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Retrograded maize starch used as a medium to enrich *Monascus* from the air in winter

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ABSTRACT

Red pigments extracted from fungus *Monascus* are used for food coloration in China. Wild-growing *Monascus* spores are usually enriched in the yeast and mold media in the air, but those media are also favorable for yeast and bacteria. In the paper, *Monascus* species have grown in retrograded maize starch lain in air outdoors in winter, molds, yeast or bacteria colonies have been absent. Then a medium of the retrograded maize starch for enriching *Monascus* in the air is explored and its physicochemical properties are determined by ordinary camera photos, NMR, SEM spectra and X-ray diffraction. The lamellar structure of frozen retrograded maize starch, whose interlamellar spacing is about 2 μm, provides a favorable condition for *Monascus* spore to germinate and grow.

1. Introduction

For centuries, *Monascus* sp. has been used for the production of red mold rice as a natural food colorant and/or spice in cooking in East Asia [1,2]. Other functional metabolites produced from the *Monascus* species include lovastatin [3], antioxidant compounds such as dimerumic acid [4] and 3-hydoxy-4-methoxy-benzoic acid [5], and some antibacterial activity compounds [6,7]. There are many media to culture and isolate *Monascus*, such as potato dextrose agar (PDA) medium, yeast and mold medium, and solid rice medium, Mizutani medium, potato carrot agar (PCA) medium, sucrose agar medium, etc. [8–15]. In order to inhibit bacterial growth, the chloramphenicol has been added into the medium [8], but those media do not work well when there exist too many yeast and bacteria in samples or there are very few *Monascus* spores in air. Occasionally, some *Monascus* have grown lonely in retrograded maize starch in winter as the retrograded maize starch was lyophilized in the air. Then a new method to enrich *Monascus* in the air is discovered.

2. Materials and methods

2.1. Materials

Maize starch (protein 0.22%, lipid 0.93%, total and apparent amylose content, 27.2% and 24.8%) was offered by Shan Dong Jin-Cheng Limited Company.

2.2. Methods

2.2.1. Preparation of retrograded maize starches

The retrograded maize starch was prepared according to our previous method [16]. Ten grams of maize starches blended with 100 mL distilled water were gelatinized for 20 min at 95 °C by continuous stirring. Gelatinized starches were autoclaved at 120 °C for 30 min and retrograded at 4 °C for 72 h. Then the wet retrograded maize starch was lyophilized in the air in winter when the atmosphere temperature was under zero. The ordinary camera photographs were taken in the sunny day.

2.2.2. NMR

The ^{1}H NMR of the samples has been determined according to the following procedure. A sample of dried starch is put into deuterated water (D_2O) at 1.5–5.0 w/w%. The mixture is heated in a water batch (60 °C) and shaken till clear homogeneity. NMR spectra of these samples were recorded on a Mercury Vx-300 MHz

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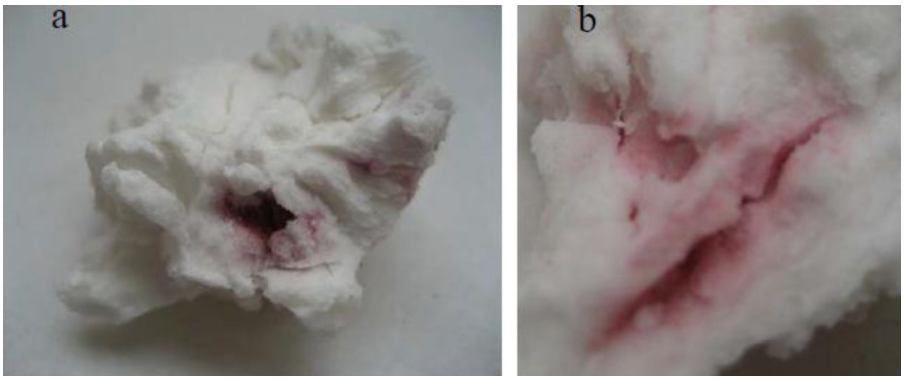

Fig. 1. The photographs of retrograded maize starch in which *Monascus* grew: (a) *Monascus* producing purple pigments; (b) *Monascus* producing red pigments. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

machine (Varian, USA) operating at 300.07 MHz for the ^{1}H nucleus, 75.45 MHz for the ^{13}C nucleus, with a 45.0° pulse and a relaxation delay time of 1.0 s.

2.2.3. Scanning electron microscopy (SEM)

Starches were suspended in absolute methanol, and a drop of the suspension was placed on silver tape, sticky side down, attached to a brass disk and sputter coated with gold/palladium (60/40). The mounted specimens were observed with a scanning electron microscope (JEOL model 1850, Tokyo, Japan).

2.2.4. X-Ray diffraction patterns

The X-ray patterns of the retrograded maize starch was obtained with copper, nickel foil filtered, $K\alpha$ radiation using a diffractometer (D-500 Siemens, Madison, WI, USA) following the method of Jane, Wong, and McPherson [13]. The diffractometer was operated at 27 mA and 50 kV. The scanning region of the diffraction angle (2θ) was from 5 to 60 at 0.04 step size with a count time of 2 s. Retrograded potato starches treated by different methods were equilibrated at 100% relative humidity for 24 h at 25 °C prior to examination.

3. Results and discussion

3.1. Photographs of retrograded maize starch in which Monascus grew

Fig. 1 shows the photographs of retrograded maize starch in which *Monascus* grew. The wet retrograded maize starch turns into sample like styrofoam when it is lyophilized in air outside for 30 d in winter (The atmosphere temperature was under zero). It infers that the water vaporization of retrograded maize starch during freeze-drying brings about the conditions for starch polymerization and inhibition of further retrogradation of starch. The *Monascus* grows from internal of retrograded starch to the outer, implying that the spores of *Monascus* germinate under comparatively hot conditions in the internal of retrograded maize starch and its mycelia can grow at frozen temperature with starch as the only nutrition. The shaggy structure of frozen retrograded maize starch provides *Monascus* with the necessary oxygen for growth. Two kinds of *Monascus* red pigments are excreted in Fig. 1. One is purplish, the other red, which might be produced by two different *Monascus* strains.

Fig. 2 shows the micrographs of *Monascus* growing in retrograded maize starch. The round spores in Fig. 2A and the cross diaphragm in mycelium in Fig. 2B, typical feature for *Monascus*, suggests that the red pigment in retrograded maize starch is surely secreted by *Monascus*. Fig. 2C shows that the mycelium is wrapped with retrograded maize starch, which infers that the strain of such *Monascus* has the ability to secrete amylase to produce glucose by hydrolysis of starch in retrograded maize starch in cold winter. The single nutrition of starch in retrograded maize starch and low temperature in winter limit the growth of most microorganisms. We have tried to isolate and culture the purple and red *Monascus*, but only small colonies grow in mold medium at 32 °C for 30 d. *Monascus* isolated in frozen retrograded maize starch probably adapt to live in a low temperature and their spores germinate very slowly.

3.2. ^{1}H NMR of maize starch and retrograded maize starch in frozen condition

Fig. 3 shows the chemical shifts of ^{1}H NMR of maize starch and retrograded ones in frozen condition. According to literature [17–20], the peaks at 3.3 and 3.6 ppm were attributed to the protons linked to the C-6 carbon of CH_2–O and CH_2–OH and to the C-1 and C-4 carbons of CH–O, respectively. The peaks at 4.0–4.7 ppm were assigned to the protons linked to the C-2, C-3, and C-5 carbons of CH–OH. As for the peak of 4.8 ppm in Fig. 2B, according to Laignel et al. [18], this signal belongs to terminal glucopyranose units. There are nearly no peaks at 5.4 or 5.0 ppm in Fig. 2A and B, being assigned

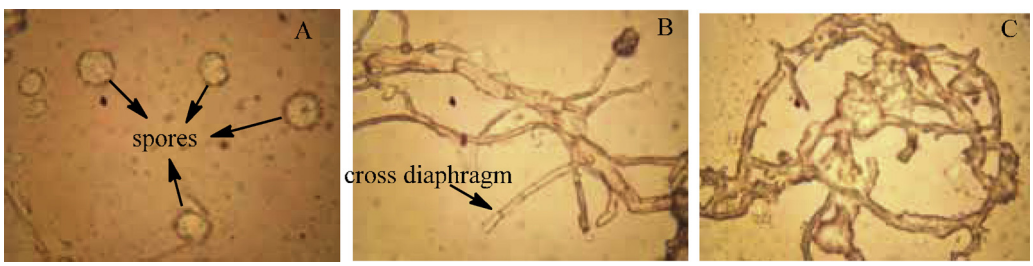

Fig. 2. Micrograph of Monascus spores (A), mycelium (B) and mycelium growing on retrograded maize starch (C) (400×). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)