Natural occurrence of toxigenic fungi and mycotoxins in rice bran

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Abstract

Thirty four samples of rice bran, of which 9 were from raw (untreated) rice (RR) and 25 from parboiled rice (PbR) were collected from commercial rice mills in and around Madras and analysed for storage mycoflora and mycotoxins. Fungi of the Aspergillus flavus group were present in 29 of the 34 samples (8 from RR and 21 from PbR) in quantities ranging from < 1-432 thousand propagules/g, though not always as the dominant mycoflora. Fungal numbers were usually higher in RR than in PbR samples. Five of the 9 RR samples and 6 of the 25 PbR samples were positive for aflatoxins. Among 29 isolates of A. flavus obtained from the bran samples, 16 isolates – 6 from RR bran and 10 from PbR bran – were found to be toxigenic in vitro. Some isolates of A. candidus also seemed to produce aflatoxin and other fluorescent substances.

Introduction

This investigation was taken up as a sequel to an earlier study in this laboratory which indicated that the aleurone layer of the rice grain, removed as bran in milling, harboured the highest population of Aspergillus flavus when compared with other parts of the grain [4]. Rice bran, which had hitherto been used in India only as cattle feed, is lately being used for edible oil extraction also. Hence it was of interest to see if the fungus produced any toxin in this substrate. Rough rice is brought for milling either raw or after parboiling. It was surmised that bran from the latter would be less supportive of fungal growth, for two reasons. Firstly, we had observed that the parboiling process effectively eliminates the grain mycoflora; and although the grain surface becomes sufficiently contaminated with fungal spores once again while it is dried in the open, the grain is usually taken for milling soon after drying, before the fungi have had sufficient time to colonize. Secondly, some of the nutrients in the aleurone layer are said to diffuse into the grain during parboiling, hence the bran may be less nutritive. In order to verify this hypothesis, a comparison was made between samples of bran from untreated rice and parboiled rice.

Materials and methods

Samples

The rice bran samples were collected from commercial rice mills situated in Nazerathpettai
(Chingleput District, 25 km Southwest of Madras) and Guindy (Southern outskirts of Madras) by random sampling method, in 150 g lots in polythene bags precleaned with alcohol, and closed tightly with rubber bands. The samples were from three different sources: (a) collected during milling as the bran came out through the outlet; (b) samples stored in the rice mill up to one week, usually in jute bags; (c) fractions of bran adhering to different parts of machinery, collected by scraping. These three categories are indicated by the same lettering (a, b and c) in the Results, while bran from raw rice and parboiled rice are indicated as RR and PbR respectively.

The samples were brought to the laboratory and their moisture content (MC) determined by hot-air-oven drying method.

Analysis of mycoflora

The mycoflora of the rice bran samples were analysed by the dilution plate method, using 10 g of sample as starter material. Czapek-Dox agar containing 500 g sucrose per litre (50% CDA) was used as the medium, and the plates were incubated at 30 ± 1°C for one week. The Aspergillus species were identified after Raper and Fennell [7], and the Penicillia, after Raper, Thom & Fennell [8]. The quantitative pattern of storage mycoflora was expressed as number per gram. The fungal isolates were maintained on slants of normal Czapek-Dox agar.

Analysis of toxins

Natural occurrence in bran

The standard AOAC method [11] was followed, using acetonitrile: 4% potassium chloride (9:1) for extraction, followed by identification and confirmation through Thin Layer Chromatography (TLC) using chloroform : acetone (95:5) as developing solvent and 15% HCl in ethanol as the spraying reagent. A standard sample of aflatoxin obtained from the National Institute of Public Health and Environmental Hygiene, Bilthoven, the Netherlands, was used for comparison. The simple screening method for aflatoxin by Seitz and Mohr [10] using methanol as an extracting solvent and developing the TLC's in chloroform : acetone (88:12), was also used in addition.

Toxigenicity of fungal isolates

To screen the fungal isolates for production of aflatoxins in vitro, the fungi were cultured in slants of an agar medium containing 2% Yeast Extract and 15% Sucrose (YES agar). Toxins were extracted from the molten agar with chloroform and assayed by TLC using toluene : ethyl acetate : 90% formic acid (6:3:1) solvent system [2].

To test the isolates of A. candidus isolated from bran for possible toxin production, the fungus was cultured on autoclaved rice as well as on YES agar, and extracted by different methods i.e., the AOAC method [11] and the use of solvents like methanol, chloroform, methanol : chloroform (3:5); acetone and acetonitrile. The extract was assayed by TLC using chloroform : acetone (95:5) or toluene : ethyl acetate : 90% formic acid (6:3:1) as the solvent system.

Results

Samples

Totally 34 rice bran samples were collected from 3 different rice mills, of which only 9 were from raw rice (RR) and 25 from parboiled rice (PbR), since paddy was usually brought to these mills in the parboiled form. With regard to source, 3 RR bran samples were collected from each category (a, b and c). Of the PbR samples, 11 were collected during milling (a), 7 from stored material (b) and 7 scraped from the machinery (c) (Table 1). The MC of the samples varied from 8% to 14%.