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Short Report

Detection of Pathogenic Yeasts from Processed Fresh Edible Sea Urchins Sold in a Fish Market

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Abstract

Yeasts of 17 processed fresh edible (raw) sea urchins obtained from seven countries were analyzed. In total, 45 to 7×10^4 colony-forming units (CFU)/g of sea urchins were recovered, and 23 yeast species were identified. Of these species, six pathogenic yeasts (*Candida albicans*, *C. sake*, *Debaryomyces hansenii*, *Pichia anomala*, *Rhodotorula mucilaginosa*, and *Trichosporon mucoides*) were detected from 11 sea urchins (65%). As these yeasts are opportunistic pathogens, infections in healthy individuals normally will not occur, but it should be understood that processed fresh edible sea urchin includes such opportunistic yeast pathogens.

Key words: processed fresh edible sea urchin, pathogenic yeast

INTRODUCTION

Raw fish and shellfish are often contaminated with *Vibrio parahaemolyticus*, which is responsible for numerous cases of food poisoning in Japan. The Food Sanitation Law provides detection values for bacteria in regard to the numbers of viable bacteria, coliform bacteria, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella*, and *V. parahaemolyticus*[1)]. Some fungal species of the genera *Aspergillus*, *Penicillium* and *Fusarium* also cause food poisoning[2, 3)]. Fungal food poisoning can result from ingesting farm products such as peanuts, corn, or wheat contaminated with mycotoxins, which are secondary fungal metabolites. Fungal food poisoning can induce chronic symptoms such as cancer, while bacterial food poisoning induces acute symptoms such as diarrhea and fever. Although no instance of fungal food poisoning resulting from the ingestion of raw fish or shellfish has been reported, it is important from the perspective of industrial hygiene to understand the fungi that contaminate these products.

In this study, we found that processed fresh edible sea urchins sold in a fish market included various pathogenic yeasts.

MATERIALS AND METHODS

Samples

In total, 17 processed fresh edible sea urchins were collected from a distributor in Tsukiji Market (Chuo-ku, Tokyo, Japan) on the days of their arrival between May and November 2006. The samples consisted of five from Japan, three from Russia, three from the United States, three from Canada, one from China, one from Korea, and one from North Korea.

Isolation of yeast from the sample

To isolate the yeasts from the samples, 1 g from each of the 17 processed fresh edible sea urchins was suspended in a 10 m*l*-sterilized physiological saline solution. A separate YM

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agar plate (Difco, Grand Island, NY, USA) containing 50 μg/ml of streptomycin, penicillin, and chloramphenicol was then inoculated with each suspension and incubated for 5 days at 27°C. At least 20 plates were used for isolation. Fifty yeast-like colonies recovered from the agar were randomly selected from each sample (850 total colonies). Another 1 g of a sample was analyzed when 50 colonies were not recovered from 1 g. Genomic DNA was extracted[4], and the D1/D2 26S rDNA of the rRNA gene was sequenced directly from the PCR products with the primer pairs NL-1 (5'-GCATATCAATAAGCGGAGGAAAAG-3') plus NL-4 (5'-GGTCCGTGTTTCAAGACGG-3')[5]. The PCR products were sequenced with an ABI 310 DNA sequencer and the BigDye Terminator Cycle Sequencing Ready kit (Perkin–Elmer Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. Strains with a 99% D1/D2 26S rRNA sequence similarity were defined as conspecific[6]. The sequence data were analyzed by the National Center for Biotechnology Information (NCBI; Bethesda, MD, USA) BLAST system (http://www.ncbi.nlm.nih.gov/BLAST/).

RESULTS AND DISCUSSION

Yeasts were recovered from all of the samples and ranged from 45 to 7×10^4 colony-forming units (CFU)/g of processed fresh edible sea urchin. The 12 overseas processed fresh edible sea urchins were all within the range of 45 to 7×10^4, while the 5 Japanese processed fresh edible sea urchins had almost uniform numbers of yeasts at 2.5×10^3 to 4×10^3. The rDNA sequences of the 850 strains recovered from the 17 processed fresh edible sea urchins were determined, and 686 were identified as known yeasts by the BLAST search. We found 12 ascomycetous yeasts (*Candida*, *Debaryomyces*, *Metschnikowia*, and *Pichia*) and 11 basidiomycetous yeasts (*Cryptococcus*, *Rhodosporidium*, *Rhodotorula*, *Sporobolomyces*, and *Trichosporon*) (Table 1). However, 164 yeast strains could not be identified as known species. Of them, 125 and 39 strains showed identical sequences. According to the rDNA sequence analysis, they were considered to be two new species that belong to the genera *Candida* (isolated from three products of Japan, DDBJ accession number; AB294180), and *Rhodotorula* (isolated from one

Table 1. Yeasts identified from 17 processed fresh edible sea urchins

Country of origin	Japan					China	North Korea	Korea	Russia			Canada			United States		
Sample number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
CFU/g	4×10^3	3×10^3	4×10^3	2.5×10^3	3×10^3	8×10^2	7×10^4	1×10^4	6×10^3	6×10^3	4×10^3	45	80	6×10^2	1×10^2	2×10^2	5×10^2
Candida albicans				3													
Candida austromariana							42										
Candida galli								10									
Candida norvegica									6								
Candida oleophila			41														
Candida saitoana								21									
Candida sake	34						8	19	44								8
Candida sp.				47	32					46							
Cryptococcus festucosus												45					
Cryptococcus longus			9														
Cryptococcus musci		43															
Cryptococcus ramirezgomezianus															11		
Cryptococcus surugaensis	3																
Debaryomyces hansenii						50							50	43			
Metschnikowia bicuspidata											29						
Pichia anomala					18												
Pichia onychis										4							
Rhodosporidium sphaerocarpum											21			7			
Rhodotorula creatinivora												5					
Rhodotorula mucilaginosa	9	7														50	
Rhodotorula sp.															39		
Sporobolomyces patagonicus																	42
Trichosporon mucoides	4																
Number of strains identified	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50

product of USA, DDBJ accession number; AB294181).

Of the 17 samples, pathogenic yeasts isolated from patients were detected in 11 samples (65%). They were *Candida albicans*, *Candida sake*, *Debaryomyces hansenii*, *Pichia anomala*, *Rhodotorula mucilaginosa*, and *Trichosporon mucoides*.

Fresh edible seafood is labeled with an expiration date, and the number of bacteria that it contains is defined by law. For processed fresh edible sea urchins, these products are judged to be "inappropriate for food" when more than 10^6/g general viable bacteria or 3×10^3/g coliform bacteria are detected[7]. The number of viable fungi is not stated, but fungi also affect the quality of these seafood products. We found several pathogenic yeasts during our analysis of the fungi of processed fresh edible sea urchins. Five of the six pathogenic yeasts were rare pathogens, with the exception of *C. albicans*.

D. hansenii has been described as an agent of catheter-related fungemia[8, 9]. This species appears to be susceptible to amphotericin B and voriconazole, but is capable of expressing high-level resistance to flucytosine and fluconazole[10]. *P. anomala* causes fungemia[11-13]. Patients receiving fluconazole treatment for *P. anomala* show good clinical outcomes[14], but treatment failures may occur in cases of breakthrough fungemias in immunocompromised patients receiving prophylaxis with fluconazole[15]. In addition, *P. anomala* has shown low susceptibility to itraconazole[16]. *Rhodotorula mucilaginosa* has been found to cause fungemia in immunocompromised patients and has shown resistance to fluconazole, itraconazole, and voriconazole[17]. Fluconazole-resistant *C. sake* is rarely isolated from patients[18]. *Trichosporon mucoides* is described as a causative agent of deep-seated trichosporonosis[19] and a causative antigen of Japanese summer-type hypersensitivity pneumonitis[20].

It is unknown whether these pathogenic fungi were components of the microflora of sea urchins or were the result of contamination from the environment or human contact during the manufacturing process. As these yeasts are opportunistic pathogens, infections in healthy individuals normally will not occur. However, it must be understood that processed fresh edible sea urchin includes opportunistic yeast pathogens.

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