The amylase production on cassava starch and identification of bacteria by 16S rRNA

Nurhayati
Department of Biology, Science and Mathematics Faculty, Diponegoro University, Semarang, Indonesia.
Email: nur_setyahayati@yahoo.com

Abstract Amylase are the most important product obtained for industrial catalyst from bacteria. The industrial dependency on enzymes and the raising demand for enzymes have increased concerns about biocatalyst. Amylase is great essential application in industries like flour industries. The objective study was to isolation, identification amylolytic bacteria and production of amylase. The isolated bacteria were characterized by morphological, physiological and biochemical tests. The highest amylase activity 5077U was achieved when the cassava concentration 1%, 30°C at pH 7 and four days incubation time without shaking. The activity of amylase was found to enhance when logarithmic phase at cassava starch as a sole carbon source. The maximum amylase enzyme activity was obtained at the logarithmic growth phase. The selected bacteria were identified based on 16S rDNA gene analysis. The 16S rRNA gene analysis revealed that amylolytic bacteria has the highest homology with Bacillus thuringiensis (99%). The selected isolate was identified as Bacillus thuringiensis after 16S rRNA gene analysis.

Keywords: amylase, Bacillus thuringiensis, 16S rRNA, cassava starch, BLAST

1. Introduction
Bacterial enzymes is important catalyst [1]. Enzymes are among the important products obtained for industrial needs such as baking, distillery, textile industry, paper, detergent [2]. In previous investigations, amylases were found in many different traditional foods. Modified starch are important flour used in industrial applications[3]. Amylases catalyzes the hydrolysis of α-D-(1,4) glycosidic linkages in starch and yields various products like smaller carbohydrate and glucose. Starch hydrolysis for industry requires of amyloglucosidase (AMG) enzymes to break down the α-1,6 glucosidic bonds of starch [4]. Amylases are of great significance in industrial applications like pharmaceutical, food, textile, detergent, clinical and paper industries [5].

The 16S rRNA gene is a marker molecular has the potential to make an essential contribution to bacteria identification by detecting the presence of bacteria. The 16S rRNA gene consists of highly conserved regions of rRNA gene. The objective of present study was to isolate and identification bacterium strain for production of amylase. In previous investigations, amylases were found in many different traditional foods. The isolation and identification of 16S rRNA gene of amylolytic bacteria screened from soil at Cangkringan, Yogyakarta, Indonesia.
2. Materials and Methods

2.1. Isolation and characterization
The Nutrient agar used for isolation and subculture. The purified strains were characterized and stored in a refrigerator. Amylolytic bacteria isolates were observed by flooding with yod in starch agar medium. The following morphological, physiological and biochemical were Gram, catalase, oxidase, indole, gelatine, urea, dextrosa, mannosa, sucrose, inositol, lactose, morphology cell.

2.2. Subculturing
Isolated bacteria were sub screened by starch agar medium to purify the amylolytic strains. Purified bacteria were cultured into nutrient agar slants. The selected bacteria were incubated in nutrient agar slant at 32°C for 24 h. The selected bacteria were reserved in the refrigerator at 4°C for further investigation.

2.3. Enzyme activity assay
The reducing sugars released by the activity of amylases on cassava starch was currently performed at 30°C. The reducing sugar was determined by Dinitrosalicylic acid (DNS). One unit of enzyme activity was defined as the amount of enzyme, required to produce reducing sugars equivalent to 1 μmol glucose/mL [6].

2.4. Substrate concentration of fermentation
The effect of cassava starch as C source on the activity of extracellular amylase was performed by reducing sugar production in medium. The effect of substrate on the activity amylase was determined at 0,1%; 0,5% and 1% cassava starch.

2.5. Molecular identification
The genomic of amylase bacteria was amplified by adding genomic isolation commercial kit. The universal primers were used to obtain a 16S rRNA gene product. Genome bacteria of selected amylolytic bacteria was extracted from the cells by using bacterial DNA extraction kit. The universal 16S rRNA primers 27F 5'-AGAGTTTGATCATGGCTCAG -3', and 1492R 5, TACGGTTACCTTGTTACGACTT -3' were used for the Polymerase Chain Reaction (Biorad Thermocycler machine) (Yan et al., 2013).

2.6. Amplification 16S rRNA gene
The genomic of strain was amplified by adding genomic isolation commercial kit. The 16S rRNA gene [6] was amplified by adding 2,5 μL DNA genom to a thermocycler microtube containing primers. Thermocycler, denaturated by heating 3 min at 95°C and subjected to 30 cycles, 55°C for 1 min and 72°C for 1 min.

3. Results and Discussion

3.1. Amylase producing bacterium
The isolation of amylase bacterium was carried out in soil of Canna edulis, Kerr rhizosphere in Cangkringan, Sleman, Yogyakarta, Indonesia. One amylase-producing bacterium was carefully chosen along with test on solid amyllum agar. Morphology, physiology, and biochemistry of the selected bacteria,16S rRNA gene were used for characterization and identification of the bacterium. The cell morphology of the amylase-producing bacterium was found in the rod. The selected amylase-producing bacterium made greater colonies due to their dominant colony. According to the tested morphological, the amylase producing bacterium was positive Gram (table 1). According to the tested biochemical, the amylase producing bacterium was amyllum positive but urea, dextrosa, gelatine, sucrose, inositol, lactosa negative (table 1). According to the tested physiological, the amylase producing bacterium was catalase positive, oxidase and indole negative (table 1).
### Table 1. Morphological, physiological and biochemical assay.

| Parameters         | result test |
|--------------------|-------------|
| Gram               | +           |
| Catalase           | +           |
| Amylum             | +           |
| Oxidase            | -           |
| Indole             | -           |
| Gelatine           | -           |
| Urea               | -           |
| Dextrosa           | -           |
| Mannosa            | -           |
| Sukrosa            | -           |
| Inositol           | -           |
| Laktosa            | -           |
| Cell morphology    | rod         |

#### 3.2. The growth of amylolytic bacterium

The logarithmic phase was observed until 96 h incubation time without shaking. The stationary phase was observed after 96 h incubation time. The advantage of amylase production by *Bacillus thuringiensis* without shaking which will reduce cost.

![Image](image1.png)

**Figure 1.** The growth of amylolytic bacterium.

#### 3.3. Effect of media concentration on amylase activity

The cassava carbon source was used for the production of amylase by *Bacillus thuringiensis*. Carbon source in the medium was used by concentrations cassava starch. The concentration of 0.1%, 0.5%, 1% cassava starch were found to be similar and showed higher amylase activity at 4 days incubation time.
Figure 2. Amylase activity of *Bacillus thuringiensis*.

3.4. The 16S ribosomal sequences detection

The chosen bacterium was identified by 16S rRNA gene, and PCR conditions were optimized for amplification of 16S rRNA gene. Basic Local alignment Statistic Tool was performed for tested the 16S rRNA sequences to find out homology with the sequence in GenBank in which 99% similarity was found with *B. thuringiensis*. The 16S rRNA gene product of the bacterium is a fragment 1492 bp. The 16S rRNA gene was comparable to references bacterial from NCBI for validating to the 16S rRNA gene sequence judgment. Based on 16S rRNA analysis, together with physiological, morphological and biochemical features, strain amylase-producing bacterium was well-known as *Bacillus*. Molecular analyze that was used for identification of bacterium is based on the 16S rRNA gene. To assign bacteria to the species, the differences in their sequences cannot exceed 3%. According to our analyses, the degree of sequence homology was achieved 99%. It can be concluded that the isolate was *Bacillus thuringiensis* with reference homology from the NCBI database using alignment of MEGA 5.0 program.

4. Conclusion

Bacterium was produced amylase at logharratmic phase at all concentration of cassava starch as C carbon source and identified strain as *Bacillus thuringiensis*.

References

[1] Adrio J and Demain A 2014 Microbial enzymes: tools for biotechnological processes *Biomolecules* **4** 1 117-39

[2] Kuddus M, Roohi, Saima and Ahmad I Z 2012 Cold-active extracellular α-amylase production from novel bacteria Microbacterium foliorum GA2 and Bacillus cereus GA6 isolated from Gangotri glacier, Western Himalaya *Journal of Genetic Engineering and Biotechnology* **10** 1 151-9

[3] Hebelstrup K H, Sagnelli D and Blennow A 2015 The future of starch bioengineering: GM microorganisms or GM plants? *Frontiers in Plant Science* **6** 247 1-6

[4] Nahampun H N, Lee C J, Jane J-L and Wang K 2013 Ectopic expression of bacterial amylotransglucanase enhances bioethanol production from maize grain *Plant Cell Rep.* **32** 9 1393-405

[5] John Ravindar D and Elangovan N 2013 Molecular identification of amylase producing Bacillus subtilis and detection of optimal conditions *Journal of Pharmacy Research* **6** 4 426-30

[6] Khannous L, Jrad M, Dammak M, Miladi R, Chaaben N, Khemakhem B, Gharsallah N and Fendri
2014 Isolation of a novel amylase and lipase-producing Pseudomonas luteola strain: study of amylase production conditions *Lipids in Health and Disease* 13 1 9