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Ethanol Fermentation of Fusant between Heterologous Transformant of *Saccharomyces cerevisiae* and *Candida tropicalis* in Pilot Scale

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Pilot Scale에서의 Fusant의 Ethanol 발효

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As the final experiment to assess the possibility of industrial application of FSC-14-75, ethanol productivity from liquefied sweet potato starch was examined in a pilot scale of 300 liters. FSC-14-75 produced 6.6%(*v/v*) of ethanol from 13.3% of liquefied sweet potato starch in 8 days, and the residual sugar was 3.15%. The corresponding efficiency was 70% of the theoretical maximum. Since we could isolate unicellular cell and flocculent cell from the fermentation broth, we designated them FSC-14-75(S) and FSC-14-75(F), respectively. We investigated ethanol productivity of FSC-14-75(F) compared with that of FSC-14-75(S) from liquefied potato starch in a mini-jar fermentor scale of 2.5 liters. FSC-14-75(F) was found more favorable than the counterpart in terms of ethanol productivity, and produced 8.1%(*v/v*) of ethanol from 15% of liquefied potato starch with an efficiency of 75%. In a pilot scale fermentation with 15% of liquefied sweet potato starch, ethanol productivity of FSC-14-75(F) reached maximum level of 7.7%(*v/v*) after 8 days, and the residual sugar was 1.9%. However, the ethanol productivity was not enhanced by a supplementary addition of Thermamyl to the fermentation broth after sterilization.

In our recent work, we attempted a transformation of the intact cells of *S. cerevisiae* by partially BamHI-digested chromosomal DNA of *S. diastaticus,* without a foreign vector, in order to develop a new brewing yeast capable of fermenting starch to ethanol directly. However, the obtained successful transformant was not suited for the direct one-step ethanol fermentation of starch because glucoamylase of the transformant, originated from *S. diastaticus,* could not hydrolize the alpha-1,6-glucosidic linkage of amylopectin(1,2).

Thus, we subsequently undertook intergeneric protoplast fusion between the transformant and *Candida tropicalis* possessing debranching activity according to the method of dead donor techniques in an attempt to introduce alpha-1,6-glucosidase, and obtained a desirable fusant FSC-14-75(3,4).

Here we examined the ethanol productivity of FSC-14-75 from liquefied sweet potato starch in a pilot scale as the final experiment to assess the possibility of industrial application.

Materials and Methods

Strains

The used strains were FSC-14-75 obtained from intergeneric protoplast fusion between TSD-14 and *Candida tropicalis* RCT-40(lys$^-$), FSC-14-75(S) and

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FSC-14-75(F) derived from FSC-14-75. (Table 1).

Media

The YPS agar medium contained 0.5% yeast extract, 1% peptone, 2% soluble starch, and 1.8% agar. The YPD medium contained 0.5% yeast extract, 1% peptone, and 2% dextrose.

Substrate

The potato starch was purchased from Junsei Co., and the sweet potato used for substrate was purchased from a grain market in Taegu. The moisture content was 20.5%.

Pilot scale fermentation

Fermentation broth; For the preparation of fermentation broth, the sweet potato starch was liquefied with the same manner as the process of Phung Gook Alcohol Industry Co.

About 20%(w/v) suspension of sweet potato starch was prepared by agitation of ca. 87 kg of sweet potato in 300 liters of tap water in the presence of antiforming agent. When the temperature reached 50 °C, 0.3% of Thermamyl (v/w, to starch content) was added and the temperature was raised to 80 °C with continuous stirring for 1 hour. The liquefied starch solution then was supplemented with 0.3% $(NH_4)_2SO_4$, 0.1% KH_2PO_4, 0.2% $MgSO_4 \cdot 7H_2O$, and 0.2% yeast extract, and sterilized at 121 °C for 20 minutes.

Fermentation conditions; Stock culture of FSC-14-75 maintained on YPD agar medium was cultured in a 250 m*l* flask containing 20 m*l* of YPD medium at 30 °C for 36 hours. This culture was then transferred to a mini-jar fermentor containing 3 liters of the same fermentation broth to develop the inoculum for the pilot scale fermentation. The incubation temperature in the fermentor was maintained at 30 °C and the culture was stirred at 300 rpm. The pH was adjusted to 4.2 with 10 N NaOH every 8 hours, and the aeration was carried out at 0.5 vvm for the initial 8 hours after seeding in order to induce the cell growth.

Table 1. List of strains used

Strain	Origin
FSC-14-75	intergeneric fusant between *C. tropicalis* RCT-40 (lys^-) and TSD-14*
FSC-14-75(S)	unicellular cell of FSC-14-75
FSC-14-75(F)	flocculent cell of FSC-14-75

*TSD-14: Heterologous transformant of *S. cerevisiae* X2180-1A by chromosomal DNA of *S. diastaticus* IFO 1046(1).

Mini-jar fermentor scale fermentation

In order to examine the ethanol productivity of FSC-14-75(S) FSC-14-75(F) from liquefied potato starch, the preparation of fermentation broth and fermentation was carried out with the same manner as the method described previously(4).

Analytical methods

The residual sugar content of fermentation broth was determined by the procedure of acid hydrolysis followed by Somogy-Nelson method(5) or Berterand method(6).

The ethanol content was measured by alcohol hydrometer after distillation(4).

Results

Ethanol productivity of FSC-14-75 in a pilot scale

As the final experiment to assess the possibility of industrial application of FSC-14-75, ethanol productivity from 13.3% of liquefied sweet potato starch (Table sugar = 14.8%) was examined in a pilot scale. In this case the inoculum was 0.6% (v/v).

As the results appeared in Fig. 1, FSC-14-75 produced 6.6% (v/v) of ethanol for 8 days, and the remaining sugar was 3.15%. The corresponding efficiency was 70.0% to total sugar and 87.5% to consumed sugar, respectively.

Ethanol productivities of FSC-14-75(F) and FSC-14-75(S) from liquefied potato starch in a mini-jar fermentor scale.

Ethanol productivity of FSC-14-75 from liquefied sweet potato starch in a pilot scale was somewhat lower than that in mini-jar fermentor scale. We could also isolate unicellular-form FSC-14-75(S) and flocculent-form FSC-14-75(F) from the fermentation broth. Thus, we examined the ethanol productivity of FSC-14-75(F) compared with that of FSC-14-75(S) in a mini-jar scale according to the method described previously.

As shown in Fig. 2, FSC-14-75(F) was more favorable than FSC-14-75(S) with respect to etha-

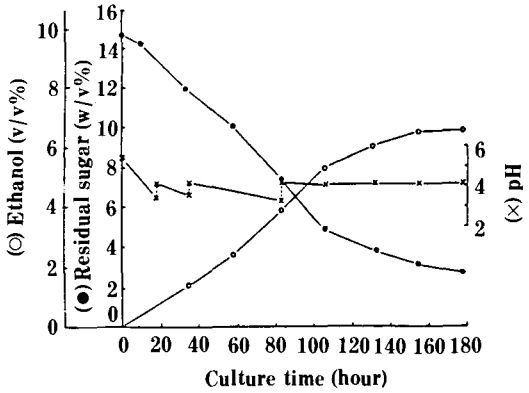

Fig. 1. Pilot scale ethanol fermentation of FSC-14-75 in liquefied sweet potato starch.

The fermentation was conducted in a pilot-scale fermentor with 300 liters of fermentation broth containing 13.3% sweet potato starch (liquefied with Thermamyl), 0.3% $(NH_4)_2SO_4$, 0.1% KH_2PO_4, 0.2% $MgSO_4 \cdot 7H_2O$, and 0.2% yeast extract at 30 °C. The seed volume was 1%, and the pH was controlled with 10N NaOH.

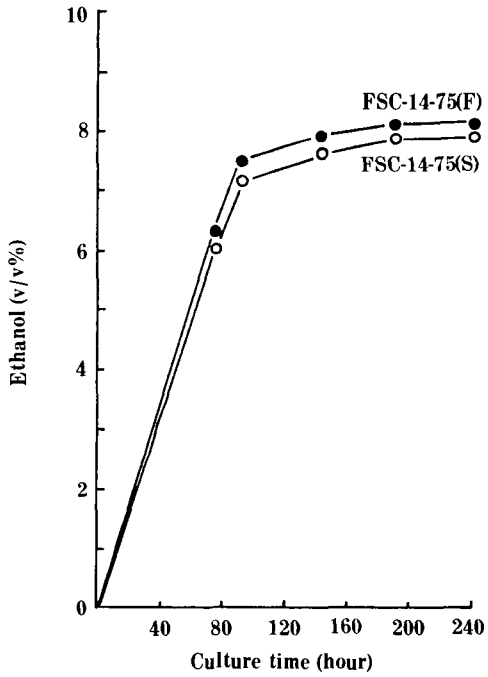

Fig. 2. Comparison of ethanol productivities of FSC-14-75(F) and FSC-14-74(F) from liquefied potato starch.

The fermentation was performed in a mini-jar fermentor with 2.5 liters of fermentation broth containing 15% potato starch (liquefied with Thermamyl), 0.3% $(NH_4)_2SO_4$, 0.1% KH_2PO_4, 0.2% $MgSO_4 \cdot 7H_2O$, and 0.2% yeast extract at 30 °C. The seed volume was 1%, and the pH was controlled to 4.2 with 2N NaOH.

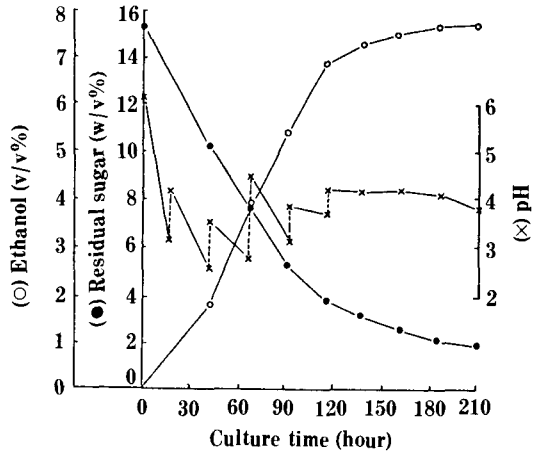

Fig. 3. Pilot scale ethanol fermentation of FSC-14-75(F) in liquefied sweet potato starch.

The fermentation was conducted in a pilot-scale fermentor with 300 liters of fermentation broth containing ca. 15% liquefied sweet potato starch (Total sugar: 15.33%), and other fermentation conditions were same as in Fig. 1.

nol productivity. FSC-14-75(F) produced 8.1% (v/v) of ethanol from 15% of liquefied potato starch, and the remaining sugar was 0.49%.

Ethanol productivity of FSC-14-75(F) in a pilot scale.

To investigate the ethanol productivity of FSC-14-75(F) originated from FSC-14-75, 15% of liquefied sweet potato starch was inoculated with 1% (v/v) of seed culture.

As showned in Fig. 3, ethanol productivity reached maximum level after 8 days, and the productivity was 7.7% (v/v) of ethanol. The remaining sugar was 1.9%.

On the other hand, as shown in Fig. 4, the addition of Thermamyl to the fermentation broth has no significant effect. The ethanol productivity of FSC-14-75(F) was reached maximum level of 7.8% (v/v) after 8 days and the residual sugar was 1.46%. However, this result was considered not so much significant compared to the case without Thermamyl.

Discussion

Because *Saccharomyces cerevisiae*, widely used for commercial production of ethanol or alcohol beverage from starchy raw materials, lacks the

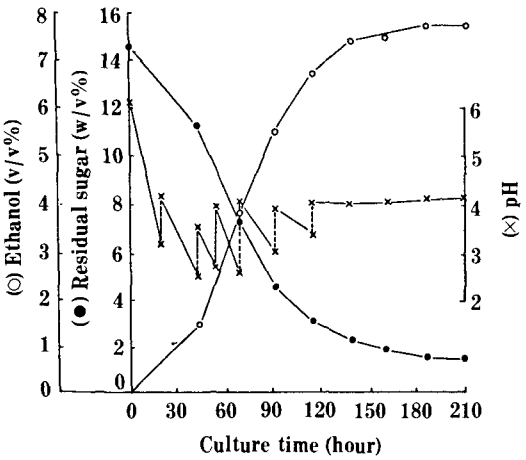

Fig. 4. Effect of Thermamyl addition to fermentation broth of FSC-14-75(F).

After the fermentation broth containing ca. 15% liquefied potato starch was sterilized, 50 m*l* of thermamyl was added together with inoculum in order to examine the effect of Thermamyl. Other fermentation conditions were as in Fig. 1, and the initial concentration of total sugar was 14.57%.

amylase enzymes necessary for starch utilization, the starchy materials must be hydrolyzed to fermentable simple sugars by the four separate steps such as cooking, liquefaction, saccharification, and fermentation(7).

Thus, to eliminate the separate saccharification step, we attempted heterologous transformation of the intact cells of *S. cerevisiae* by Bam HI-digests of chromosomal DNA of *S. diastaticus*(1) and subsequently the transformant was fused with *C. tropicalis* in the presence of PEG and $CaCl_2$ according to the method of dead donor techniques(8), and finally obtained a desirable fusant FSC-14-75(3). Then we examined the ethanol productivities of FSC-14-75 from liquefied potato starch in flask and mini-jar fermentor scale(4). Consequently, the results showed that FSC-14-75 had sufficient potentiality to be used for direct fermentation of liquefied potato starch to ethanol.

In this study, we examined ethanol productivity of FSC-14-75 from liquefied sweet potato starch in a pilot scale as the final experiment to assess the possibility of industrial application.

The fusant FSC-14-75 produced 6.6% (v/v) of ethanol for 8 days from 13.3% of liquefied sweet potato starch, and the residual sugar was 3.15%. This result was somewhat worse than the case in the mini-jar fermentor scale which was reported previously(4), and might be due to the difference of substrates.

On the other hand, since we could isolate unicellular cell and flocculent cell from the fermentation broth, we designated them FSC-14-75(S) and FSC-14-75(F), respectively. As the result that we investigated the ethanol productivity of FSC-14-75(F) compared with that of FSC-14-75(S) in a mini-jar fermentor scale, FSC-14-75(F) was more favorable than the counterpart with respect to ethanol productivity. It produced 8.1%(v/v) of ethanol from 15% of liquefied potato starch with an efficiency of 75%.

In a pilot scale fermentation of 15% of liquefied sweet potato starch by FSC-14-75(F), ethanol productivity reached maximum level of 7.7%(v/v) after 8 days, and the remaining sugar was 1.9%. The ethanol productivity was not enhanced as much as expected, although 50 m*l* of Thermamyl was added to the fermentation broth together with inoculum after sterilization. These results may suggest that the residual sugars are mainly composed of low molecular weight oligosaccharides possesing alpha-1,6-glucosidic linkage. Hence it is thought that the fusant FSC-14-75 could be sufficiently suited for commercial ethanol production from liquefied starch by supplementation of alpha-1,6-glucosidase.

요 약

Fusant FSC-14-75의 산업적 이용가능성을 검토하기 위한 최종실험으로서 working volume이 300 liters인 pilot scale에서의 발효력을 조사하였다.

13.3%의 liquefied sweet potato starch(Total sugar=14.8%)을 ethanol 발효원으로 함유한 fermentation broth 300 liters를 0.6% seed로 발효시킨 결과 발효 최종일인 8일만에 6.6%(v/v)의 ethanol을 생성하였고 이때 잔당은 3.15%이었으므로 발효율은 총당에 대해서는 70%, 소비당에 대해서는 87.5%로 나타났다.

한편 fermentation broth로부터 FSC-14-75 유래의 unicellular cell과 flocculent cell을 분리할 수 있었으며 이를 각각 FSC-14-75(S)와 FSC-14-75(F)로 명명하였고, 이들 두 균주의 ethanol 발효능을 mini-jar fermentor scale에서 조사한 결과 FSC-14-75(F)가 ethanol productivity에 있어서 우

수하였으며 15%의 liquefied potato starch로부터 발효 10일만에 8.1%(v/v)의 ethanol을 생성하여 발효율은 총당에 대해 75%였다.

FSC-14-75(F)의 ethanol productivity를 pilot scale에서 조사하기 위해 총당 15.33%의 liquefied sweet potato starch를 함유한 fermentation broth 300 liters를 1% seed로써 발효시킨 결과 ethanol 생성은 배양 8일만에 최대 7.7%(v/v)였으며 이때 잔당은 1.9%였다. 또한 FSC-14-75(F) 균주의 발효과정중 α-amylase의 계속적인 작용효과를 검토하기 위하여 300 liters의 fermentation broth를 살균후 1% seed와 동시에 50 ml의 Thermamyl을 재차 첨가하고 ethanol 생성을 조사한 결과 발효율 및 잔당은 첨가하지 않은 경우와 별다른 차이가 없었다.

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