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Construction of a microbial community for effective hydrolysis of the cellulosic and lignocellulosic biomass

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Research aims

In recent years, bioethanol has become an important product. Cellulosic and lignocellulosic biomass has high potential as feedstock for bioethanol, and has been the focus of active research and development. Wood wastes, rice straws and rice husks, etc. are suitable biomass materials for bioethanol production because use of these materials for ethanol production does not compete with food production. However, they are complex mixtures of cellulose, hemicellulose (including xylan) and lignin, and cannot be decomposed easily. Microorganisms have been found to decompose cellulose and lignin with high efficiency previously[1]. However, microorganisms which can decompose xylan in the same environment have not been reported. During our analysis of microbial community composition in the composting reactor, we detected cellulose-degrading bacteria *Thermobifida fusca*, lignin-degrading bacteria *Ureibacillus thermosphaericus,* and xylan-degrading bacteria *Thermobacillus composti* in the same compost sample. Therefore, these three bacteria may be possible symbiotic microorganisms. In this study, to construct the microbial community for effective hydrolysis of the cellulosic and lignocellulosic biomass, we carried out the following three investigations: 1) searching for optimum conditions for co-culturing *T. fusca*, *U. thermosphaericus* and *T. composti* in the same reactor, 2) screening of microorganisms, from the same compost sample, that enhance the hydrolysis efficiency and improve the stability of microbial community composition, and 3) characterization of xylanase derived from *T. composti*.

Methods

Optimum conditions for culturing three microorganisms in the same reactor

T. fusca (DSM43792), *U. thermosphaericus* (DSM10633) and *T. composti* (DSM18247) were obtained from DSMZ (The German Resource Centre for Biological Material). *T. composti* is the most difficult to grow among these bacterial species. Therefore, according to the optimal growth conditions for *T. composti*, the modified Brock's basal salts (MBS)[2] supplemented with 0.5% yeast extract was used as a growth medium. And the growth potentials of the three bacterial species in the medium with various initial pH were determined at 50°C. *U. thermosphaericus* and *T. composti* were cultured in the liquid medium. Their growth was monitored by the optical cell density at 600 nm by using a spectrophotometer. *T. fusca* is an actinomycetous bacterium. *T. fusca* forms aggregates, not dispersed uniformly in the liquid medium. Since its optical cell density could not be measured by using spectrophotometer, the optimum growth conditions for *T. fusca* were determined by counting colonies on solid medium.

Cellulosic and lignocellulosic biomass glycosylation

Rice husks were used as cellulosic and lignocellulosic biomass. They were milled by using a household use kitchen mixer. After size fractionation (>1 mm, 1 mm–0.1 mm, <0.1 mm) by using sieve, milled rice husks were autoclaved twice. MBS (pH 8.0) supplemented with 0.5% rice husks or MBS (pH 8.0) supplemented with 0.5% rice husks and 0.5% yeast extract was used as medium. Microorganisms were inoculated into the medium and static cultivation was performed at 50°C for 2 weeks. Experiments were conducted under the following eight conditions: growth medium without inoculation (blank), cultures inoculated individually with single species, mixed cultures inoculated with two species (*T. fusca* and *U. thermosphaericus*, *T. fusca* and *T. composti*, *U. thermosphaericus* and *T. composti*), and mixed cultures inoculated with all the three species. Released reducing sugar was measured by using DNS method[3]. To confirm the presence of the three species of bacteria in cultures, RFLP analysis was conducted as follows: genomic DNA mixture was extracted from bacterial cells grown in liquid culture and used as template DNA for polymerase chain reactions (PCR). 16S rDNAs were amplified by PCR. PCR products were digested with restriction enzymes *Sac*I and *Pst*I.

Screening of microorganisms from compost sample

The composting sample was added into MBS (pH 8) supplemented with 0.5% yeast extract, and incubated at 50°C for 24 hours. This enrichment culture was plated onto solid medium for isolation of microorganisms. After single colony isolations, purified isolates were analyzed by 16S rDNA sequencing.

Characterization of xylanase derived from *Thermobacillus composti*

T. composti was cultured in MBS (pH 9) supplemented with 0.5% beechwood xylan, with shaking at 150 rpm at 50°C, for 3 days. The culture was separated into the supernatant and bacterial cells by centrifugation. Proteins in the supernatant were precipitated by ammonium sulfate precipitation. After centrifugation, the proteins were resuspended in buffer. A crude enzyme solution was obtained after desalting treatment. For analysis of xylanase activity, the crude enzyme solution was added to a buffer solution containing 0.5% beechwood xylan. After incubation for 1 hour at various temperatures, the amount of produced xylose was quantitated by the DNS method.

Results

The optimum growth pH for *T. fusca*, *U. thermosphaericus*, and *T. composti* were 8.0, 7.5–8.0, and 8.0–9.0, respectively. These results showed that pH 8.0 is a suitable growth pH for co-culturing these three species in the same medium. Therefore, for effective hydrolysis of the cellulosic and lignocellulosic biomass, the mixed cultures with various combinations of the three species were incubated at pH 8.0. Examination of biomass hydrolysis efficiency with the each culture showed that the mixed culture containing all of the three bacterial species released a larger amounts of reducing sugars (total of C6 and C5 sugars) than any other mixed cultures, regardless of presence of yeast extract, at the end of experiments (after 2 weeks). When rice husks with a particle size of <0.1 mm were used, hydrolysis efficiency was about 65% (reducing sugars calculated as glucose). The hydrolysis efficiency observed with the mixed culture containing all of the three bacterial species was about 1.5-times higher than that of the culture containing only *T. fusca*, and about 1.1–1.8 times higher than those observed with the mixed cultures containing two bacterial species out of the three species. These results showed that combination of the tree different bacterial species could enhance hydrolysis efficiency of cellulosic and lignocellulosic biomass.

The 16S rDNA sequencing analysis showed that the microorganisms isolated from the same compost sample belonged to *Geobacillus thermoglucosidasius* and *Geobacillus toebii*. It was reported that certain *G. thermoglucosidasius* strain can ferment major C6 and C5 sugars in cellulosic biomass and can tolerate high concentrations of ethanol[4]. From cellulosic and lignocellulosic biomass, the microbial community constructed in this study was found to release both C6 and C5 sugars. Therefore, there is a possibility that inclusion of *G. thermoglucosidasius* in the mixed microbial community containing the three bacterial species might lead to more efficient production of ethanol from cellulosic and lignocellulosic biomass. In addition, the optimum temperature of xylanase derived from *T. composti* was estimated at 55–60°C.

Conclusion

In this study, a microbial community capable of effective hydrolysis of the cellulosic and lignocellulosic biomass was successfully constructed. In addition, the optimum temperature of xylanase derived from *T. composti* was estimated.

References

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