A Comparative Study of Various Staining Techniques for Determination of Extracellular Cellulase Activity on CMC Agar Plates

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ABSTRACT

Introduction: The most prevalent biomass on Earth is Cellulose. Plant cell walls are mostly made of cellulose, a crystalline polymer of D-glucose residues connected by β-1, 4 glycosidic bonds and i the most prevalent carbohydrate in nature. Enzymes, often known as biocatalysts, are intricate protein molecules produced by living things. Both the reactions they employ, referred to as substrates, and the processes they catalyze are extremely selective [1]. Several extracellular enzymes of significant industrial importance are produced by microbes. Among them, cellulase is one. In the present work we have isolated bacteria from soil for the production of cellulase and for selection, selective screening of the isolated bacteria was done.

Objective:

- Compare the efficacy of the different staining methods, (Congo red, Gram Iodine, Saffranin and Coomassie Brilliant Blue), to visualize hydrolytic zone of different stain for determination of extracellular cellulase activity of microbial cultures grown on agar plates supplemented with CMC.
- Compare the visualized hydrolysis zones from each staining method with quantitative cellulase activity levels (if measured), to verify the accuracy of each method in reflecting actual cellulase production.

Methodology: The most significant industrial enzymes are cellulases. They are an accessible enzyme for a range of commercial applications because to their particularity, cost-effectiveness, and catalytic potency for different substrates. In this study 3 strains of bacteria were isolated from soil by serial dilution method.

For the selective screening of these bacteria, a number of techniques are employed. One of the most widely used techniques is to staining plate method by using different dyes. Then in next step we stained all strains by using different dyes including Congo red, gram iodine solution, safranin and Coomassie brilliant blue.

The zone of clearance created by cellulase activity was observed. It is generally acknowledged that the bacteria's capacity to hydrolyze cellulose is indicated by the diameter of the zone of clearance.

Result: Cellulases are known to transform carboxymethyl cellulose (CMC), a soluble type of cellulose, into monomeric or dimeric structures.Clear zones encircling bacterial colonies are formed by cellulase activity on CMC agar; wider zones denote greater activity. Safranin and Coomassie Brilliant Blue increase sensitivity but result in lesser halos, whilst Congo red and gramme iodine yield crisp findings which table no. 1 describes.

Conclusion: The staining methods used to measure extracellular cellulase activity on CMC agar are compared in this study. Congo red is perfect for qualitative cellulase activity measurement because it enables good visualisation and specificity, while gramme iodine delivers high contrast. When screening industrial and environmental samples for cellulase-producing bacteria, Congo red works very well.

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