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Short Communication

New combination of xylanolytic bacteria isolated from the lignocellulose degradation microbial consortium XDC-2 with enhanced xylanase activity

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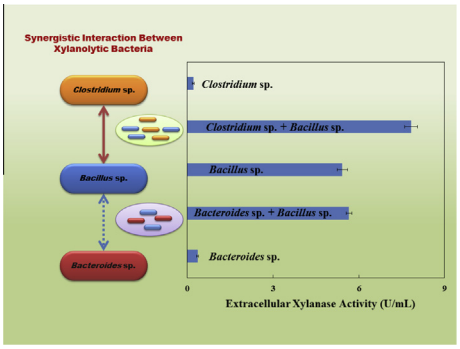
HIGHLIGHTS

- An aerobic xylanolytic strain was combined with anaerobic xylanolytic strains.
- Xylanase activity was enhanced by combining *Bacillus* sp. and *Clostridium* sp.
- Not all xylanolytic bacterium can coexist with xylanase production enhancement.
- *Bacillus* sp. enabled the growth of anaerobic *Clostridium* sp., not *Bacteroides* sp.

GRAPHICAL ABSTRACT



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ABSTRACT

Three bacterial strains with extracellular xylanase activity were isolated from the microbial consortium XDC-2. The aerobic strain A7, belonging to *Bacillus* sp., was combined with the anaerobe *Clostridium* sp. strain AA3 and/or *Bacteroides* sp. strain AA4 to obtain an efficient natural xylanolytic complex enzyme. The synthetic microbial community M1 consisting of strains *Bacillus* and *Clostridium* showed enhanced extracellular xylanase activity and production, and higher lignocelluloses degradation capability than any of the pure cultures and other synthetic microbial communities. Neither corn straw degradation nor extracellular xylanase activity was enhanced in the other synthetic microbial communities, *Bacillus*, *Bacteroides* with or without *Clostridium*. Quantitative polymerase chain reaction showed that the aerobic strain *Bacillus* enabled the growth of the anaerobic strain *Clostridium*, but not that of the anaerobic strain *Bacteroides*. These findings suggest that strains *Bacillus* and *Clostridium* can coexist well and have a positive synergistic interaction for extracellular xylanase secretion and lignocellulose degradation.

1. Introduction

Lignocellulose is the most abundant renewable biomass resource and has significant potential for the production of bioethanol, methane, and forage (Kaparaju et al., 2009; Guo et al., 2011). Lignocellulosic biomass is hydrolyzed to sugars, which can

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then be converted to bioenergy. However, only a small portion of the biomass is used to produce animal feeds, paper, and bioenergy, with the remainder directly burnt in the field. The most significant technological and economical limitation for lignocellulosic biomass utilization is lignocellulose hydrolysis, because cellulose is strongly bound to hemicellulose and lignin in nature (Himmel et al., 2007). Therefore, enzymatic hydrolysis has been proposed as an alternative strategy, because it is usually conducted under mild conditions and does not have a corrosion problem (Singh et al., 2009).

The abilities of pure-culture microorganisms to degrade celluloses and hemicelluloses are generally limited. The majority of pure-culture isolates with high lignocellulolytic activities can only degrade substrates with relatively simple structures and compositions, such as artificial xylan and carboxymethyl starch, but are unable to use natural lignocelluloses (Levin et al., 2006; Kato et al., 2004). Several studies have evaluated the potential for using mixtures of different microorganisms to improve the capability of natural lignocelluloses degradation (Haruta et al., 2009; Guo et al., 2010; Wang et al., 2011). Of particular note, the microbial consortium XDC-2 has been shown to degrade natural lignocelluloses, and also secreted extracellular xylanase efficiently in liquid culture under static conditions at room temperature (Guo et al., 2010). The extracellular enzyme showed high hydrolysis for natural lignocelluloses, and degraded most of the hemicelluloses (89.5% weight loss for the core of decorticated corn stalk and 77.1% for corn stalk) (Guo et al., 2010). Hemicellulose degradation and breakdown is very important to improve lignocellulose utilization. Compared with cell-associated enzymes, extracellular enzymes are easier to extract and are more available. However, little is known about the functional bacteria with xylanase activity in the consortium XDC-2, or the mechanisms of synergistic interaction, mutual coordination, and restraint between these xylanolytic bacteria. Therefore, resolving these uncertainties could help to achieve better control for the practical application of a microbial consortium for enzyme production.

XDC-2 can maintain high xylanase activity during incubation from an initial aerobic condition to the subsequent anoxic condition (Guo et al., 2010). Studies have shown that the coexistence of anaerobic and aerobic bacteria is important for effective cellulose degradation (Kato et al., 2004). Accordingly, three bacterial strains with xylanase activity were isolated from the microbial consortium XDC-2 under aerobic and anaerobic conditions and combined with an anaerobe/anaerobes complex to obtain an efficient natural xylanolytic complex enzyme. However, not all members of XDC-2 can be completely identified. Therefore, establishing a microbial combination from isolated bacteria is the first step in clarifying the role of each bacterium and their interactions in secreting extracellular xylanase.

In the present study, the extracellular xylanase and corn stalk degradation abilities of the three pure cultures and synthetic microbial communities were assayed and compared. The results of this study should contribute to the understanding of the mechanisms of the high extracellular xylanase activity in XDC-2, and be helpful for promoting the industrialized production of extracellular xylanase.

2. Materials and methods

2.1. Preparation of lignocellulosic materials

Corn stalks obtained locally from Wuhan, China were air-dried and then submerged in 1% (w/v) sodium hydroxide at room temperature for 24 h, washed with tap water to neutral pH, and oven-dried at 80 °C. The dried straws were milled to pass through a 1-mm sieve for further use.

2.2. Isolation of bacterial strains from original microbial consortium XDC-2

The bacteria were aerobically and anaerobically isolated with peptone cellulose solution (PCS) medium (Guo et al., 2011) and DSM 122 medium at 35 °C, respectively. After appropriate incubation periods, active cultures were transferred (5%, v/v) to fresh medium with 1% (w/v) rice straw as a carbon source. Phylogenetic analysis was detailed in the Supplementary Material.

2.3. Synthetic microbial communities

DSM 122 medium was used for the synthetic microbial community experiments. Each isolate was precultivated to the stationary phase under the conditions described above. When the OD (optical density) value of aerobe (strain A7) and anaerobes (strain AA3 and AA4) reached to 1.2 and 0.8, respectively, 250 μL of each preculture solution was inoculated into 5 mL of DSM 122 medium in various microbial combinations (1st generation). The OD of sample was determined by using UV spectrophotometer (Bio-Spes Mini, Shimadzu, Japan) at 600 nm. When the corn stalk in the medium started to degrade, 250 μL of culture solutions were transferred to 5 mL of the same medium (2nd generation). The lignocellulose degradation and extracellular xylanase activity in the synthetic microbial communities were assessed after at least four transfers of the culture into new medium. Synthetic microbial communities were established by combining an aerobe (strain A7) with the following anaerobes: strain AA3 (designated as M1), strain AA4 (M2), and both strains AA3 and AA4 (M3). Unless stated otherwise, all of these cultures were incubated under static conditions with a loose cap at 35 °C in a 10-mL serum bottle. All of the experiments were performed in triplicate.

2.4. Determination of enzyme activities and weight loss

In the cultures, xylanase activities were assayed according to Bailey et al. (1992). Samples were centrifuged at 12,000*g* for 10 min at 4 °C and the supernatants were used as extracellular enzyme samples. The weight losses of corn straw and lignocellulosic components were determined using a gravimetrical analysis method (Guo et al., 2010).

2.5. Real-time quantitative polymerase chain reaction (qPCR)

The abundance of *Bacillus subtilis*, *Clostridium sartagoforme*, and *Bacteroides graminisolvens* in the synthetic microbial communities were estimated by qPCR. The specific primer sets used were Baci1233F (5–cagcgaaaccgcgaggttaag–3) and Baci1404R (5–ttacct caccgacttcgggtg–3) for *B. subtilis*, Clos157F (5–acattacattttcgcat gaag–3) and Clos404R (5– ctgaagacagagctttacgat–3) for *C. sartagoforme*, and Bact992F (5–gtgaaggtgctgcatggttgtc–3) and Bact1108R (5–cctcacatcttacgacggcagt–3) for *B. graminisolvens*. The primer sets were designed specifically for this study. qPCRs were performed using a LightCycler system (Roche Diagnostics, Mannheim, Germany) and the LightCycler Fast-start DNA Master SYBR green I Kit (Roche Molecular Biochemicals, Indianapolis, IN, USA) as previously described (Zhang et al., 2014).