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The Comparative Study Between Cellulase Enzyme Extracted From Goat and Gold Fish

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Abstract: *Rumen is an excellent environment for microbial growth consisting of bacteria, fungi and protozoa which are widely known to play important role in the fermentation process of ruminant cattle feed [1]. Cellulase is an enzyme produced by cellulolytic microbes capable of hydrolizing β-1,4 glycoside bond in cellulose, a polysaccharide structure often found in plants Cellulose degradation by cellulolytic bacteria is a product of synergy in a group of cellulase enzymes. Cellulase enzyme system consists of three groups of hydrolytic enzymes, i.e. (1) endo-(1,4)-β-D-glucanase (endoglucanases), (2) exo-(1,4)-β-D-glucanase (exoglucanases), and (3) β -glucosidase Endo-(1,4)-β-D-glucanase enzyme hydrolyzes β bonds randomly in a morphous regions of cellulose fibers [4], generates oligosaccharides of different lengths, and can form a new chainend [5]. Exo-(1,4)-β-D-glucanase enzyme works towards reducing and non-reducing end of polysaccharide chains, especially on crystalline cellulose region, and liberates glucose as the main product resulted by β-glucosidase enzyme. Hydrolysis of crystal line cellulose part can only be done efficiently by exoglucanase enzyme. The synergy between endoglucanases and exoglucanases enzymes produces cellobiose molecules. Cellulose hydrolysis effectively requires an enzyme (β-glucosidase) that breaks down cellobiose into two molecules of glucose. Lignocellulosic materials are the most abundant resource for the production of renewable bioenergy and fermented products. Cellulosic materials need to be first hydrolyzed into fermentable sugars since they are not useful in their polysaccharide form (Li et al., 2009). The biohydrolysis of cellulose through the use of cellulolytic microorganisms is an attractive approach since the degradation of cellulose by chemical agents produces environmental pollution. Fungal species have been primarily used commercially for cellulase production because of their capacity to secrete cellulolytic enzymes into their medium, which allows for easy purification and extraction (Maki et al., 2009). Among the cellulolytic fungi, Trichoderma spp. and Aspergillus spp. have been extensively investigated since they can produce all three types of cellulose-degrading enzymes (Wang et al., 2008). However, bacterial cellulases have several advantages. First, bacteria have higher growth rates than fungi and can easily grow to high cell densities in inexpensive nutrient sources (Maki et al., 2009). Second, the enzyme expression system of bacteria is more convenient. Third, bacteria can not only survive harsh conditions but can also excrete enzymes that are stable under extreme conditions of high temperature and low or high pH.*

Keywords: Lignocellulosic Material, Cellulolytic Fungi And Bacteria , Degradation, Cellulose

I. INTRODUCTION

Cellulose is an organic compound and a polysaccharide consisting of a linear chain of several hundred to many thousands of β(1→4) linked D-glucose units. Cellulose is an important structural component of the primary cell wall of green plants, many forms of algae and the oomycetes. Some species of bacteria secrete it to form biofilms. Cellulose is the most abundant organic polymer on Earth Cellulose is mainly used to produce paperboard and paper. Smaller quantities are converted into a wide variety of derivative products such as cellophane and rayon. Conversion of cellulose from energy crops into biofuels such as cellulosic ethanol is under development as a renewable fuel source. Some animals, particularly ruminants and termites, can digest cellulose with the help of symbiotic micro-organisms that live in their guts, such as Trichonympha. In human nutrition, cellulose is a non-digestible constituent of insoluble dietary fiber, acting as a hydrophilic bulking agent for feces and potentially aiding in defecation Cellulase (4-β-D-glucan 4-glucanohydrolase) is any of several enzymes produced chiefly by fungi, bacteria, and protozoans that

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catalyze cellulolysis, Most mammals have only very limited ability to digest dietary fibres like cellulose by themselves. In many herbivorous animals such as ruminants like cattle and sheep and hindgut fermenters like horses, cellulases are produced by symbiotic bacteria. Endogenous cellulases are produced by a few types of metazoan animals, such as some termites, snails,and earthworms.

Recently, cellulases have also been found in green microalgae (Chlamydomonas reinhardtii, Gonium pectorale and Volvox carteri) and their catalytic domains (CD) belonging to GH9 Family show highest sequence homology to metazoan endogenous cellulases. Algal cellulases are modular, consisting of putative novel cysteine-rich carbohydrate-binding modules (CBMs), proline/serine-(PS) rich linkers in addition to putative Ig-like and unknown domains in some members. Cellulase from Gonium pectorale consisted of two CDs separated by linkers and with a C-terminal Cellulolytic bacteria have been found in a wide range of habitats and environments, such as animal digestive tracts [13,14], decaying organic matter [15–17], herbivore dung mangrove sediments [20,21], manure [22,23], terrestrial soils [24,25], and wetlasoils [26,27]. However, few studies have focused on the cellulolytic microbes isolated from lake environments [28,29]. Recently, the cellulolytic bacteria isolated from freshwater lakes were identified as belonging to the genera Aneurinibacillus, Bacillus, Klebsiella, Micromonospora,Proteus, Pseudomonas, and Streptomyces [29–31]. These cellulolytic bacteria are mainly related to the carbon cycle of organic matter in freshwater lake ecosystems and are responsible for the hydrolysis of lignocellulosic biomass to fermentable sugars by their cellulolytic enzymes [27,32]. Cellulolytic enzymes, generally called cellulases, comprise endoglucanases or carboxymethylcellulases (E.C. 3.2.1.4), exoglucanases or cellobiohydrolases (E.C. 3.2.1.91), and β-glucosidases (E.C. 3.2.1.21), which synergistically work to hydrolyze the β-1,4 glycosidic linkages of cellulose polymer in lignocellulosic biomass [27,33,34]. Nowadays, cellulases account for 20% of the global enzyme market and they have biotechnological potential in various industries [35,36]. Therefore, the isolation and screening of cellulolytic microbes from various environments are some of the important approaches for obtaining novel cellulases

Several different kinds of cellulases are known, which differ structurally and mechanistically. Synonyms, derivatives, and specific enzymes associated with the name “cellulase” include endo-1,4-β-D-glucanase (β-1,4-glucanase, β-1,4-endoglucan hydrolase, endoglucanase D, 1,4-(1,3;1,4)-β-D-glucan 4-glucanohydrolase), carboxymethyl cellulase (CMCase), avicelase, celludextrinase, cellulase A, cellulosin AP, alkali cellulase, cellulase A 3, 9.5 cellulase, and pancellase SS. Enzymes that cleave lignin have occasionally been called cellulases, but this old usage is deprecated; they are lignin-modifying enzymes. Lignocellulosic materials are the most abundant resource for the production of renewable bioenergy and fermented products. Cellulosic

materials need to be first hydrolyzed into fermentable sugars since they are not useful in their polysaccharide form (Li et al., 2009). The biohydrolysis of cellulose through the use of cellulolytic microorganisms is an attractive approach since the degradation of cellulose by chemical agents produces environmental pollution

II. METHODOLOGY

Cellulolytic enzyme activity Media used in this study were nutrient broath(Peptone0.5g ,Yeast Extract 0.2 g ,Sodium Chloride0.5 g ,Agar1.5 g ,pH 7), sodium hydroxide (NaOH) , cellulose extract

- To determine cellulose activity Bacteria isolated from intestine of goat and fish were grown in nutrient broath in a test tubes. the tubes were incubated at 37C for 24hours
- NaOH is add in 1ml of culture from both the samples for cell lysis with the help of vortex machine. This method uses sodium hydroxide (NaOH) to simultaneously extract and lyse cultured cells. NaOH facilitates lysis by locally changing the pH on the cells, thus facilitating denaturation of cellular components.
- Cellulose extract added in a both the test tubes containing lysed cells and kept for one day to check enzymatic activity
- the optical density was measured at 540 nm. One unit of endo-β-1,4-glucanase activity was defined as the amount of enzyme that

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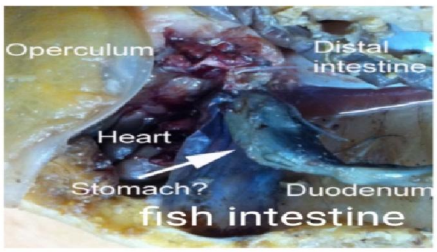

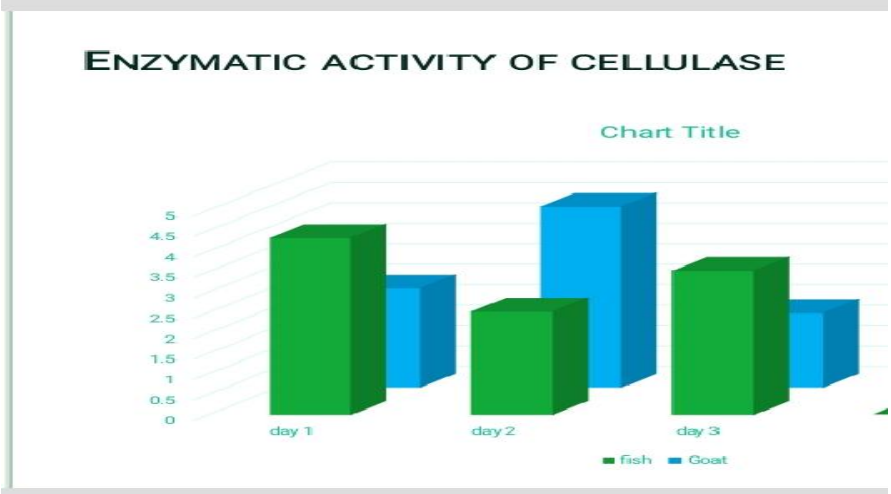

III. RESULT

The comparative study between the goat intestine and fish intestine led to a conclusion that the cellulolytic activity of goat's intestine is greater than that of fish.

Therefore, this study was conducted to screening, optimization, purification and characterization of cellulase from cellulase producing bacteria present in intestine of gold fish and goat

IV. DISCUSSION

Growth curve describes gradual growth process of a microorganism, from the beginning until the end of activity. This consists of four main phases: Lag, exponential, stationary, and death [10]. During this phase mass or cell accretion has not happened yet. Therefore, phase curve is generally flat. Lag phase interval depends on the compatibility between activity and environment setting. In this research, this phase occurs before in the first 2 h of E. cloacae WPL 214 isolate growth and followed by exponential phase.

Exponential or logarithmic phase is a phase when transformation activity increases and the accretion of microorganism growth reaches maximum speed so that the curve is in exponential form. This increasing activity should be offset by many factors among others: Biological factors, such as shape and nature of the microorganism to its environment, life association between related organism, and non-biological factors, such as temperature, pH, and nutrient content in the growth medium. Therefore, this study was conducted to screening, optimization, purification and characterization of cellulase from cellulase producing bacteria present in intestine of gold fish and goat

V. CONCLUSION

Based on the research results, it can be concluded that cellulolytic enzyme having activity of endo-(1,4)-β-D-glucanase, exo-(1,4)-β-D-glucanase and β-glucosidase can be produced from cellulolytic isolates from goat and fish

Nowadays, cellulases account for 20% of the global enzyme market and they have biotechnological potential in various industries .Therefore, the isolation and screening of cellulolytic microbes from various environments are some of the

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important approaches for obtaining novel cellulases Most bacteria in nature cannot be isolated and cultivated by traditional culture-based methods The isolation of cellulolytic bacteria from unique environments is a challenge for the acquisition of novel cellulases. Freshwater lakes provide a unique habitat for diverse bacteria because they differ from other aquatic habitats such as rivers and oceans. This study initially describes the diversity and cellulolytic activity of culturable aquatic bacteria from gold fish.

Rumen is an excellent environment for microbial growth consisting of bacteria, fungi and protozoa which are widely known to play important role in the fermentation process of ruminant cattle feed

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