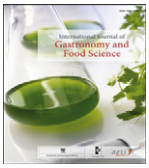

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Defining microbial terroir: The use of native fungi for the study of traditional fermentative processes

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Abstract

In any fermentative process, the impact of the native microbial community is hugely important. The present study examines the far-reaching implications of harvesting and isolating specific native fungi and bacteria to use as inoculum for new forms of traditional techniques. As a chef one not only has the chance to understand their craft on a cellular level, but to connect more deeply to the indigenous life of their environment, their ''microbial terroir.'' In the course of developing *butabushi*, *koji* and *miso*, DNA analysis has been performed throughout to understand the impact of our native microbes and to propagate them in controlled environments. It is a rare moment in an urban environment when a chef can grow anything, and rarer still to be able to connect with terroir. The goal of this project is to create truly indigenous products, through stewardship of our native microorganisms.

Keywords: Microbiology; Terroir; *Katsuobushi*; *Koji*; *Miso*; Fermentation

Introduction

This study began with *Katsuobushi*. A traditional component of *dashi* in Japan, it has been produced for hundreds of years, and imparts an immense amount of umami when used in *dashi* or as garnish (McGee, 2004). Traditional *katsuobushi* is made through a process by which the fish is boiled, smoked, inoculated with a specific mold used throughout Japanese cooking and then undergoes a lengthy aging and drying process. The resulting product is intensely flavored, and contains high levels of the amino acids responsible for umami (McGee, 2004).

With *katsuobushi* as the inspiration, an idea formed to begin work on developing a similar product using pork, termed *butabushi*. As the technique and product developed, a good deal of fungi grew on its surface, and the question of safety for consumption became hugely important. To answer this question, samples were sent to microbiologists at Harvard University who did DNA sequencing and analysis. Through the process of sequencing and assembling the phylogeny, it was found that fungi inherent to our environment were present that may have had an impact on the end product. To determine the most replicable method for creating the *butabushi*, testing began using un-inoculated pork as the control, and inoculating other samples with isolates of both our ''in-house'' mold, as well as a traditional Japanese mold used for a number of fermented products, *Aspergillus orzyae*. Strains of this fungal genus *Aspergillus* are used in *sake*, *soy* and *miso* production (Wada et al., 1991; Steinkraus, 1996).

This recognition of native fungi and microbial terroir has been the catalyst for examining the complex subject that is the focus of this study. While continuing work on the *butabushi*, we also began testing other traditional fermented products. The idea began with a hope that the rice used as a drying element in our *butabushi* would be a viable option for making *koji*, and ultimately miso. *Koji* is a fermented grain,

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traditionally rice or barley, inoculated with *A. orzyae* and left to colonize (Katz, 2010). It is then used in a number of fermented products as its enzymatic production facilitates a starch to sugar conversion, as well as peptide and amino acid development in starchy, protein rich environments (Shurtleff and Aoyagi, 2001). With this in mind, two simultaneous paths emerged: one to understand and replicate traditional fermentations, and also to sample and harvest native microorganisms from our environment.

The microbiological implications in modern cooking draws an obvious comparison to the process by which hydrocolloids became common in kitchens around the world. The need to understand on a cellular level what is being done in kitchens is of more vital importance now than ever before. Around the world cooks are engaged in fermentative processes that they do not fully understand, but with this comes the opportunity to develop a new lexicon and set of techniques to share. The goal in each part of this study is to examine the traditional fermentative processes thoroughly enough to facilitate the use of native fungi exclusively. Additional experiments have been conducted in each area to try and determine the efficacy of fungal colonization, consumption and enzymatic production.

Materials and methods

Materials

For the *butabushi* preparation the following ingredients were used: pork tenderloin, hanger steak and pork loin were purchased from Pat La Frieda Meats (USA). Activa RM (transglutaminase) from Ajinimoto Brand (USA). *A. orzyae* molds were obtained by Gem Cultures (USA) and Vision Brewing (AUS). Sushi Rice and Wood Chips were obtained from NY Mutual Trading (USA) and Natural Grilling and Fuel Company (USA), respectively.

For the *koji* preparation, Hanami short grain Japanese rice were obtained by NY Mutual Trading (USA). *A. orzyae* strains were also used in both procedures and were purchased by Gem Cultures (USA) and Vision Brewing (AUS). For the miso elaboration, soybeans were purchased from Laura Soybeans (USA).

Methods

Butabushi preparation

For Pork and Beef, connective tissue and excess fat were removed. All samples were then steamed in Combi-Oven for 30 min at 100 °C. Following cooking, samples were transferred to a smoker, and smoked for 6 h with hickory and apple chips. Afterwards samples were placed in dehydrator on racks for 8 h at 40 °C. Both inoculated and un-inoculated pork were placed on raw sushi rice in foil-lined containers and sealed. Inoculum used were both fresh and freeze dried isolates of mold native to our environment, as well as *A. orzyae*. Fungal isolates were made into separate slurries with warm water, and brushed onto surface of pork and beef. Meat was left to age for 3 months in a dark chamber at 30–33 °C. When checked at 2 week intervals, mold growth was scraped with the back of a knife, or rubbed with gloved hands. Depending on size/moisture content of pork, at the three-month mark removed from rice, scraped fully and sealed in vacuum bags (Fig. 1).

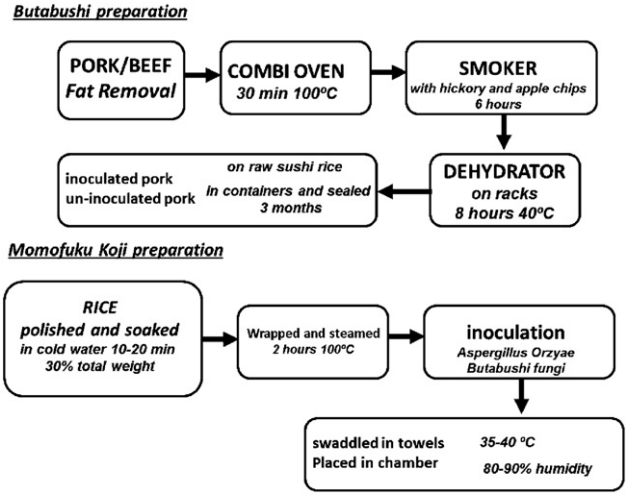

Fig. 1. Schematic diagram of butabushi and koji preparation.

Momofuku koji preparation

Raw sushi rice was polished thoroughly with intentional kernel fracture and then soaked in cold water 10–20 min until 30% of total weight was absorbed. Rice was weighed at each stage: raw, soaked and cooked. It was then wrapped in muslin, and steamed at 100 °C for 2 h, flipping packet at 30 min intervals. Next it was removed from steamer and left to sit until temperature reached 40–45 °C, then inoculated with varying percentages of *A. orzyae* spores, or with isolates from *butabushi* fungi. 1 g of *Aspergillus* spores can inoculate up to 45 kg of cooked rice (Shurtleff and Aoyagi, 2001). A small amount of All Purpose flour was sterilized, and mixed with inoculum to aid in even dispersal. Inoculated rice was swaddled in towels and placed in chamber with constant temperature between 35 and 40 °C and optimum humidity range of 80–90%. Rice was stirred at 24 h mark, clumps broken up, and when even colonization was apparent, rice was split into furrows to maintain temperature as mold generates heat during later colonization phase. At 50 h mark, rice was removed from chamber; and is ready for immediate use, dehydration or refrigeration for storage.

Momofuku miso

Soybeans were rinsed and soaked overnight at room temperature. Hulls that float to surface were discarded. Beans were then cooked in water for 3–4 h at 80 °C. Cooking liquid was strained and reserved. Beans were then pureed until smooth with addition of 1/3 of their weight of cooking liquid. This mixture is left to cool to 45 °C, at this point mixed by hand with varying percentages of *koji* and salt. This mixture was then formed into balls and packed into a sterilized