2.1 Chemicals required: Cellulose, congored, NaCl, peptone, beef extract, agar and all chemicals were procured from Himedia.

2.2 Collection of soil samples: Soil samples were collected from various local area of ishanpur, bulandshahar. Two soil samples were collected individually and stored for further use in the laboratory. Soil samples has been passed through sieve in order to remove all impurities, before carry out the experiments.

2.3 Preparation of culture media: For the isolation cellulase producing isolates, Nutrient agar medium (NAM) was used. The Nutrient agar medium composed of agar 15 g, beef extarct: 3 g, NaCl: 5.0 g, peptone: 5 g per 1000 ml of distilled water. This media was enriched with 0.5-1 % cellulose for the of cellulase isolates. The pH of the medium was set at 7.0 and sterilized in autoclave for 15- 20 minutes at 121℃. After autoclaving, media was cool down and transferred into sterile petri plates. After pouring the media into the petri plates, the plates were left for the solidification of the media.

2.4 Preparation of serial dilutions:For the preparation of the serial dilutions, sterile and clean test tubes were taken. For serial dilution, 1 g of the soil sample was taken in one of the test tubes with 9 ml of the distilled water, which denotes the dilution number 10^{-1}. After that, 1 ml of the sample was taken from the test tube 1 and transferred to test tube 2 containing the 9ml of distilled water to make dilution 10^{-2}. This process is repeated for the preparation of serial dilutions from 10^{-1} to 10^{-9}.

2.5 Transfer of serial dilutions onto NAM plates:Each of the serial dilution was used for isolation of cellulase producing bacteria. NAM. For isolation, 100-200µl of serially diluted samples (10^{-1} - 10^{-9}) was spreaded onto NAM plates. Triplicates of plates was used for each dilution to avoid the error. The plates were labelled according to the serial dilutions i.e. plate 10^{-1}, 10^{-2} and so on. Plates were incubated in incubator at 37 ^{o}C for 24-48 h.

2.6 Isolation of isolates:After 24-48 hours plates were observed for the growth of bacteria. Bacterial growth was in the form of colonies. These bacterial colonies were isolated by the streaking method, and were transferred to the fresh NAM medium containing 1% celluose for the selective and discrete growth of the colonies. After that secondary screening was performed for the above developed colonies on the nutrient agar plates. The same process was repeated until the formation of discrete colonies.

2.7 Screening of Cellulase producing isolates:The diameter of zone of different colonies onto agar plates was visualized after staining with congo red. Plates containing discrete colonies were also stored at 4^{o}C for further identification and screening of cellulase producing bacteria.

3 RESULTS AND DISCUSSION

Isolation and screening of the various isolates was performed using serial dilution and pour plate method. Selection of the isolates was done on the basis of the discrete colonies and further used for screening. In primary screening formation of clear zone after staining with congo red indicates that these colonies are cellulase producer. Isolates producing transparent zone on nutrient agar plate containing cellulose represents the substrate hydrolysis.

Out of 2 soil samples named, soil sample 1 was selected, as maximum bacterial growth was observed on this sample. All the isolates with no zone formation were discarded after primary screening. Out of total 10 isolates, 5 was selected due the presence of cellulose hydrolysis zone diameter greater than the 5 mm These isolates were further taken for the secondary screening of the cellulase producing bacteria. Maximum diameter of hydrolysis zone observed in isolate SHI III on the nutrient agar medium containing cellulose was with the diameter of 9 ± 1 (Table 1). Use of cellulose containing nutrient agar plates for the screening of cellulase producing bacteria have been reported by several workers (Irfan et al., 2012; Korra et al., 2014; Ghimire et al.,

2016; Lokande and Pethe, 2017; Maravi and Kumar, 2020).

Table 1. Various isolates along with their zones of substate hydrolysis.

Sr. No.	Isolates	Diameter of zone on plates containing NAM +Cellulose
1	SHI I	7 ± 1
2	SHI II	6 ± 1
3	SHI III	9 ± 1
4	SHI IV	5 ± 1
5	SHI V	8 ± 2

4 CONCLUSION

Soil is a rich source of microbial flora. Isolation of cellulase producing microbe from soil is one of the old and famous technique. Various fungal sources are available in literature for the production of cellulase enzyme with enzyme titre. Bacterial sources for the production of cellulase enzyme are very few in the literature. So, this study shows a very simple methodology for isolating cellulolytic micro-organisms.

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