ISOLATION IDENTIFICATION AND CHARACTERIZATION OF AMYLASE-PRODUCING BACTERIA FROM COW DUNG

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Abstract: Cattle dung offers enormous economic potential in various bioengineering applications, including nutrition and manufacturing. Amylase is a protein that breaks starch polymers but does not release free sugars, resulting in less homogenous starch gluten. Chemicals are necessary for the survival of living things because they speed up biological and organic processes. As a result, the term "biocatalyst" was coined to refer to proteins. Amylose is a water-insoluble glucose polymer linked by \(1, 4\) glycosidic linkages. Amylopectin is a polysaccharide that is branched and water-soluble. Proteins that are hydrolyzed by starch might be endo- or exo-acting. The dairy animal compost suspension is arranged using a serial dilution technique. Different bacterial societies are decontaminated on agar media using a streak plate approach. Antibacterial enzyme assays are used to identify bacteria that can produce various exoenzymes.

Introduction

Amylase has significant economic potential in various bioengineering applications, from nutrition to manufacturing. Two chemical delivery techniques (SSFs) employed in the synthesis of enzymes are liquid maturation (SMF) and solid maturation (SMF). The latter is mainly utilized to make proteins like amylase. Solid State Maturation (SSF) has replaced Submerged Maturation (SMF) since it matches microorganisms' natural environment. SSF uses a variety of agricultural wastes as substrates for amylase synthesis (Vijayaraghavan et al., 2015b). Cattle breeding is a historical technique that benefits farmers and the economy worldwide.

Cattle dung is utilized as fertilizer in Indian subcontinent agriculture for thousands of years (Singh et al., 2013). Dairy fertilizers enhance soil mineral condition, boost plant resilience to pests and diseases, and promote other positive activities such as plant development, sulfur oxidation, and phosphorus solubility. Water makes up the majority of dairy cow excrement, which supports a lattice of undigested plant fiber-rich in vitamins, microbes, and by-products (Sharma & Singh, 2015a).

In most countries, there is a lack of knowledge on biogas generation. Emerging countries demand clean, renewable energy sources to exhibit and fulfill their potential. Biogas innovation is a realistic local energy demand solution that can benefit both people and the biological system (Preliminary Biological Screening of Microbes Isolated.Pdf, n.d.).

Literature review

Amylase

Amylase is a protein that breaks down starch polymers but does not release free sugars, making starch gluten less uniform quickly. Saccharified amylase and liquefied amylase are two kinds of amylase with varying degrees of hydrolysis. The latter decomposes the starch polymer but does not release free sugar, causing the starch gluten to become less dense more quickly. Saccharified amylase creates free sugar and lowers the thickness of the starch adhesive more slowly than released sugar (Frontiers | Bacterial and
Organic processes. Their role is to function as a chemical catalyst, reducing the time it takes for these processes to occur both within and outside of an organism (Semwal et al., 2018). Chemicals are essential for the survival of living things. Protein has been employed in making cheese for years. Wilhelm Friedrich Kühne from the University of Heidelberg invented the word "enzyme" and Humans have been using enzymes for years (Gupta & Rana, 2016).

Starch is considered a glucose polymer produced by many plant species. Crystals include 98–99 percent of their dry weight in two types of -glucans, amylase and amyllopectin (Semwal et al., 2018; Ya’aba & Ramalan, n.d.). Amylose is a glycosidic bond-linked water-insoluble glucose polymer. Amylopectin is a water-soluble branching polysaccharide having 10–60 glucose units in short chains.

**Bacterial**

Bacillus of licheniformis, amyloliquefaciens and stearothermophilus are among the bacteria that have been proven to produce substantial quantities of -amylase for mechanical applications. In severe conditions, certain bacteria may create alpha-amylase. At high temperatures, some thermophilic microbes, for example, generate (Bagge et al., 2010). Most starch treatment methods need high temperatures, such as saccharification, gelatinization, and liquefaction. Hence thermostable -amylase aids in the process under these harsh circumstances. Geobacillus isolated from Manikaran hot springs, a prevalent source of thermostable amylase. Thermophilic -amylase (BLA) is more fundamentally flexible than mesophilic amylase (BAA). Despite the ideal temperature for protein being 80°C, amylases are resistant to other harsh conditions, especially during the natural occurrences. Halophilic-amylase can withstand saline and high temperatures (Islam, 2016; Sharma & Singh, 2015b; Vijayaraghavan et al., 2015a).

Furthermore, this protein is resistant to natural solvents and maintains mobility under low-water situations. *Nesterenkonia* sp. strain F is a halophilic bacterial source of alpha-amylase, producing proteins where natural solvents including chloroform, benzene, cyclohexane, and toluene have catalytic activity. Due to acidic buildups on the surface of halophilic alpha-amylase, the molecule is stable in moo water. (Elyasi Far et al., 2020). Aspergillus flavus NSH9 is a suitable source of -amylase for commercial uses since it assures extracellular quantities of proteins easily accessible from microbial growth conditions. Other species' parasites have been characterized as having some particular traits that make them suited for mechanical targeting. Table 1 lists different sources of -amylase-producing bacteria.
MATERIAL AND METHODS

A serial dilution procedure was used to arrange the dairy animal compost suspension. A 1 g bovine fecal test collected and labeled was combined with 10 ml sterile phosphate buffer and aggressively vortexed for 2 minutes for adequate test mixing. All tests were incubated at 370 °C for 3040 minutes before placing in a microbial staging hatchery. Each sample's decay is separated following incubation using standard dilution procedures and sterile pipettes. The method is used to make a phosphate compartment. 9 mL of sterile phosphate buffer is included in each container. A milliliter of the active component is aseptically added to tubing number 1, and 1 ml of sample is replaced after the labeled tubing is put on the tubing platform (Sharma & Singh, 2015a).

Table 1. Source and types of microbial.

<table>
<thead>
<tr>
<th>Source</th>
<th>Microbial type</th>
<th>Feature of alpha-amylase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus stearothermophilus</td>
<td>Bacteria</td>
<td>Thermophile alpha-amylase</td>
</tr>
<tr>
<td>Geobacillus bacterium</td>
<td>Bacteria</td>
<td>Thermophile alpha-amylase</td>
</tr>
<tr>
<td>Nesterenkonia sp. strain F</td>
<td>Bacteria</td>
<td>Halophilic enzyme</td>
</tr>
<tr>
<td>Bacterium Pseudoalteromonas sp. M175</td>
<td>Bacteria</td>
<td>Cold-active alpha-amylase</td>
</tr>
<tr>
<td>Nocardiosis aegyptia</td>
<td>Actinomycetes</td>
<td>Cold-active alpha-amylase</td>
</tr>
<tr>
<td>Streptomyces fragilis</td>
<td>Actinomycetes</td>
<td>Cold-active alpha-amylase</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>Fungi</td>
<td>Commercial production</td>
</tr>
<tr>
<td>Aspergillus awamori</td>
<td>Fungi</td>
<td>Commercial production</td>
</tr>
<tr>
<td>Aspergillus oryzae</td>
<td>Fungi</td>
<td>Commercial production</td>
</tr>
<tr>
<td>Aspergillus flavus</td>
<td>Fungi</td>
<td>Thermophile alpha-amylase</td>
</tr>
</tbody>
</table>

Table 2. Cow manure microorganism count.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Dilution</th>
<th>Method used</th>
<th>Total bacteria count</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sample 1</td>
</tr>
<tr>
<td>1</td>
<td>10⁻⁴</td>
<td>Serial dilution</td>
<td>192.5x10⁻²</td>
</tr>
<tr>
<td>2</td>
<td>10⁻⁵</td>
<td></td>
<td>141.5x10⁻³</td>
</tr>
<tr>
<td>3</td>
<td>10⁻⁶</td>
<td></td>
<td>80.5x10⁻⁴</td>
</tr>
<tr>
<td>4</td>
<td>10⁻⁷</td>
<td></td>
<td>24.0x10⁻⁵</td>
</tr>
</tbody>
</table>
Results

The different bacterial societies are decontaminated using a streak plate method on Supplement agar media. Collect the colony off the spread plate with a sterile vaccinating circle and crisscross the loop over the surface of another vessel. Set the container to 900 degrees and moved the circle over the previously streaked region. After fixing the loop over the fire and repeating the same preparation, all plates were hatched for 24 hours (Bagge et al., 2010). The first part saw the most development, and the third segment included the most isolated colonies. The process is repeated several times until pure colonies are obtained.

![Figure 2. Recovered Enzymes.](image)

The bacterial cultures decontaminated were saved (Singh et al., 2013). Following the unadulterated improved technique, the separated microorganism colonies were observed for colony morphological assurance; color, shape, size, surface, edges, edges, and height. These cultures were differentiated by a variety of recoloring, including Gram's staining, endospore recoloring, and so on (Isolation of Amylase Producing Bacteria from Soil and Identification by 16S rRNA Gene Sequencing and Characterization of Amylase, n.d.). Antibacterial enzyme tests investigate bacteria capable of producing many exoenzymes and their hydrolytic characteristics. An enlarged zone represents the unprotected stage: resistance (R) or vulnerability (S) (R). Reactivity of the constituent is calculated by dividing its location of hydrolysis by colony size.

Discussion

The capacity of bacillus strains to produce cellulose is exciting in terms of bioengineering and breaking down agricultural waste left in fields after harvest. Bacillus produces amylase, which is used in various mechanical processes, most notably in the starch industry. The Antibacterial Vulnerability Test (AST) determines the most effective antibacterial agent for treating bacterial infections in vivo. Bacillus cocci are Gram-positive cocci, whereas B4 is a Gram-negative bacillus. Colonies can vary in shape, height, surface area, and color. Attenuation 104 revealed the most significant number of bacterial populations in the 60.5x10^4 to 175x10^4 CFU / ml range. Enzymes that catalyze the degradation of proteins into amino acids are Proteases. Esterases and esterase-lipases are hydrolases that degrade esters into acids and alcohols. Proteases are hydrolases that use water to break down high molecular weight compounds (polysaccharides, lipids, proteins) into smaller cell components such as glucose. Proteases account for approximately 40% of global enzyme sales.

Conclusion

Dairy manure is high in microbial flora, which may be utilized as a probiotic or live microbial supplement to change the gut microbiota. The potential of Bacillus strains to produce cellulose is exciting in terms of bioengineering and the breakdown of agricultural waste. The Antibacterial Vulnerability Test (AST)
determines the most effective antibacterial agent for treating bacterial infections in vivo. An improved approach for identifying bacteria capable of generating a wide range of exoenzymes and their hydrolytic properties exists. Antibacterial enzyme assays are used to identify bacteria that can produce a variety of biofuels, such as anthocyanins and amylases.

References


*Preliminary biological screening of microbes isolated.pdf.* (n.d.).


