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DIRECT FERMENTATION OF CELLULOSE TO ETHANOL BY A CELLULOLYTIC FILAMENTOUS FUNGUS, *MONILIA SP.*

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SUMMARY

A saprophytic filamentous fungus, *Monilia sp.*, isolated from bagasse compost was found to utilize many polysaccharides (including cellulose) and to produce cellulases and hemicellulases. *Monilia sp.* also fermented glucose, xylose and cellulosic materials to ethanol. Over 60% of the solid cellulose substrate added to *Monilia sp.* cultures was converted to ethanol as the major fermentation product. These results indicate that *Monilia sp.* is a potential organism for the direct conversion of cellulosic biomass to ethanol.

INTRODUCTION

Renewable biomass contains cellulose, hemicellulose, lignins and pectic materials. Converting cellulosic materials directly to ethanol has not been satisfactory in the past. Many prokaryotes and eukaryotes have been found to produce extracellular cellulase when grown in a medium containing cellulose or cellulase inducers (Gong and Tsao, 1979), yet they cannot ferment sugars to ethanol. On the other hand, many microorganisms that are able to ferment sugars to ethanol lack the genetic information to produce the hydrolytic enzymes needed to break down cellulose.

Methods combining the enzyme production and fermentation ability of organisms have been used to produce ethanol from cellulosic materials. One method is a coupled saccharification-fermentation process that consists of cellulose, cellulase enzyme, yeast and nutrients (Blotkamp, et al., 1978; Meyers, 1978) but, separate steps for cellulase production and concentration are required. A mixed culture of a cellulolytic fungus and a glucose fermenting yeast has also been examined (Peitersen, 1975). Combining these two, a single step, saccharification-fermentation,

mixed-culture process has been examined by mixing a cellulolytic bacterium *Thermoactinomyces* and a fermenting bacterium *Clostridium thermocellum* to produce ethanol from cellulosic biomass (Pye and Humphrey, 1979).

An Attempt for the direct fermentation of cellulosic materials to ethanol has been made with a thermophilic anaerobic bacterium *C. thermocellum* which can hydrolyze and ferment cellulose to ethanol (Cooney, et al., 1979) in combination with a thermophilic pentose fermenting anaerobe, *C. thermosaccharolyticum*. This mixed culture has been shown to ferment both Solka-Floc and corn stover to a mixture of ethanol, acetic acid and lactic acid (Wang, et al., 1979; Brooks, et al., 1979). This communication reports on a cellulolytic filamentous fungus, *Monilia sp.* that is able to directly ferment cellulose to ethanol.

MATERIALS AND METHODS

Monilia sp. was isolated from bagasse compost located in Clewiston, Florida and purified by single-spore isolation. In all experiments a YME medium was used which contained Bacto-Yeast extract, 3.0 gm; Bacto-Malt extract, 3.0 gm; Bacto-Peptone 5.0 gm per liter, and the carbon sources were added as required. The media was autoclaved at 121°C for 20 minutes and all inoculations were made from spore suspensions.

Dry Weight Measurements: Cultures were grown in 100 ml YME in 250 ml Erlenmeyer flasks with 1% of various polysaccharides added and incubated 4 days at 30°C on a reciprocal incubator-shaker. The mycelia were washed and filtered through a Buchner funnel and dried in a gravity-convection incubator at 70°C till a constant weight was maintained.

Fermentation of Cellulose Substrates: Cultures were grown aerobically in 100 ml YME in 250 ml Erlenmeyer flasks with 1% cellulose for 24 hours

at 26°C. Additional substrates were then added and the flasks were incubated under non-aerated conditions to carry out the cellulose fermentation.

Cellulase and Xylanase: Cultures were grown in 300 ml YME in 500 ml Erylenmeyer flasks with 2% Avicel, 2% Solka-Floc or 1% xylan at 30°C in an incubator shaker. Samples were taken daily and enzymes were precipitated by 75% saturation of ammonium sulfate. Cellulase activity was measured in terms of the amount of reducing sugars produced from carboxylmethyl cellulose (Gong, et al., 1979). Xylanase activity was measured as the amount of reducing sugars produced from xylan (Sigma). The dinitrosalicylic acid reagent method was used to measure the reducing sugars (Miller, 1959).

Ethanol Production: The concentration was measured by gas chromatography.

Sugar Consumption: Liquid chromatography was used to analyze and quantify sugar consumption (Ladisch, and Tsao, 1978).

RESULTS

Growth on Polysaccharides: The results shown in Table 1 indicate that *Monilia sp.* is able to utilize various polysaccharides as sole carbon and energy sources.

TABLE 1

VEGETATIVE GROWTH OF *MONILIA SP.* IN LIQUID MEDIUM WITH VARIOUS POLYSACCHARIDES

Substrates	Mycelia Dry Weight (mg/100ml)
D-Glucose	478
D-Xylose	613
Avicel (Crystalline Cellulose)	625
Bagasse	470
Acid Treated Bagasse	455
Ground Corn Cob	454
Xylan	528
Starch	434
Pectin	407
Chitin	377

Cellulase and Xylanase Production: When *Monilia sp.* was grown in 2% Avicel as the sole carbon source, cellulase activity was detectable in the culture broth after 48 hours growth. The enzyme concentration increased linearly for 10 days of incubation. Xylanase was also detected in this culture broth (Figure 1). These results indicated both cellulase and xylanase are produced when cellulose is used as a substrate. When xylan was used as a sole carbon source, only xylanase was detected (Figure 2). Sugars, such as glucose, fructose and xylose, served as good carbon sources for fungal growth, but the production of cellulase in the culture broth was minimal.

Fermentation of D-Glucose and D-Xylose to Ethanol: The results of ethanol produced by *Monilia sp.* from D-glucose and D-xylose are shown in Figure 3. The data show *Monilia sp.* is able to ferment D-glucose readily, but D-xylose fermentation is at a much slower rate. After seven days of incubation, however, over 40 per cent of the D-xylose had been converted to ethanol. These results indicate that *Monilia sp.* is able to ferment both D-glucose and D-xylose to ethanol.

Direct Fermentation of Cellulose to Ethanol: Experiments on the direct fermentation of cellulose to ethanol were done using Avicel(PH 101, FMC Corp.) and Solka-Floc (BW-40, Brown Co.) as substrates under anaerobic conditions. As shown in Figure 4, ethanol was produced from both substrates. Higher amounts of ethanol were obtained from Solka-Floc fermentation. Based on the ethanol produced under experimental conditions, over 70% of the Solka-Floc and over 60% of the Avicel were converted to ethanol.

DISCUSSION

In the bioconversion of renewable biomass to liquid fuels, it is desirable to use a single organism to carry out the simultaneous

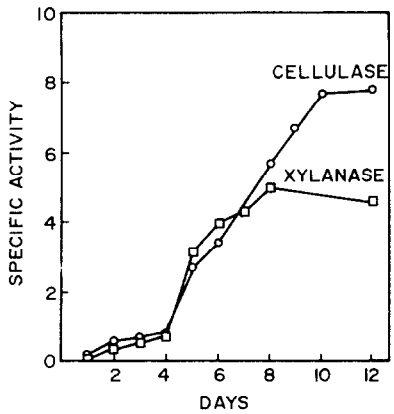

Figure 1. Cellulase and xylanase production by *Monilia sp.* in the presence of cellulose (Avicel). Specific activity: reducing sugars (mg glucose equivalent) produced per ml culture filtrate per min.

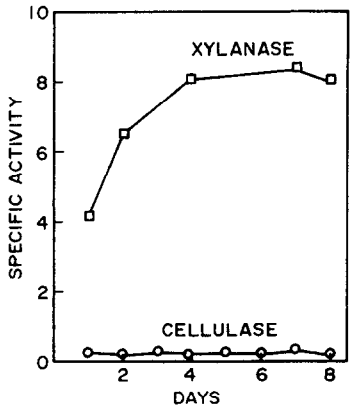

Figure 2. Xylanase production by *Monilia sp.* in the presence of xylan.

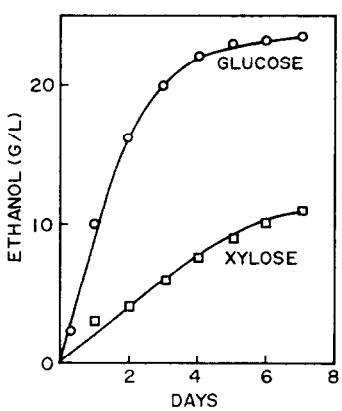

Figure 3. D-Glucose and D-xylose fermentation by *Monilia sp.* The initial sugar concentration was 50 gm/l.

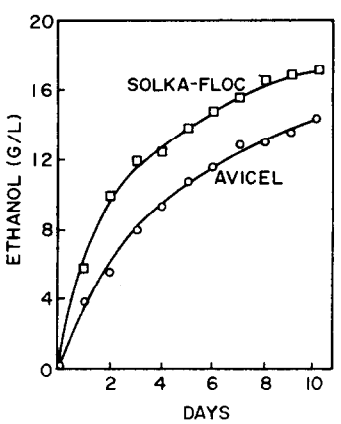

Figure 4. Fermentation of celluloses to ethanol by *Monilia sp.* The initial cellulose concentration was 50 gm/l.

hydrolysis of polysaccharides and subsequent fermentation of hydrolyzates to ethanol. The results of this study show that *Monilia sp.* is able to utilize cellulose, hemicellulose, pectic materials and various other polysaccharides as carbon sources. Furthermore, the ability of *Monilia sp.* to ferment D-xylose to ethanol is especially significant because D-xylose is a major constituent of cellulosic biomass which is presently under-utilized due to the lack of a suitable organism to ferment it efficiently.

Ethanol production from cellulose requires that an organism be able to produce hydrolytic enzymes as well as to ferment the hydrolyzate. The accumulation of ethanol that resulted from the incubation of *Monilia sp.* with crystalline cellulose or Solka-Floc indicates that *Monilia sp.* can actively hydrolyze cellulosic materials and ferment them to ethanol. From this study it is evident that *Monilia sp.* is a potential organism for the direct conversion of renewable biomass to ethanol.

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