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Effect of quantity of food residues on resistance to desiccation, disinfectants, and UV-C irradiation of spoilage yeasts adhered to a stainless steel surface

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Although *Saccharomyces cerevisiae* and *Debaryomyces hansenii* are important for food fermentation, they are considered as spoilage yeasts in various processed foods. In this study, we investigated the quantity-dependent effects of milk, egg yolk, and orange juice on the survival rates of food-spoilage yeasts when desiccated on a stainless steel surface. The protective effects on yeast cells before and after drying against disinfectants and UV-C irradiation were also investigated. Microbial suspensions (0.1 mL) were placed on a stainless steel dish (50 mmφ). After drying for 2 h at room temperature in the absence of food residue, viable cell counts of *S. cerevisiae* and *D. hansenii* decreased from 6 to <2 and 3–4 log CFU/dish, respectively. Even small quantities of milk (1 μL), egg yolk (0.1 μL), and orange juice (1 μL) per dish of yeast suspension protected the yeast cells from desiccation. Milk and egg yolk also protected the yeasts when treated with 0.02% w/v sodium hypochlorous acid and 0.2% w/v benzalkonium chloride. Yeast cells were protected from UV-C irradiation by milk, egg yolk, and orange juice. These results suggest that small sediments of food increase the resistance of surface adherent spoilage yeasts against desiccation, disinfectants, and UV-C irradiation.

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1. Introduction

Cross-contamination of microorganisms from harvesting and processing environments to final food products may lead to spoilage and quality deterioration during distribution and storage. Among undesirable microorganisms, psychrotrophic Gram-negative bacteria such as *Pseudomonas* has been regarded as a contaminant in raw materials and foods stored at low temperature (Caldera et al., 2016; Pinto, Ipplito, & Baruzzi, 2015). However, in case of some foods processed by incomplete pasteurization, acidification, modified atmosphere packaged (MAP) and/or fermented foods, lactic acid bacteria (LAB) and/or yeasts are major contaminants, owing to their tolerance levels (Golomb et al., 2013; Kabisch et al., 2016; Kuda, Koyanagi, Shibata, Takahashi, & Kimura, 2016). *Saccharomyces cerevisiae* and *Debaryomyces hansenii* are regarded as beneficial yeasts for production of various brewed and fermented foods (Padilla, Manzanares, & Belloch, 2014; Rogers, Veatch, Covey, Staton, & Bochman, 2016). However, these yeasts contaminate various processed foods and lead to undesirable ethanol and gas productions and as a consequence, add other flavours and colour (Martorell, Querol, & Fernández-Espinar, 2005; Westall & Filtenborg, 1998).

Biofilm formation on surfaces is a potential route of contamination for pathogenic and spoilage microorganisms in the food-processing industry (Jiménez-Pichardo et al., 2016; Simões, Simões, & Vieirab, 2010) and domestic environment (Haysom & Sharp, 2005). When food-contaminating agents such as *Salmonella*, *Staphylococcus aureus*, and *Listeria monocytogenes* were dried in the presence of nutrient-rich food residues, they exhibited resistance to desiccation, disinfection by surfactants such as benzalkonium chloride (BC), and irradiation with 254-nm ultraviolet (UV-C) light (Kuda et al., 2012; Li, Kuda, & Yano, 2014). In addition, it has been reported that food residues protect LAB cross-contamination against desiccation (Kuda, Nakano, Takahashi, & Kimura, 2016). However, the protective effects on spoilage yeasts against stresses such as desiccation, disinfection, or UV-C radiation remain unclear.

In this study, we investigated the quantity-dependent effects of milk, egg, and orange juice on the survival rates of the food-

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spoilage yeasts *S. cerevisiae* and *D. hansenii* when desiccated on a stainless steel surface. Moreover, protective effects of food residues on yeast cells before and after drying against disinfectants and UV-C irradiation were investigated. Effects of food residues on yeast biofilm formation were also determined.

2. Materials and methods

2.1. Yeast culture and food material

In this study, *S. cerevisiae* Misaki-1 (Accession No. LC093859) and *D. hansenii* NBRC 0083 were employed. To produce cultures, a loop of yeast cells from Potato Dextrose Agar (PDA: Nissui Pharmaceutical, Tokyo, Japan) for *S. cerevisiae* and PDA containing 3%w/v NaCl for *D. hansenii* were inoculated into 20 mL of Glucose Peptone Yeast broth (GPY: 10 g glucose, 10 g peptone and 5 g yeast extract per 1000 mL distilled water [DW]) and the 3% NaCl added GPY broth, respectively. Then the inoculated cultures were incubated at 30 °C for 24 h (*S. cerevisiae*) or 48 h (*D. hansenii*) with shaking (120 rpm). The cultures reached stationary phase.

Dairy (cow) milk, soymilk, fresh raw egg (whole-egg, egg-yolk, egg-white) and five juice products made from orange, grape, apple, grape-fruit and tomato were purchased from a retail shop in Tokyo. Among the food samples, dairy milk, egg-yolk and orange juice samples were serial-diluted by 10-fold increments in distilled water (DW) to a maximum dilution of 10^{-4} (10000-fold).

2.2. Adhesion of the yeast cells to stainless surface

Fifty-millimeter diameter SUS304 stainless steel dishes were purchased from As One (Osaka, Japan) and used as experimental surfaces. Prior to use, in order to equalize the effect of the surface conditions on the survival cell count, the steel dishes were ultrasonicated twice for 15 min with neutral detergent, brushed for 60 s with tap water, washed with DW and autoclaved at 121 °C for 15 min.

The yeast cells were placed in the dish and attached as previously reported (Kuda, Shibata, Takahashi, & Kimura, 2015), with slight modifications. Briefly, yeast cells in the GPY broth culture were washed by centrifugation at 5000 × g for 5 min at 4 °C, and re-suspended in DW. This washing process was repeated twice. The cells were finally re-suspended in 2 mL of DW. Then 0.05 mL of this cell suspension was added to 1 mL of the diluted food samples or DW (control) that the final cell concentration was approximately 8–9 log cfu/mL.

A yeast suspension (0.1 mL) was spread over an area of about 10 mm diameter in the center of the dish ($n = 3$) and dried for 120 min at room temperature (20–24 °C) in a bio-safety cabinet (Class IIA; Airtech Japan; Tokyo, Japan) with ventilation. After drying, the adhered cells were detached by rubbing for 30 s using a sterile cotton swab and re-suspended in 3 mL PBS containing 0.1% agar (PBS-A). The detached cell suspension (30 mL) was immediately diluted with PBS-A, spread on PDA or 3% NaCl-PDA and incubated at 30 °C for 24 h or 48 h. The viable count of cell suspension with DW on the steel surface before drying was also determined.

2.3. Microscopic observation

For observation, 3 μL of the yeast cell and food residue suspensions, prepared as described, were adhered by semi-drying onto a cover slip. The adhered cells and food residues were observed using low-vacuum table-top scanning electron microscope (SEM: TM3030; Hitach-Tschnologies, Tokyo, Japan) in low vacuum and charge reducing mode (Kuda, Nakano, et al., 2016). The samples were prepared without any vapour deposition or staining treatments.

2.4. Disinfectant treatment and enumeration of viable cells on stainless steel dishes

To determine the sanitation effect of the disinfectants, the dried and adhered yeast cells in dairy milk, egg-yolk and orange juice solutions and on the stainless steel surface, prepared as above, were covered with 0.2 mL of 0.02% w/v sodium hypochlorous acid (HC; Wako Pure Chemical, Osaka, Japan) or 0.2% w/v benzalkonium chloride (BC; Wako Pure Chemical) solutions, which is the highest recommended concentration indicated by the makers. After 10 min at room temperature, 3 mL of TSB was added. Then, cell viability was determined as above.

2.5. UV-C treatment and enumeration of viable cells on dishes

To determine the sanitation effect of the UV-C treatment, the yeast cells on the stainless steel surface before (wet) and after the drying, prepared as above, were irradiated UV-C (254 nm) using a UV-C light (CSL-15C; Cosmo Bio, Tokyo, Japan) for 1 min (Kuda et al., 2012). The distance between stainless steel surface and the light was set as 22 cm and the UV strength at the surface was 0.162 ± 0.016 mW/cm^2. After the irradiation, 3 mL of TSB was added immediately onto the surface to re-collect cells. Then, cell viability was determined as above.

2.6. Biofilm formation of the yeast cells with the food residues on polystyrene surface

Biofilm assay was determined with method of O'Toole (2011), modified slightly. The serial food residue dilutions with or without yeast cells (0.2 mL) were put into a 96 well polystyrene microplate (CLS3595; Sigma-Aldrich, St. Louis, MO). After incubation at 30 °C for 7 days in a polyethylene pouch to avoid the desiccation, the suspensions in the microplate were removed. Then, the microplate was washed by filling each three times with DW. The remained food residue and the cells were stained with 0.1 mL/well of 0.2%w/v Victoria blue solution (Nissui Pharmaceutical) for 5 min. After washing with DW three times, the remained pigment was dissolved with 0.2 mL of 99.5%v/v ethanol (Wako Pure Chemical). Optical density at 617 nm was determined by a grating microplate reader (SH-1000 Lab; Corona Electric, Hitachinaka; Ibaraki, Japan).

2.7. Statistical analysis

The cell viability data was expressed as the mean and SEM of log cfu/dish ($n = 3$). Statistical analysis was performed using the software EXCEL Statistic 6.0 (Esumi Co. Ltd. Tokyo, Japan). One-way ANOVA was used to assess differences. Then, individual means were compared using Tukey's test. Significant differences were accepted at $p < 0.05$.

3. Results and discussion

3.1. Effect of milk, egg, and juice products on S. cerevisiae dried on the stainless steel surface

In our previous study, it was noted that the sensitivity of logarithmic or early stationary growth-phase bacterial cells to drying was higher than that of stationary growth-phase cells (Kuda, Yano, & Kuda, 2008). It is known that sigma factors produced in the stationary phase, in prokaryotes and eukaryotes, lead to the generation of self-defence proteins that protect the cells against acidic

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