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Analysis of Endo-1,4-β-Glucanase (eglS) Gene in Cellulose-Degrading Bacteria (Bacillus) Based on In Silico Research

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Abstract. The process of cellulose degradation naturally requires the help of microorganisms. The group of Bacillus bacteria is one of the cellulolytic bacteria that can break down cellulose. The mechanism of cellulose degradation in Bacillus is a complex system and was regulated by a number of genes. One of Bacillus genes that has mechanism of cellulose degradation is eg1S gene. This gene is responsible to hydrolyze the internal glycosidic bonds to reduce the long chain of cellulose. This research examines the comparasion of endo-1,4-β-glucanase gene homology in Bacillus that can degrade cellulose. The method of research is in sillico-based research by using National Center for Biotechnology Information (NCBI) website, Clustal Omega website, and MEGA-X software. The results obtained in this research are the identity of endo-1,4-β-glucanase gene, phylogenetic tree, and similarity index in Bacillus that can degrade cellulose. From the research that has been done, it can be concluded that the highest homology ratio of endo-1,4-β-glucanase gene is occupied by *Bacillus subtilis strain AuChE413* and *Bacillus subtilis strain IARI-SP-1* with 100% identical similarities.

Keywords : Bacillus, Homology eglS gen, Cellulose degradation

Running Title : Analysis of Endo-1,4-β-Glucanase (eglS) Gene

INTRODUCTION

Indonesia is one of the megabiodiversity countries. This is compatible with the abundance of species in Indonesia, both flora, fauna, and microbes. One of the species that has the highest spread in the nature is bacteria. This is related to the habibat microbes that are able to live in all places (ubiquitous). The presence of bacteria plays important role in the cycles that exist in nature. The bacteria act in decomposition, untangle compounds into simplercompounds, and produce certain substances that are required in their life (Hatmanti, 2000).

The most bacteria are known to have cellulolitic activity. Cellulolitic bacteria is a type of bacteria that capable degrade substrates that containing cellulose (Mulyasari *et al.*, 2015). Cellulose has a very abundant availability in nature, and usually cellulose was found in organic matter such as herbs, leaf litter, weathered wood, etc. As an organic material that has abundant availability, cellulose has constraints in terms of their digested. Cellulose degradation constraints in nature will be very effective if using the help of cellulolitic bacteria (Kurniawan *et al*., 2019). These bacteria will produce cellulose enzymes that will break down cellulose into simple sugar. The resulting sugar would be processed into a source of carbon for bacterial growth. (Zhang & Zhang, 2013).

The used of cellulolitic bacteria has been widely developed in various industrial sectors based on biotechnology, e.g. food, beverage and pharmaceutical industry and industrial waste management (Hatmanti, 2000). The application of cellulolitic bacteria in the industrial field is considered highly effective, because the production costs are cheap, short production time, multi-enzyme production,and tends to be stable under extreme conditions (Laderia et *al.* , 2015) .

According to Nofu *et al.*, (2014) some bacteria that have been shown to be able to degrade cellulose are *Bacillus subtilis* and *Bacillus amyloliquefaciens*. This type of bacteria is one of the largest extracellular enzyme-producing bacteria compared to other bacteria. The most of bacillus bacteria are capable to producing cellulose enzymes. Besides that, Bacillus bacteria also produce several types of extracellular enzymes, such as amylase enzyme, protease, etc. Cellulose degradation mechanisms in bacteria generally was regulated by a number of genes. The gene that responsible for cellulose degradation mechanism is the gene of endo-1,4-β-glucanase (eglS). This gene that responsible for hydrolysis of internal glycosidic bonds to reduce the long chain of cellulose. Judging by the benefits of cellulolitic bacteria, bacteria that capable to expressing this gene were looking for by many industrial sectors to help accelerate production at a lower cost.

Previous studies have been dominated by in vitro based or laboratory scale. Bioinformatics approach is carried out to determine the characteristics and homology of bacillus bacteria strain that has endo-1,4-β-glucanase (eglS) genes. So, the research of homology endo-1,4-β-glucanase (eglS) gene analysis in cellulose-degrading bacteria is important to do.

MATERIALS AND METHODS

The research method was conducted based on library research about cellulose-degrading bacteria that has the endo-1,4-β-glucanase (eglS) gene. Then continued in silico based research by utilizing genome sequence data obtained from GenBank NCBI (National Center for Biotechnologi Information). The resulting data were processed using MEGA-X software for multiple alignment and phylogenetic tree construction with neighbor-joining method. The highest level of sequence similarity can be done by using clustal omega website to obtain identity matrix.

RESULTS AND DISCUSSIONS

Cellulose-Degrading Bacteria

Cellulose is one of the abundant biopolymer in nature (Arifin *et al.*, 2019). According to Nofu *et al.* (2014) cellulose can be categorized as one of the main carbohydrates synthesized by plants and has a percentage of almost 60% of the constituent components of plant structure. The availability of cellulose in nature can be found in some organic materials such as herbs, leaf litter, and weathered wood. The process of cellulose decomposition naturally requires the help of microorganism that capable of producing extracellular enzymes. One of those microorganisms are known as cellulolytic bacteria (Kurniawan et *al.*, 2019).

Cellulolytic bacteria is a type of bacteria that has the ability to degrade a cellulose substrate (Mulyasari et *al.*, 2015). Cellulose-degrading bacteria were widely isolated from various sources such as soil, rotten plants, organic materials, compost, even in animal digestion (Irfan *et al.*, 2012). The presence of bacteria are abundant in nature, widely used by the industrial, e.g. food, beverage and pharmaceutical industry and industrial waste management. This encourages the application of bacteria in industry that is environmentally friendly and low cost.

The Process of Cellulose Degradation

According to Faiqoh (2016) cellulose degradation is divided into 2, that are enzymatic and chemical. The mechanism of degradation enzimatically has a greater advantage then used the chemical degradation mechanism. Some of the advantages are there is no hydrolysis of sugar from hydrolysis result, producing products with a softer condition, potentially to produce higher yields, and has lower-cost (Jayus *et al.* , 2019).

According to Nofu *et al.*, (2014) the largest bacteria that capable to degrade cellulose is the bacillus bacteria group. The genus of Bacillus is one of the bacteria that has a wide variety of abilities. Bacillus is also very potential to be developed in the biotechnology industry because it has characters such as, has a wide range of growth temperatures, spore-forming, cosmopolitan, resistant to antiseptic compounds, aerobic or facultative anaerobic character, has diverse enzymatic capabilities, and some of them are capable of biodegradation of many compounds (Hatmanti, 2000).

Table 1. Bacillus species data that has endo-1,4-β-glucanase (eglS) gene

No.	Species Name
1.	Bacillus subtilis strain AuChE413
2.	Bacillus subtilis strain IARI
3.	Bacillus amyloliquefaciens strain IARI
4.	Bacillus subtilis strain VSDB5
5.	Bacillus subtilis strain BS-02
6.	Bacillus subtilis strain BY-02
7.	Bacillus subtilis
8.	Bacillus subtilis strain 168 trpC2

Previous research who was conducted by Ulfa et *al.*, (2014) it shown that Bacillus bacteria have the best results to degrade cellulose than Nocardia and Pseudomonas bacteria. This is shown by the fact that Bacillus bacteria can survive for the longest time in the incubation process, and it shown with the most degradation ability than other bacteria. So, the Bacillus bacteria were selected in this research.

The degradation mechanism in bacteria is regulated by a number of genes. The gene that has responsible for the degradation mechanism in bakteri bacteria is endo-1,4-β-glucanase (eglS) gene. This gene responsibles for the mechanism of endoglucanase activity that occurs in bacteria. That gene would hydrolyze internal glycosic bonds to reduce the long chain of cellulose. So, cellulose would break down into a simple sugars and can be used bacteria for the growth process (Nofu et *al.*, 2014). The identity of endo-1,4-β-glucanase (eglS) gene can be seen in Table 2 to support informations about that gene.

The exploration of the Bacillus bacteria group that has endo-1,4-β-glucanase (eglS) gene was carried out in-silico using the NCBI (National Center for Biotechnology Information) website. The results of exploration of Bacillus bacteria that have endo-1,4-β-glucanase (eglS) gene were obtained 8 species that derived from *Bacillus subtilis* species and *Bacillus amyloliquefaciens* with several strains (Table 1).

No.	Comparison	Identity
1.	Gene symbol	eglS
2.	Gene Description	endo-1,4-beta-glucanase
3.	Locus tag	BSU_18130
4.	Gene type	Protein coding
5.	RefSeq status	Provisional
6.	Organism	*Bacillus subtilis subsp. saeed A. 168 (strain: 168, sub-species: subtilis)*
7.	Lineage	Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae; Bacillus
8.	Old locus tag	BSU18130

Table 2. Identity of Endo-1.4-β-glucanase (eglS) gene in Bacillus Bacteria

The samples were obtained then were searched for the complete genome (nucleotide base), then the sequence alignment was carried out using the multiple alignment method. The results of alignment can be displayed in the form of phylogenetic tree construction and identity matrix to know the homology of endo-1,4-β-glucanase (eglS) gene in the 8 samples.

Construction of Phylogenetic Tree Bacillus Species Spesies

Phylogenetic tree construction is the most important and interesting thing in bioinformatics studies. There are several methods for construction of phylogenetic trees from molecular data (nucleotide or amino acid) (Sitou & May, 2009) .

When the nucleotide sequences or proteins of two different species have similarities, it is assumed that they are derived from a common ancestor sequences. Sequences that have been aligned will show the sequences position were unchanged or *conserved* and it were *divergent* or develops differently from the *common*

ancestor (Mount, 2001).The purpose of the sequencing or alignment process is to match homologous characters that indicate the gene has a similar function (Blackshelds & Wallace, 2006). So, it can be seen which species have the highest similarity.

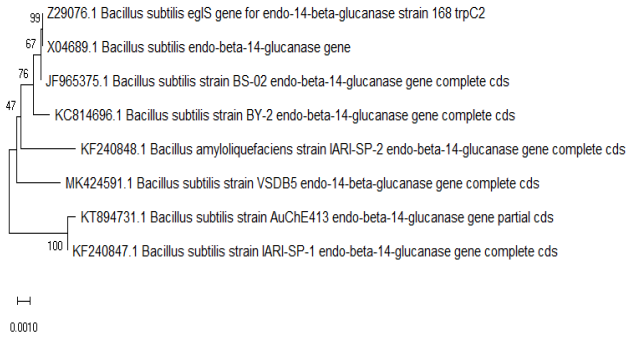

Figure 1. Construction of neighbor-joining phylogenetic tree genome sequences of bacillus bacteria that have endo-1,4-β-glucanase (eglS) gene

In this research was taken 8 sequences of the *Bacillus subtilis* species from various strains and *Bacil lus amyloliquefaciens*. The selection of these sequences were based on literature studies which have stated that Bacillus bacteria have an endo-1,4-β-glucanase (eglS) gene. This gene had a role in the degradation process of cellulose. In addition, the Bacillus *subtilis* and *Bacillus amyloliquefaciens* species belong to the same genus, which allows these species to have not too much difference in homologous characters.

Phylogenetic tree construction was carried out using Molecular Evolutionary Genetics Analysis (MEGA-X) software. The method that used in phylogenetic tree construction is neighbor-joining method. From the research that has been done, the results showed that the species of *Bacillus subtilis strain AuChE413* has high similarity to the *Bacillus subtilis strain IARI-SP-1* (Figure 1). This could indicate that endo-1,4-β-glucanase (eglS) gene can be found in *Bacillus subtilis strains AuChE413* and *Bacillus subtilis strains IARI-SP-1* are homologous or similar.

Table 3. Identity Matrix (Heat Map) Percentage of Genome Sequences of Bacillus Bacteria

No.	Nama Spesies	1	2	3	4	5	6	7	8
1	Bacillus subtilis strain AuChE413		100,00%	99.07%	99.13%	99.40%	99.01%	99.01%	99.20%
2	Bacillus subtilis strain IARI	100,00%		99.07%	99.13%	99.40%	99.40%	99.40%	99.20%
3	Bacillus amyloliquefaciens strain IARI	99.07%	99.07%		99.13%	99.40%	99.40%	99.40%	99.33%
4	Bacillus subtilis strain VSDB5	99.13%	99.13%	99.13%		99.47%	99.47%	99.47%	99.40%
5	Bacillus subtilis strain BS-02	99.40%	99.40%	99.40%	99.47%		100,00%	100,00%	99.80%
6	Bacillus subtilis strain BY-02	99.01%	99.40%	99.40%	99.47%	100,00%		100,00%	99.80%
7	Bacillus subtilis	99.01%	99.40%	99.40%	99.47%	100,00%	100,00%		99.80%
8	Bacillus subtilis strain 168 trpC2	99.20%	99.20%	99.33%	99.40%	99.80%	99.80%	99.80%	

The results of the alignment of the selected genome sequences with Clustal-W multiple alignment, were obtained an identity matrix (Table 3) which showed the degree of similarity genome sequences from bacteria that was analyzed. From those results indicate that in general, there are have high similarity (above 99%) of all analyzed samples. The highest level of similarity was shown by a gray heat map with a number of 100% (table 3). In addition, the highest gene homology similarity can be shown in the phylogenetic tree with the shortest branching (Figure 1). These show that the *Bacillus subtilis strain AuChE413* bacteria has an endo-1,4-β-glucanase (eglS) gene homology which is identical to *Bacillus subtilis strain IARI-SP-1* which is shown with a 100% identity matrix.

CONCLUSIONS

From the in silico-based research that has been done, it shows that the endo-1,4-β-glucanase (eglS) gene in the *Bacillus subtilis strain* AuChE413 species has a high similarity with the *Bacillus subtilis strain IARI-SP-1*. This is compatible with the phylogenetic tree construction in the samples was analyzed that has short branching. In addition, the alignment results obtained a identity matrix which indicated that the bacteria were identical with 100% percentage. So, the gene of endo-1,4-β-glucanase (eglS) in the *Bacillus subtilis strain AuChE413* bacteria has the same homology as the *Bacillus subtilis strain IARI-SP-1*.

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