การคัดเลือกแอคติโนมัยสีทเอนโดไฟต์ที่ย่อยบีตา-กลูแคนและวิเคราะห์ยีนที่เกี่ยวข้อง

Screening of β-glucan degrading activities from endophytic actinomycetes and characterization of their genes

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[1]*หน่วยวิจัยแอคติโนแบคทีเรีย ภาควิชาพันธุศาสตร์ คณะวิทยาศาสตร์ มหาวิทยาลัยเกษตรศาสตร์ กรุงเทพ 10900;* [2]*ห้องปฏิบัติการเทคโนโลยีเอนไซม์ หน่วยวิจัยเทคโนโลยีทรัพยากรชีวภาพ ศูนย์พันธุวิศวกรรมและทคโนโลยีชีวภาพแห่งชาติ อุทยานวิทยาศาสตร์ ปทุมธานี 12120*

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บทคัดย่อ

การทดสอบความสามารถในการย่อยสลายลิกโนเซลลูโลสของแอคติโนมัยสีทเอนโดไฟต์จำนวน 308 สายพันธุ์ กับซับสเตรท 3 ชนิด คือ ไซแลน เซลลูโลส และบีตา-กลูแคน ผลการทดลองพบว่า แอคติโนมัยสีท 81 สายพันธุ์ สามารถสร้างเอนไซม์ย่อยลิกโนเซลลูโลสได้ คัดเลือกสายพันธุ์ที่สามารถย่อยบีตา-กลูแคนมาทดสอบภายใต้สภาวะความเป็นกรดและอุณหภูมิสูง และออกแบบไพรเมอร์เพื่อเพิ่มปริมาณยีนบีตา-กลูคาเนส ในกลุ่มไกลโคไซด์ ไฮโดรเลส เมื่อสร้าง phylogenetic tree พบว่าเอนไซม์บีตา-กลูคาเนส แบ่งออกเป็น 4 กลุ่ม ยีนบีตา-กลูคาเนสจากสายพันธุ์ที่ทนความเป็นกรดและอุณหภูมิสูงจะถูกนำไปศึกษาต่อไป

ABSTRACT

Three hundred and eight strains of endophytic actinomycetes were determined for lignocellulose degradation activity i.e. xylanase, cellulase and β-glucanase. The results showed that 81 strains could degrade lignocellolosic polymers. The β-glucanase producing strains were investigated under extreme condition. The primers of some GH gene families that related in β-glucan degradation were designed to achieve PCR amplification of the corresponding genes. Phylogenetic analysis revealed that β-glucanases were clustered in 4 GH families. The genes from β-glucanase producing strains under extreme condition will be characterized further.

คำสำคัญ: แอคติโนมัยสีท, เอนโดไฟต์, บีตา-กลูคาเนส, ไกลโคไซด์ ไฮโดรเลส

Keywords: actinomycete, endophyte, beta-glucanase, glycoside hydrolase

INTRODUCTION

Degradation of lignocellulose involves a complex interplay between different lignocellulolytic enzymes including cellulose, xylanase and glucanase (Malherbe and Cloete, 2003). Actinomycetes represent the important part of the microbial community reliable for the degradation and recycling of natural biopolymers such as cellulose. Many *Streptomyces* strains secrete large quantities of extracellular enzymes such as cellulases and xylanases to degrade lignin, hemicellulose, and cellulose (McCarthy and Williams, 1992).

In this study, we have determined the ability of endophytic actinomycetes in the hydrolysis of lignocellulosic substrates particularly, β-glucan under extreme conditions. Genes involved in β-glucanase activity were also amplified and characterized.

MATERIALS AND METHODS

1. Screening of lignocellulose degrading activities and selective strains under extreme conditions

Endophytic actinomycetes from GKU and GMKU culture collections were grown in starch-casein (SC) medium at 28°C, 200 rpm for 7 days and were subcultured to basal medium supplemented with 0.5% CMC-cellulose (Sigma) and grown for 5 days. The cell-free cultures were verified on AZCL-xylan, AZCL-CMC and AZCL-β-glucan plates.

The β-glucanase positive strains were screened further under extreme conditions by overlaying the plates with AZCL-β-glucan and incubated at high temperatures (45°C, 55°C, 60°C and 65°C) for 12 hr. In case of low-pH condition (pH 4), the substrate plates were adjusted at pH 4 and incubated at 28°C.

2. Primer design and amplification of β-glucanase genes

The conserve amino acid regions of 4 glycoside hydrolase (GH) gene families (i.e. GH5, GH6, GH9 and GH16) that encoding β-glucanases were determined and designed for 4 pairs of specific primers. Genomic DNAs of actinomycetes were extracted according to Kieser *et al*. (2000). The PCR mixture was prepared and incubated the reaction according to the manufacturer's instructions for DNA polymerase (Thermo scientific, USA). The PCR products were purified using the PCR purification kit (Geneaid, Korea) and directly sequenced by Macrogen, Korea.

3. Phylogenetic analysis

Phylogenetic tree was constructed using ClustalX2.0.12 (Larkin *et al*., 2007) and deduced using the neighbor-joining method by MEGA4 (Tamura *et al*., 2007).

RESULTS AND DISCUSSION

1. Pre-screening and investigation under extreme conditions

In this study, 308 strains of endophytic actinomycetes were investigated for lignocellulose degrading activities such as xylanase, cellulase and β-glucanase. The results revealed that 81 strains produced lignocellulose degrading activities. Sixty-nine strains degraded xylan (85%), 7 strains degraded cellulose (8%) and 22 strains degraded β-glucan (27%).

β-glucanase positive strains were then tested under the extreme conditions. Surprisingly, the results showed that 9 strains were able to degrade β-glucan at pH4, 60 °C.

2. Amplification of β-glucanase genes and phylogenetic analysis

PCR products from β-glucanase genes in different GH family were generated by specific primers. The PCR products were obtained and showed sizes of 542, 543, 559 and 609 bp corresponding to β-glucanases in GH5, GH6, GH9 and GH16 gene family, respectively (Fig. 1a). The PCR products from all positive strains were subsequently sequenced and conceptually translated and compared with those β-glucanases available in GenBank database.

The phylogenetic tree of all β-glucanases was constructed and revealed that the β-glucanases of endophytic actinomycetes were clustered into 4 groups of GH gene family (Fig. 1b). β-glucanase from *Paenibacillus provencensis* (Fu *et al*., 2010) was clustered in GH9 family with those of endophytic actinomycetes while β-glucanase from *Bacillus subtilis* (Qiao *et al*., 2009) was separated from those of GH16 family (Fig. 1b). Interestingly, β-glucanases of *Streptomyces* sp. GMKU 311, GMKU 312, GMKU 322 and GMKU 323 that active at pH4, 60°C were tightly grouped together in all GH families (Fig. 1b).

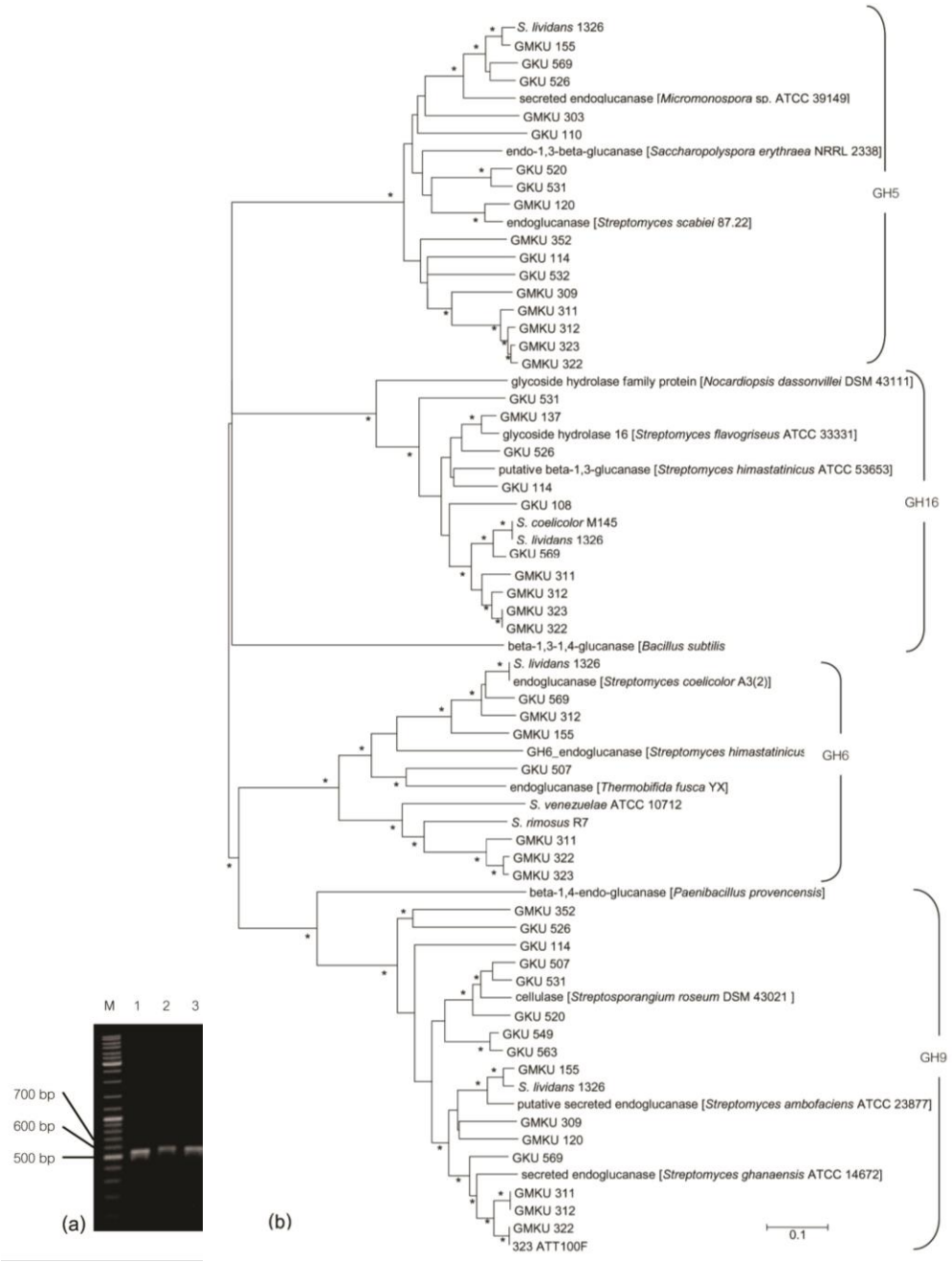

Figure 1 (a) PCR products of β-glucanases from *Streptomyces* sp. GMKU 322. Lane M: 1 kb DNA ladder (0.1-10 kb), Lane 1: GH5 (542 bp), Lane 2: GH6 (543 bp), Lane 3: GH9 (559 bp), Lane 4: GH16 (609 bp). (b) Phylogenetic analysis of β-glucanases of endophytic actinomycetes in GH5, GH6, GH9 and GH16 family. Asterisks indicate levels of bootstrap support above 50%.

CONCLUSION

Endophytic actinomycetes in this study revealed a good source for lignocellose degrading enzymes. Moreover, some β-glucanase producing strains were able to degrade glucan under low pH and high temperature condition. These strains carried β-glucanase genes in GH5, GH6, GH9 and GH16 gene family. Phylogenetic analysis of those β-glucanases revealed that they were clustered into 4 groups accordingly. Gene disruption and overexpression of β-glucanases are on the way to elucidate their function.

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