Extraction of Two Active Polysaccharides from the Yeast Cell Wall

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Yeast cell wall matrix particles are composed entirely of β-glucan and mannoprotein. Alkali-insoluble (1→3)-β-D-glucan was extracted from the yeast cell wall by an alkaline-acid method. IR spectra analysis showed that the product was chemically pure glucan, that is to say, it contained no other carbohydrates and proteins. So, the alkaline-acid method was ideal for extracting (1→3)-β-D-glucan from the yeast cell wall. We also purified and analyzed mannan oligosaccharides by the dilute alkali-Sevage method from the yeast cell wall.

Key words: (1→3)-β-D-Glucan, Mannan Oligosaccharides, Yeast Cell Wall

Introduction

The yeast cell wall is a nonspecific stimulator of the immune system of both man and animals (Cabib *et al.*, 2001). Yeast is applied in the wine industry: its ability to bind undesirable components allows to prevent and cure stuck fermentations. The yeast cell wall consists of 30–60% polysaccharides (β-glucan and mannan oligosaccharides) (Huang *et al.*, 2004, 2005), 15–30% proteins, 5–20% lipids and a small amount of chitin. Most of the protein is linked to the mannan oligosaccharides and is referred to as the mannoprotein complex. β-Glucan can stimulate the cells of the immune system (macrophages) and helps to overcome bacterial infections. Mannan oligosaccharide has been demonstrated to prevent diarrhoea in weaning pigs. It binds to pathogenic bacteria in the gut and carries them through and out of the intestinal tract. Mannan oligosaccharide also has prebiotic activity and can serve as a nutrient source for the growth of beneficial bacteria in the colon. Based on the important biological functions of yeast cell walls, the extraction methods for alkali-insoluble (1→3)-β-D-glucan and mannan oligosaccharide from the yeast cell wall have been studied.

Results and Discussion

IR spectrum analysis of (1→3)-β-D-glucan

The IR spectrum of (1→3)-β-D-glucan (Fig. 1) shows the typical spectral pattern of (1→3)-β-D-glucan, that is to say, it contains absorption bands arising from the ν(CC) and the ν(COC) stretching vibrations at 1161 cm^{-1}, two partially overlapped bands at 1078 and 1044 cm^{-1} attributable to ring and C–OH side group stretchings, a band at 891 cm^{-1} assigned to the β-glycosidic C_1–H deformation mode, and the highest intensity of the ν(OH) band at lower frequency (3380 cm^{-1}). The presence of amide I and amide II bands at 1654 and 1637 cm^{-1} accords with the residual protein content (1.6%) of the glucan.

IR spectrum analysis of mannan oligosaccharides

Fig. 2 shows the IR spectrum of mannan oligosaccharides. It contains absorption bands arising from the ν(CC) and the ν(COC) stretching vibrations at 1140 cm^{-1}, a band at 846 cm^{-1} assigned to the α-glycosidic C_1–H deformation mode, and the highest intensity of the ν(OH) band at lower frequency (3456 cm^{-1}). The presence of a carbonyl group band at 1688 cm^{-1} proved to have the residual protein in the mannan oligosaccharide sample.

The alkaline-acid method for extracting (1→3)-β-D-glucan and the dilute alkali-Sevage method for extracting mannan oligosaccharides

Yeast cell wall matrix particles are composed entirely of β-glucan and mannoprotein. (1→3)-β-D-Glucan that was extracted is alkali-insoluble, but the mannoprotein is alkali-soluble (Fujii *et al.*, 1999). In the process of the alkaline-acid method, we used 4% phosphoric acid to remove (1→6)-β-D-glucan, which is linked to alkali-insoluble (1→3)-β-D-glucan (Bron, 1996). The Sevage method was used to remove the residual protein.

Materials and Methods

Materials

Yeast cell walls were purchased from Anqi Company (Yichang, China). IR spectra were recorded with an FT-IR apparatus, and wavenumbers are reported in cm^{-1}.

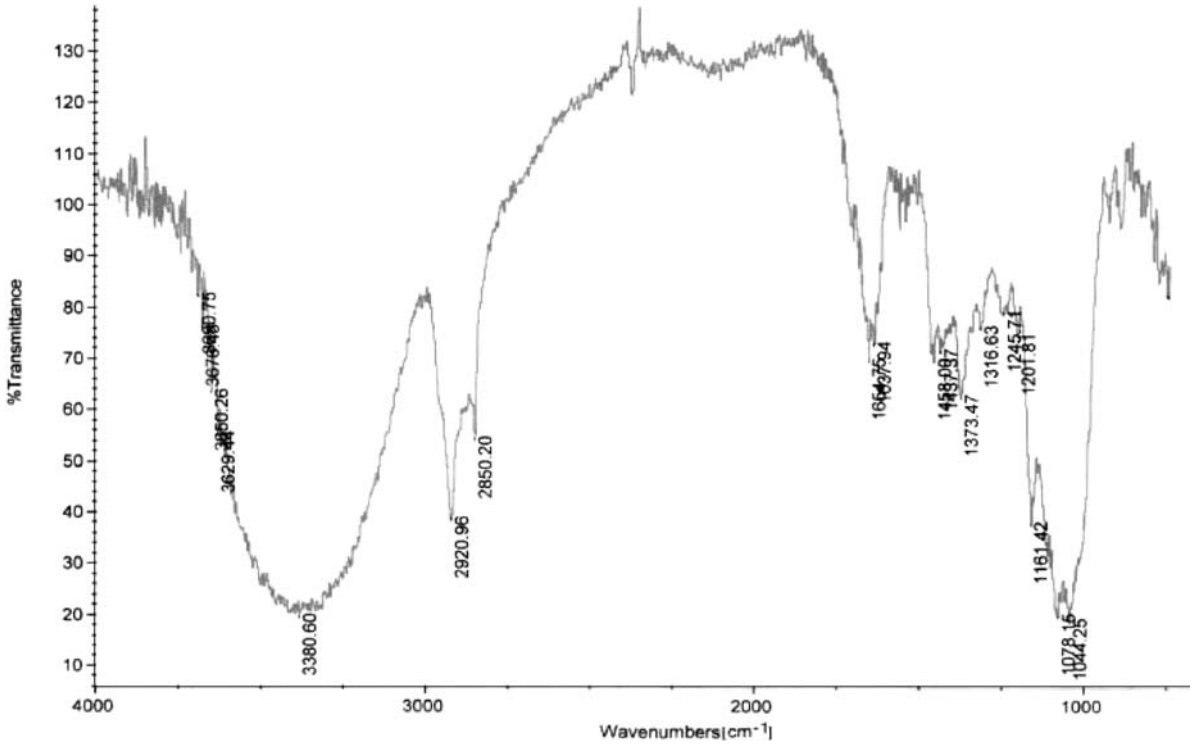

Fig. 1. IR spectrum (in KBr) of alkali-insoluble (1→3)-β-D-glucan.

Extraction of alkali-insoluble (1→3)-β-D-glucan

Alkali-insoluble (1→3)-β-D-glucan was obtained from yeast cell walls by extraction with 6% NaOH at 60 °C for 4 h. Distilled water was added to the dispersion, and the insoluble part, after stirring for 30 min, was collected by centrifugation. The sediment was suspended in 3% NaOH and heated at 90 °C for 2 h. The insoluble material was recovered by centrifugation, washed three times with distilled water, and subsequently extracted twice with 4% phosphoric acid at room temperature for 2 h. The insoluble residue, representing the cell wall (1→3)-β-D-glucan, was separated by centrifugation, resuspended in distilled water, and decanted with water until pH 7. The aqueous suspension was taken for the recovery of the particulate glucan using the technique of spraying dryness. The yield was 13.5%.

Extraction of mannan oligosaccharides

The water-soluble mannan oligosaccharides were obtained from 5 g yeast cell walls by extraction with 1% NaOH (50 mL) at 100 °C for 2 h, cooling and neutralizing to pH 7 with dilute HCl solution. After filtration, the mannan oligosaccharides were precipitated by adding 200 mL (4 volumes) of absolute ethanol. The precipitate was washed with absolute ethanol and diethyl ether, respectively. The residual protein was further removed by the Sevage method (Staub, 1965).

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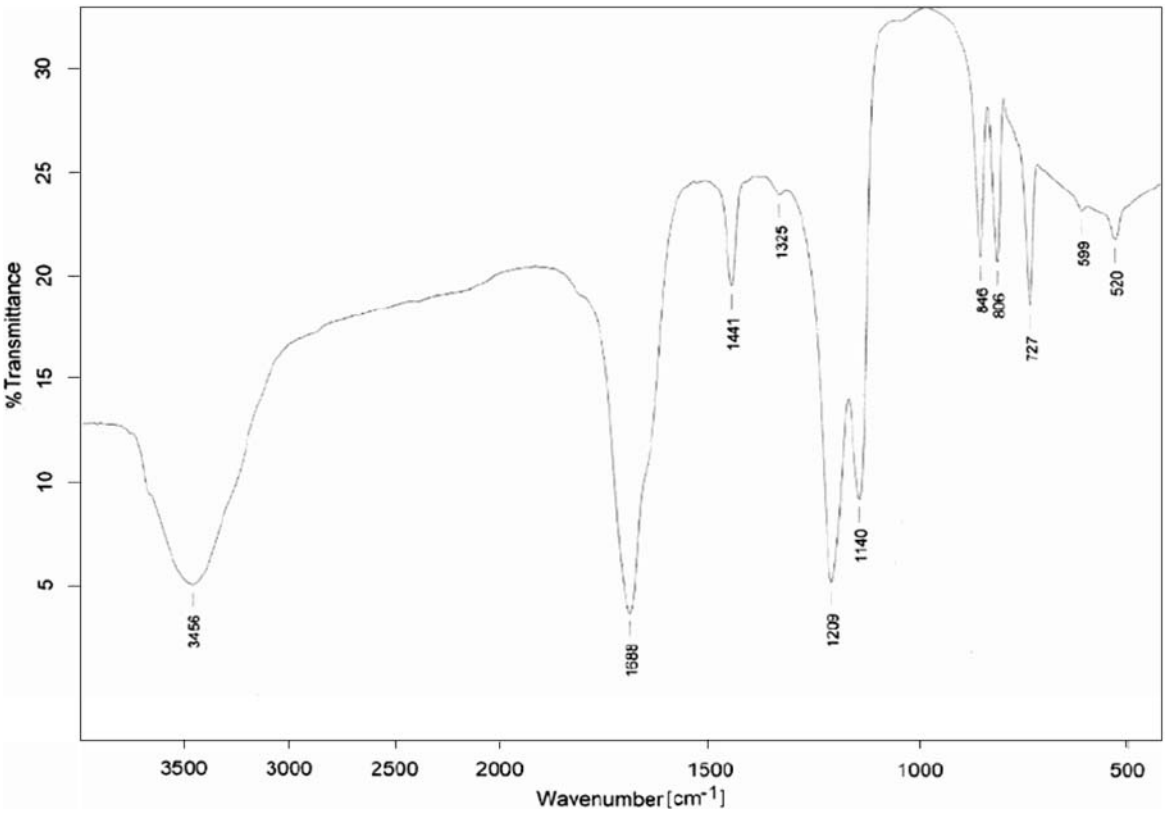

Fig. 2. IR spectrum (in KBr) of the mannan oligosaccharides.

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