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# Wild toxicity, cultivated safety: aflatoxin and kōji classification as knowledge infrastructure

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## ABSTRACT

In 1960, the trajectory of aflatoxin as one of the earliest and best studied cases of a naturally occurring carcinogen in food intersected with the trajectory of an industrial microbe known in the Japanese vernacular as kōji, used for centuries in Japan to make sake, soy sauce, and miso. Over about two decades, the aflatoxin crisis spurred the emergence of a new evolutionary narrative of kōji, *Aspergillus oryzae*, as a domesticated, non-toxigenic species unique to the Japanese brewery that was clearly distinguishable from its wild, commonly found in nature, and aflatoxin-producing close relative, *Aspergillus flavus*. It was a shift that came hand-in-hand with the reconstruction of kōji classification. This essay examines the challenges of microbial classification after 1960. By asking how mycologists made a scientific narrative that originated in the interests of Japanese national industries convincing internationally, it explores the knowledge infrastructure that underlay both manufacturing issues and knowledge in microbiology.

## KEYWORDS

Biotechnology;  
microbiology; classification;  
traditional foods;  
environmental health

## Introduction

Kōji, *Aspergillus oryzae*, enzyme maker extraordinaire, and gastronomic mold responsible for the brewing of sake, soy sauce, and miso, is today the ‘national fungus’ (*kokkin*) of Japan.<sup>1</sup> Its identity, however, was not always so stable. This essay focuses on a moment of crisis beginning in 1960 that redefined kōji identity, a moment when the perception of this mold as a helpful, edible, living worker changed. In 1960, over 100,000 poultry in England died from an unknown disease named Turkey X. Investigators linked the disease to the peanut meal in the turkeys’ industrial feed, and identified the cause as a mycotoxin produced by the fungus *Aspergillus flavus*, secreted while the mold grew on nuts and grains during storage. Named ‘aflatoxin’, the toxin soon emerged as one of the most powerful carcinogens known in food, which caused proliferation of bile duct epithelial cells in the liver when consumed by rodents, fish, birds, or primates. Kōji – which was closely related to *A. flavus* and taxonomically part of the *Aspergillus flavus-oryzae* group at the time – came to be viewed in a new light, as a hazard and potential source of environmental contamination in the body.<sup>2</sup>

Fermentation science (*hakkōgaku*) differentiates between what is a desired, valuable, useful metabolic product of a microbe, and what is an undesired, harmful, or simply wasteful and useless product, distinctions which in turn are used to understand the physiological nature of the microbes themselves. As the field began to develop in early twentieth-century Japan, scientists and brewing technicians valued different kinds of molds with respect to their usefulness (*yūeki*) and harmfulness (*yūgai*) in brewing, by collecting, preserving, and classifying microbes on the basis of their morphological and physiological properties, paying particular attention to the properties affecting mass consumption.<sup>3</sup> In the wake of the 1960 aflatoxin crisis that threw the fundamental quality of *kōji* as an edible good into question, and cast its perception as a hazard, Japanese fermentation scientists faced the thorny task of revaluing the fungus economically and culturally. Over the following decades, they constructed a new evolutionary narrative in which Japanese industries had created, through centuries of use and selection, a domesticated, non-toxic species *Aspergillus oryzae* that could *only* be found in the Japanese brewery, and that was different than its closely related – but wild, commonly found in nature, and sometimes potently toxigenic – relative *Aspergillus flavus*.<sup>4</sup>

At the heart of this story of crisis response is the science of microbial taxonomy and systematics.<sup>5</sup> Between the 1960s and 1980s, a scientific narrative about *kōji*'s uniqueness that originated in the interests of Japanese national industries was made convincing internationally.<sup>6</sup> How this happened is especially revealing of the social and material infrastructures underpinning scientific knowledge, particularly infrastructures of microbial classification. Due to their material basis, *kōji* classification systems are necessarily a result of 'partial perspective'.<sup>7</sup> The question, then, is how such perspectives are made persuasive more broadly. This article builds on the work of other historians of life sciences that has focused on the role of technological infrastructure in generating new scientific knowledge. Here, I take analytic inspiration from the historiography of technology to foreground and explore instead the role of infrastructure in stabilizing and maintaining existent microbial knowledge.<sup>8</sup>

The first part of this article looks at how the 1960 aflatoxin scare changed the problem landscapes in which *kōji* identity existed, as the perception of *kōji* shifted from helpful worker to subtle poisoner. The Turkey X outbreak came at a time when medical thinking on cancer began to make new connections between daily life and chronic exposure to low levels of carcinogenic substances over time, through diet.<sup>9</sup> *Kōji* fell within the scope of the connection between familiar foods and carcinogenicity. The second part of the article examines infrastructures of *kōji* classification after 1960, and traces the elements that Japanese scientists mobilized in order to rebuild and maintain classificatory systems of the *kōji* mold. Aflatoxin as a hazard changed the scientific characterization of a mold whose biochemistry and physiology had already been well studied in relation to food processing, brewing, and enzyme production. Within that change, classification practices were key, and were central to characterizing the nature of the new hazard.

A new focus in classification emerged that sought to understand varieties not only in relation to classic characters useful in brewing, but in relation specifically to aflatoxin production. Which molds were toxigenic, and which molds were non-toxigenic? Classification of microbes is a field where the question of the stabilization of knowledge infrastructure is especially revealing because of the peculiar challenges of microbial classification for disciplinary biology, including the asexual nature of microbial

reproduction, as well as microorganisms' mutability and pleomorphy. In the period under study, the 1960s to 1980s, classification work relied primarily on phenotypic analysis: examining and comparing the morphological, metabolic, physiological, and chemical characteristics of strains. These challenges of microbial classification were compounded by the problem of relations between industry and science, and between national and international systems, which had a material basis.

All of the challenges in stabilizing infrastructures of microbial knowledge bear on the question that this article explores: how was a scientific narrative that originated in the interests of Japanese national industries *made convincing internationally*? In this evolutionary narrative, Japanese industries had created – through centuries of use and selection – a domesticated, non-toxic species, *A. oryzae*, that could *only* be found in the Japanese brewery, and that was different than its sometimes potentially toxigenic wild relative, *A. flavus*. In this shift, the 'machinery of life' is apparent in two forms: the industrial context in which scientists worked when addressing the microbial classification problem, and the infrastructure of microbial classification that underlay both manufacturing issues and knowledge in microbiology.<sup>10</sup> Resonant with scholarship in the history of life sciences, I consider the continuing significance of local practices amid standardization of tools.<sup>11</sup> At the same time, I draw on approaches from the history of technology to examine the process of how users shape and change international infrastructure as they respond to problems at the national level.

## The problem of aflatoxin

Aflatoxin, a potent carcinogen, first surfaced as a hazard to consumers and as a variable in determining the futures of industrial food manufacturers in 1960. It showed up dramatically in the context of industrial agriculture, particularly the processing of crops for animal feed, with a series of outbreaks in farm animals. In farms in the south and east of England, large numbers of turkeys died over a few months. Investigating scientists named the disease 'Turkey X' because the origin of the disease was initially unknown, although it rapidly became clear that a mold growing on the peanut meal that had sourced the turkey feed made by one feed company was responsible. The mold produced a toxin that resulted in liver lesions when fed to young poultry. In the early investigation of this outbreak, day-old ducklings became the biological assay for the toxin, while a chemical assay was developed that used paper chromatography. As scientists subsequently linked other liver cancer outbreaks in American trout as well as Kenyan and Ugandan ducklings to the same toxin, they realized that the toxin affected a wide range of animals other than birds and could potentially affect humans. Moreover, experiments found that dairy animals fed the mycotoxin excreted a variant of the toxin in their milk (M1) that had similar effects on ducklings. The toxin was named 'aflatoxin' after the mold responsible for the Turkey X outbreak, *Aspergillus flavus* Link ex Fries. In this way, aflatoxin, a metabolic product of a mold, *Aspergillus flavus*, became known as a potent carcinogen.<sup>12</sup>

The Turkey X outbreak coincided with a newly rising consciousness that components of *food* could cause cancer.<sup>13</sup> Anxiety over environmental factors in cancer had originated partly in investigations on radiation in the midst of post-World War Two debates over the environmental effects of nuclear testing, and in 1958, an amendment to the U.S.

Food, Drug and Cosmetic Act set a standard for regulating carcinogenic additives in food. Initially, anxiety over carcinogens in food had focused on synthetic additives. However, the discovery of aflatoxin as a hazard signaled the beginning of a growing awareness of carcinogens of natural origin. In 1969, a U.S. Department of Agriculture researcher wrote the following regarding precautionary measures to be undertaken in the laboratory: 'Although nothing is yet known as to the effect of the aflatoxins on man, because of the extreme toxicity and carcinogenicity of these aflatoxins to a number of warm-blooded animals extreme care should be taken in the handling of materials contaminated with these potent toxins ... It would be well to exercise the same precautions in handling the aflatoxins as those commonly used in the handling of radioactive materials'.<sup>14</sup>

The anxiety about aflatoxin as it affected farm animals and laboratory animals immediately translated into concerns about human health, and experts outlined possible routes of exposure for humans. As they did, they came to approach aflatoxin as a problem of global environmental health. U.S.-based researchers surveyed the aflatoxin literature in a book-length review published in 1969. There they described *Aspergillus flavus* as a 'storage fungus', invading stored nuts and grains after harvest, and which might sometimes include aflatoxin-producing strains. Studies in the 1960s had found aflatoxin in peanut meal from at least 13 peanut-producing countries, in peanut butter in the United States, and in 'many agricultural commodities from various parts of the world, including cassava, corn, cottonseed meal ... peas, rice, soybeans, and wheat'.<sup>15</sup> These experts believed that warm and humid climates could combine with a lack of sufficient preventive measures in food processing and storage to heighten the problem of the fungus. They noted that 'toxigenic strains can grow and produce aflatoxins on most if not all products of agriculture'. This was because 'one must consider that the mold *Aspergillus flavus* Link is ubiquitous'.<sup>16</sup>

Convinced that 'the cause of most cancers must be in the environment' rather than genetic, researchers in the United States began to link aflatoxin risk to everyday dietary patterns, looking at different regions of the world.<sup>17</sup> For example, scientists working at the U.S. Food and Drug Administration argued for connections between the global geographical distribution of hepatoma (liver cancer) and local dietary patterns involving ingestion of grains that might be contaminated with mold toxins, noting 'the higher incidence of this disease in parts of Africa south of the Sahara, Southeast Asia, Japan and southern India'.<sup>18</sup> Inviting a direct link between eating traditional foods fermented by mold action and being at risk of ingesting mold toxins, they stated: 'The incidence of hepatoma in Indonesia is among the highest in the world. A recent interesting observation concerns the consumption of fermented peanut presscake, a very popular food in Western Java. This material supplies the protein source in the diet and varies in aflatoxin content from 0.1 to 16 ppm [parts per million]'.<sup>19</sup> Elsewhere, the essay noted how 'in certain areas of the world, molds have been used to convert rice, soya, and fish into foods of unusual texture and flavors ... In general, the possibility that mycotoxin contamination of food is endangering the health of human beings is of great concern in the Far East as well as in Africa'.<sup>20</sup>

The problem for mycologists was that *A. flavus* microbes were not as well delineated taxonomically as the new aflatoxin crisis seemed to demand. The authority in classification at the time was Raper and Fennell's 1965 publication, *The Genus Aspergillus*. Both

there and among mycologists, *A. flavus* was the collective name for a group of closely associated microbial isolates, as well as the name of a species. When used as a group name, it included strains from, most notably, the species *A. oryzae* – or *kōji*, the mold used in the traditional Japanese brewing industries – as well as, among others, *A. parasiticus*, *A. tamarii*, *A. flavus* var. *columnaris*, *A. parasiticus* var. *globosus*, and of course *A. flavus*. As one author in the aforementioned 1969 review put it, ‘confusion exists as to the identity of the *Aspergillus* species reported in the literature’.<sup>21</sup> The association of the brewing mold, *kōji*, with the *A. flavus* group immediately raised the question of whether *kōji*, too, produced aflatoxin, and whether *kōji* was subtly poisoning consumers who were ingesting quantities of aflatoxin in sake, soy sauce, miso, and other *kōji*-brewed goods over their lifetimes.

Studies of aflatoxin along with its producer *A. flavus* were among the earliest studies on natural, rather than synthetic, carcinogens in foods. Prior to the aflatoxin crisis of 1960, Japanese experts had studied natural fungal hazards before, including *Penicillium* molds that yellowed rice and produced various toxic metabolites as they grew.<sup>22</sup> But after the Turkey X outbreak of 1960, fungi and their metabolites, especially aflatoxin, gained a much higher profile as agents of cancer. Later, in the 1970s, the scientific link between natural foods and cancer would strengthen. Japanese medical scientists took a leading role in this work. Their investigation of everyday foods and cooking, using the Ames test for mutagenicity, highlighted the carcinogenicity of compounds in grilled fish and meats, and connected the high incidence of stomach cancer in Japan to the consumption of nitrosamines in fermented foods.<sup>23</sup>

Yet, consumer movements protesting food contaminants in Japan, which gathered on a large scale in the 1960s and grew through the 1970s, continued to focus on synthetic additives and the petrochemical industry. Among the most notorious food poisoning cases were the Morinaga milk poisoning case of 1955, where contamination by arsenic was the result of a milk additive, and the Kanemi rice-oil episode of 1968, where the cooking oil was found to contain polychlorinated biphenyls (PCBs).<sup>24</sup> In the 1970s, monosodium glutamate (MSG) sales fell in Japan for the first time in history, following anxieties that the flavoring product was made from petroleum and contained toxic additives. In response, the main manufacturer Ajinomoto reoriented their marketing, altering the phrase used to describe the flavor enhancer from ‘chemical seasoning’ (*kagaku chōmiryō*) to the more natural-sounding ‘umami seasoning’.<sup>25</sup> All this occurred against the backdrop of slow but landmark legal victories in the ‘Big Four’ (as they were known in Japan) pollution cases in the early 1970s, when victims won the right to compensation for mercury poisoning in Minamata as well as Niigata, cadmium poisoning in Toyama, and air pollution in Yokkaichi.<sup>26</sup> One result of the heightening environmental anxieties directed against the chemical industries in Japan was the growth of a culture of nostalgia centered on rural, ‘native-place’ (*furusato*), traditional food. Another was a robust organic farming industry buttressed by consumer cooperatives from the 1970s onward. Both trends were also reactions against the spread of a standardized, urban, middle-class mass consumer culture spurred on by Japan’s high-speed economic growth from the 1950s to the 1970s.<sup>27</sup>

At that particular historical moment, the discovery of aflatoxin in 1960 and its association with the brewing mold, *kōji*, should have been well poised to threaten Japanese consumers’ turn to natural, nostalgic, traditional food. Such a reversal in

consumer culture would have paralleled the shift that was taking place in biomedical research, from a focus solely on synthetic carcinogens to natural carcinogens as well. Instead, sake, soy sauce, miso, and other brewed products that relied on *kōji* became valued culturally as culinary symbols of ‘native-place’ traditions that spanned millennia, at the same time as they continued to yield massive economic returns in the food industry. According to leading Japanese fermentation scientist Sakaguchi Kin’ichirō, around 1972, fermented goods accounted for close to 2% of Japan’s gross national product.<sup>28</sup> In the wake of the aflatoxin crisis, the fact that *kōji* identity folded easily into the national celebration of domestically produced foods, which were lauded for their ‘high quality, safety and perfection of form’ in contrast to imported foods, cannot be taken for granted.<sup>29</sup> Rather, it was a result of Japanese microbiologists’ efforts over the period of about a decade to reclassify the yellow-green *kōji* microbe as something quite different than its wild, commonly found in nature, and sometimes potently toxigenic yellow-green relative, *Aspergillus flavus*.

### Infrastructures of classification

If the root of the aflatoxin hazard was the mold itself, how could the hazard be identified, or the presence or absence of the toxigenic molds known? By the 1960s, scientists certainly had an awareness that molds could make ordinary foods toxic, and a growing awareness that everyday foods could be carcinogenic. Japanese medical researchers played a key role in pioneering some of this work. But in the case of *A. oryzae* – one of the species classified under the *A. flavus* group – here was a fungus that had been specifically preserved, selected, and cultivated as mold starters in Japanese breweries for centuries. Twentieth-century mold starter companies increasingly used sophisticated technologies, from pure culture to induced mutation, in order to enhance the product quality of *A. oryzae* and create mold starter spores with an edge over their competitors.<sup>30</sup> To suggest that this celebrated, deliberately cultivated microbe could be poisonous due to aflatoxin production, by association with the *A. flavus* group, immediately raised a problem for the Japanese brewing industries – from sake to soy sauce, miso to vinegar.

Aflatoxin as a new hazard changed the conception of the *kōji* mold, which had been well-studied as a beneficent fungus and then had to be defended by the industries concerned in light of this new risk. *Kōji* had already been the subject of intensive taxonomic and biochemical study for decades, but such studies had been carried out with a focus on the characteristics that were useful in brewing, rather than the detrimental aspects of aflatoxin production. Therefore, it was an urgent problem for mycologists in Japan to clarify the relationship between *kōji* varieties and *A. flavus*. By looking at *kōji* work since 1960, we can trace how the perception of risk in food, and fungal classification practices in science, defined and shaped each other.<sup>31</sup>

In the mid-1960s, the question of whether *kōji* preparations contained aflatoxin was open. C. W. Hesseltine and others, working at the Northern Regional Research Laboratory of the U.S. Department of Agriculture (USDA) in Peoria, Illinois, tested 53 strains of *A. oryzae* that were used in food fermentation.<sup>32</sup> Most of the strains were isolated from commercial *kōji* mold starters in Japan, while a handful of strains came from mold preparations in China as well as Chinese black beans in Taiwan. The USDA scientists found that none of the 53 strains produced aflatoxin. At the same time, they



cautioned that the strains they had studied had all come from pure-cultured products, 'and not from poor tane koji or starters used in home fermentations that contain many kinds of molds'. Poor-quality starters, they warned, 'can be expected to be contaminated with *A. flavus*'. Rice, an ideal substrate for aflatoxin production, is used to produce koji'.<sup>33</sup> In Japan, news articles appeared through the late 1960s and beginning of the 1970s reporting that aflatoxin could be found in commonplace foods in home kitchens, including wheat, azuki bean flour, rice exported to Southeast Asia, soba, and pork, as well as homemade miso paste.<sup>34</sup> Yet, no news items appeared attacking the Japanese fermentation industries themselves. The national media suspended doubt for the time being when it came to Japanese commercial brewers of *kōji*, while mycologists worked to reclassify this economically and culturally important mold, and alter the understanding of its relations to *A. flavus*.

Through classification, experts could objectify the scope of the hazard and localize the cause. However, *kōji* classification was an especially difficult question because of the inherent challenges of microbial classification, or the task of dividing the microbial world into species, and elevating species differences above the differences between varieties. Not only did asexual reproduction preclude using reproductive isolation to define a 'species' for microbes. Unlike a plant, where the individual phenotype could be studied easily, for a microorganism the individual organism was a cell. Thus microbial classification practices necessarily relied on characterizing not one cell as an individual, but a cultured colony of clones that could collectively represent an 'individual' strain. Yet among the cells in that colony, one could find widely varying forms, and depending on the culture medium one could also find genetic differences. Moreover, since microbes generally lacked a fossil record, there was little history to supplement information from present microbes beyond culture collections (which did not go back much further than about a hundred years). The variability and sheer mutability of microbes made it difficult to choose reliable species-demarcating characteristics, and the problem could be further complicated by the phenomenon of pleomorphism, or the existence of multiple forms of the same type of microbe at different stages in its life cycle.<sup>35</sup> Microbial classification systems were ultimately defined by the international rules that had gradually been put into place, with significant room for the judgment of the individual researcher, for example regarding where to place the microbial isolates that possessed intermediate characteristics between species.

For *kōji*, the challenges of microbial classification were compounded by the relations between industry and science, and between national and international systems. Japanese industries had developed their own classification systems, based on their own microbial collections within brewing companies. There were also vernacular names in Japanese for understanding the *kōji* microbes (*kōji-kin*) that were widely used by scientists and brewers alike. How could the knowledge of experts in national industries be reconciled with the species names used in international classification systems? Clearly, there was a strong material dimension to this question, since *kōji* as a sake brewing microbe was restricted to the Japanese brewery; other Asian liquor brewing industries used different molds to create their products. The problem of reconciling local industrial knowledge with formal international systems, then, was also a problem of reconciling physical collections of strains in different locations, and of comparing strains in one location with strains held in another – since comparison with other strains formed the basis of



microbial classification. The material question of the number and kind of strains that were physically held in any one collection mattered, and the material differences between collections would have a bearing on how different perspectives on identification and variation could be reconciled. It was only through material exchange that a scientific narrative that had originated in the interests of Japanese national industries could be made convincing internationally – the evolutionary narrative in which Japanese industries had created, through centuries of use and selection, a domesticated, non-toxic species *Aspergillus oryzae* that was unique to the Japanese brewery, and that was different from the closely related, but wild, ubiquitous in nature, and sometimes potentially toxigenic species *Aspergillus flavus*.

The authoritative *Aspergillus* classification text, Kenneth B. Raper and Dorothy I. Fennell's *The Genus Aspergillus* (published in 1965), proposed 18 representative groups including *A. flavus* (which at the time included the species *A. oryzae*, although today the *A. oryzae* species is in an *A. oryzae* group). In Japan on the other hand, a detailed vernacular understanding of some of these microbes in industry, and among scientists linked to industry, preceded the larger classification schemes and differed from them. What the vernacular name *kōji* referred to was not simply a species. The name *kōji* itself was older, and on its own described the entire mold growth with its mix of strains that brewing specialists created at a particular stage in the brewing process. But *kōji-kin*, or *kōji* microbe, was a late nineteenth-century neologism, and referred only to the useful microbes responsible for the power of the *kōji* mold.<sup>36</sup> Japanese industries called one group of *kōji* 'yellow *kōji* microbes' (used in sake, soy sauce, miso, and vinegar) for their yellow-green color, and another group of *kōji* 'black *kōji* microbes' (used in distilled liquors like *shōchū*, *awamori*, or industrial alcohol) for their black color. From the perspective of the scientific classification systems of that time, those yellow-green *kōji* and black *kōji* would fall into the *A. flavus* and *A. niger* groups respectively. As Murakami Hideya – microbiologist at the National Research Institute of Brewing in Japan and the preeminent figure who was involved in taxonomic work on *kōji* – noted emphatically, the *A. flavus* group was represented by the *A. flavus* Link strain, while the *A. niger* group was represented by the *A. niger* von Tieghem strain; yet, within the microbes known as *kōji*, not one strain existed that corresponded to either of these two representative strains, and in each of the two groups, many of the strains varied a great deal from *kōji*.<sup>37</sup>

Microbial culture collections in Japan began in breweries, as specialist *kōji* spore starter makers had preserved and evaluated molds since the thirteenth century. Only with the introduction of pure culture techniques in the late nineteenth century, however, could brewers as well as an emerging group of scientists culture and propagate single microbial strains as a pure culture in the laboratory. They could thus maintain and preserve particular strains of well-performing microbe indefinitely. Collections of strains were built up in many sites. In Japan, these included brewing companies for commercial manufacture, university laboratories for research purposes, national collections and government-supported brewing experiment stations as a service to academic or industrial laboratories, and pharmaceutical or chemical companies in order to make antibiotics and other products. *Kōji* research in the 1960s involved not only commercial experts, some of who held Ph.D.'s, but also experts in state-supported as well as privately supported national-scale institutions, which were designed to mediate between academia, industry, and government

ministries. By the 1960s, the largest collection in Japan was the Institute for Fermentation, Osaka (IFO). The National Research Institute of Brewing (RIB) in Tokyo also held a substantial collection of *kōji* strains included commercial *kōji* starter cultures sent from breweries since, historically, the institute had performed microbial research in order to aid Japanese brewing enterprises.<sup>38</sup>

Across the world, other important culture collections included the Centraalbureau voor Schimmelcultures (CBS; now the Westerdijk Institute) in Utrecht, the Netherlands, as well as the American Type Culture Collections (ATCC) in Manassas, Virginia, and Northern Regional Research Laboratory (NRRL) in Peoria, Illinois, in the United States.<sup>39</sup> An international network of culture collections, associated with UNESCO, was established in the 1960s. As Hasegawa Takeji, who was for many years director of the IFO in Japan, writes: 'World-wide damage of culture collections of microorganisms during the Second World War paved the way for the establishment of an international union'.<sup>40</sup> Japanese microbiologists played a central role in putting forward the proposal of an international network to promote culture collections.<sup>41</sup>

Work in microbial classification relied on reference to – and comparison with – the strains maintained in such culture collections. What is the broader significance of calling something a species? It is not a purely intellectual exercise, but closely linked to the microbe's function in a practical context. One of the purposes of classification is to be able to say definitively what the microbe does or does not do in that context. Where a medical microbiologist may ask whether a microbe causes a particular disease in humans, an industrial microbiologist may ask, with regard to brewing, whether a microbe is a strong producer of amylase enzymes that convert starch into sugar (good for sake, such as *Aspergillus oryzae*), or whether it is a strong producer of protease enzymes that break down protein (good for soy sauce, like *Aspergillus sojae*), or whether it is likely to make a tasty end-product.<sup>42</sup> In the 1960s, microbiologists faced a new question: does the microbe produce aflatoxin, or is it safe?

Classification practices for *kōji* at the time were primarily based on comparing strains to named and preserved 'standard strains', using set characters or 'keys' to do so. For *Aspergillus*, classification was difficult because the character often varied continuously between a large group of different strains. Under such circumstances, classification practices relied on looking at numerous characteristics, and possessing a large collection of strains for comparison. Characters included both morphological features as could be seen under a microscope, and physiological features such as metabolic products. Between the 1960s and 1980s, scientific work in classification made crucial aspects of Japanese narratives about *kōji* persuasive internationally so that they were incorporated into classification schemes outside Japan. The new international classification schemes differed from their earlier counterparts in making a clear distinction between *A. oryzae* (the 'sake *kōji*' microbe) and *A. flavus*, as well as between *A. sojae* (the 'soy-sauce *kōji*' microbe) and *A. parasiticus*. In the new system, *A. oryzae* and *A. sojae* were part of the *A. oryzae* group, which never produced aflatoxin, while the *A. flavus* and *A. parasiticus* species, both part of the *A. flavus* group, included some strongly aflatoxin-producing strains.

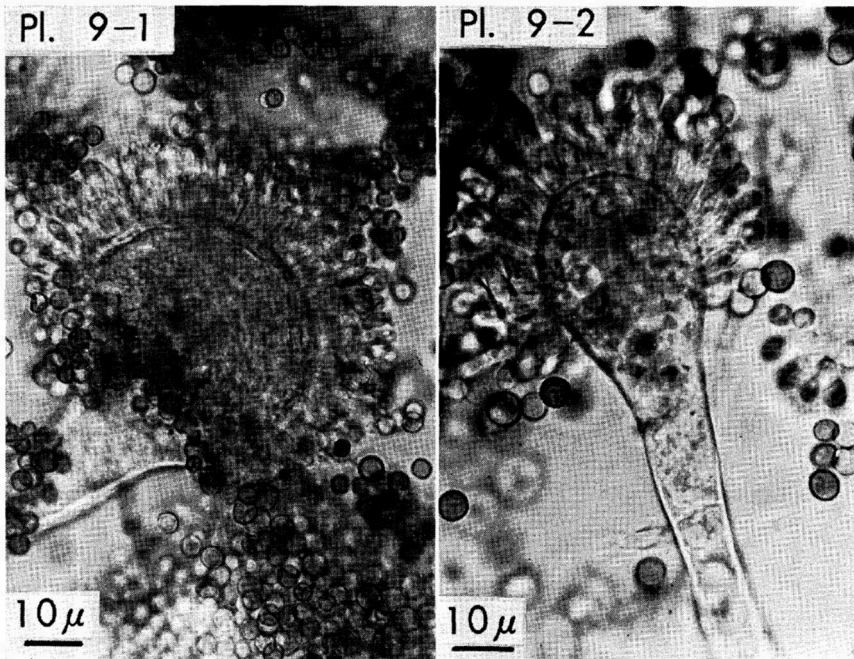
Material exchange of strains played a vital role in this process, as well as reconciling the history of exchanges that had created the reference standard strains in global collections. Here, the material differences between the microbial world in Japanese

industry and abroad are clearly visible. The predecessor to Raper and Fennell's *The Genus Aspergillus* was Charles Thom and Margaret Brooks Church's 1926 reference text, *The Aspergilli*. In 1921, these U.S.-based researchers received 16 strains of *kōji* microbes in the mail, sent by Japan-based scientist Takahashi Teizō, who had been investigating *kōji-kin* collected from mold starter companies. Thom and Church identified one of Takahashi's strains as being similar to the standard strain known as *A. oryzae* (Thom No. 113), and another as being like the standard strain called *A. flavus* (Thom No. 108). In their 1926 reference text, Thom and Church combined them into an *A. flavus-oryzae* group and placed a number of Takahashi's *kōji* strains there (*A. flavus* and *A. oryzae* are now two separate groups in current taxonomy, neither of which are equivalent to the earlier groups; this is the main taxonomic change that is discussed below). Other strains sent by Takahashi were placed into a different group, *A. tamarii*. Thus were Japan's *kōji-kin* characterized in the 1926 international reference text.<sup>43</sup>

Following the 1960 Turkey X outbreak and the discovery of aflatoxin production by *A. flavus*, Murakami Hideya at Japan's National Research Institute of Brewing in Tokyo traced the history of two of the standard *A. oryzae* as well as *A. flavus* strains kept in international collections. A 1971 English-language paper summarizes the key findings of his work, in which he painstakingly elucidated the pedigrees of Thom No. 113 and Thom No. 108, and studied as many as 406 strains.<sup>44</sup> (See [Figure 1](#).)

Here his driving questions echoed those of earlier Japanese researchers such as botanist Saitō Kendō. Writing in the late 1940s, Saitō emphasized that Japanese experts had used a great deal of *kōji* for a long time, and microbiologists both Japanese and European who worked in Japan had studied *kōji* morphology and physiology in detail. From the earliest studies, it was clear to researchers working in Japan that *A. oryzae* was different from *A. flavus*, the latter of which was well-known and recognizable in the wild. *A. oryzae*, on the other hand, was not found in the 'natural world'. Saitō argued that Thom had only ever apparently isolated *A. oryzae* from a rotting Brazil nut once, yet it was often isolated from East Asian-produced fermented goods. Thom had reasoned that intermediate varieties filled the gaps between the characters of the standard strain for *A. oryzae* and the standard strain for *A. flavus*, and therefore chose to treat the two together as one group. Yet, Saitō asserted, at the time of Takahashi Teizō's investigations it was already clear that the strain Wehmer had called *A. flavus* (Wehmer's *A. flavus* was used by Thom and Church as the standard strain Thom No. 108) was a similar-seeming, but different strain that Wehmer had erroneously conflated with *A. flavus*. In any case, Saitō wrote, these were purely academic names, and in actual practice on the brewery floor, there was nothing wrong with using another system of classification. But as a question of broader interest, Saitō thought the problem worthy of further work. Why had so many *kōji* varieties been created? Were they simply derived from special strains that had been set aside and cultivated for fermented goods, or did they come from a geographically distinct origin?<sup>45</sup>

What Murakami Hideya found in his 1960s and 1970s investigations of the two standard strains, Thom No. 113 (then *A. oryzae* (Ahlburg) Cohn) and Thom No. 108 (then *A. flavus* Link), confirmed Saitō's doubts about Thom and Church's 1926 system as it concerned *kōji* microbes.<sup>46</sup> The 1920s system had been built on a much sparser collection of *kōji* strains than were used by brewers in Japan on a day-to-day basis. At that point, there had been little incentive to question further the identification of *kōji*-



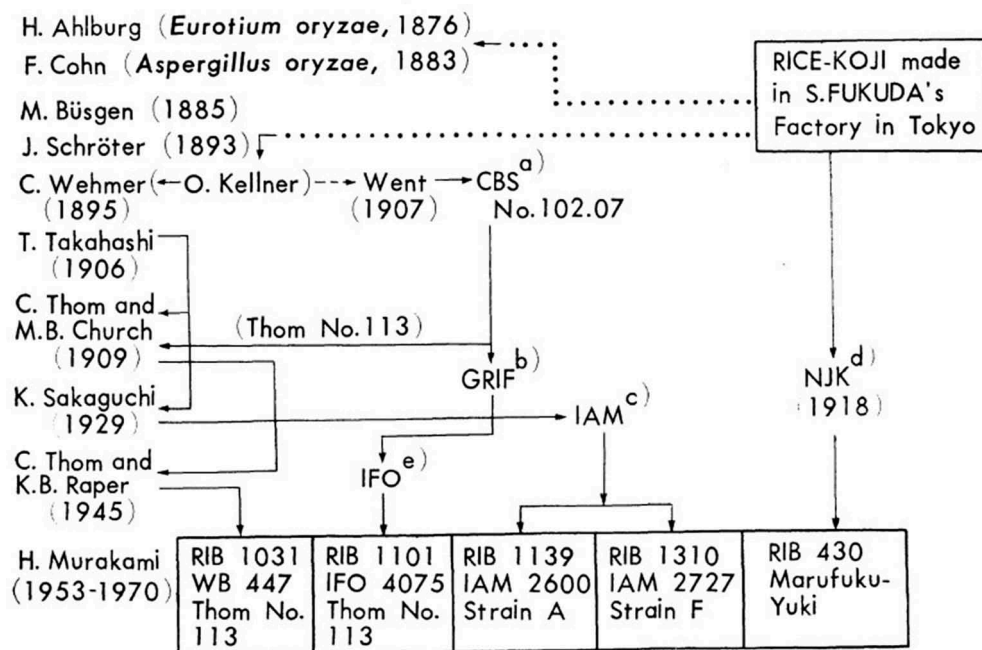
**Figure 1.** Examples of photographs from Murakami Hideya's paper showing the appearance of *kōji* strains. The photographs focus on the conidial head with sterigmata in: (Pl. 9-1) *A. oryzae* RIB 609, abundant sterigmata in a large head, and (Pl. 9-2) *A. oryzae* RIB 537, small number of sterigmata with predominance of uniseriate sterigmata in the small head. Reproduced from Murakami, 'Classification of *Kōji* Mold', 289 with permission of Terrapub.

related standard strains – which, in turn, marked the reference points for comparison in classification work – since, at the time, the way in which *kōji* microbes were classified internationally had little bearing on their practical use either in Japan or anywhere else.

Considering the history of the standard *A. oryzae* strain, Murakami confirmed that Thom No. 113, which had been isolated by Wehmer in the late nineteenth century, was indeed the original *A. oryzae* strain that had been isolated by Ahlburg and Cohn (see Figure 2).<sup>47</sup> Ahlburg working at the Tokyo Medical School had isolated a microbe from rice *kōji* which he judged to be different from the known *Aspergillus flavus* Link in 1876, and Cohn soon after changed its classification to *Aspergillus oryzae*. A decade later Kellner, working at the Komaba Agricultural School, sent a sample of Japanese *kōji* to Dutch microbiologist Wehmer, who isolated a strain from it and gave the strain the same name as Ahlburg's of *Aspergillus oryzae* (Ahlburg) Cohn. Later Wehmer sent his strain to be preserved in the Netherlands as CBS No. 102.07, and to Thom in the United States where it was held in the ATCC as Thom No. 113. Ahlburg's original strain went to the mold starter company Nippon Jōzō Kōgyō in Tokyo, where it sold very well under their brand name Marufuku Yukijirushi. The same *kōji* strain appeared to have been sent from the mold starter company later to the National Research Institute of Brewing, where it became preserved as RIB 430.

Considering the history of the standard *A. flavus* strain Thom No. 108, on the other hand, Murakami argued that it had been erroneously identified. The original standard



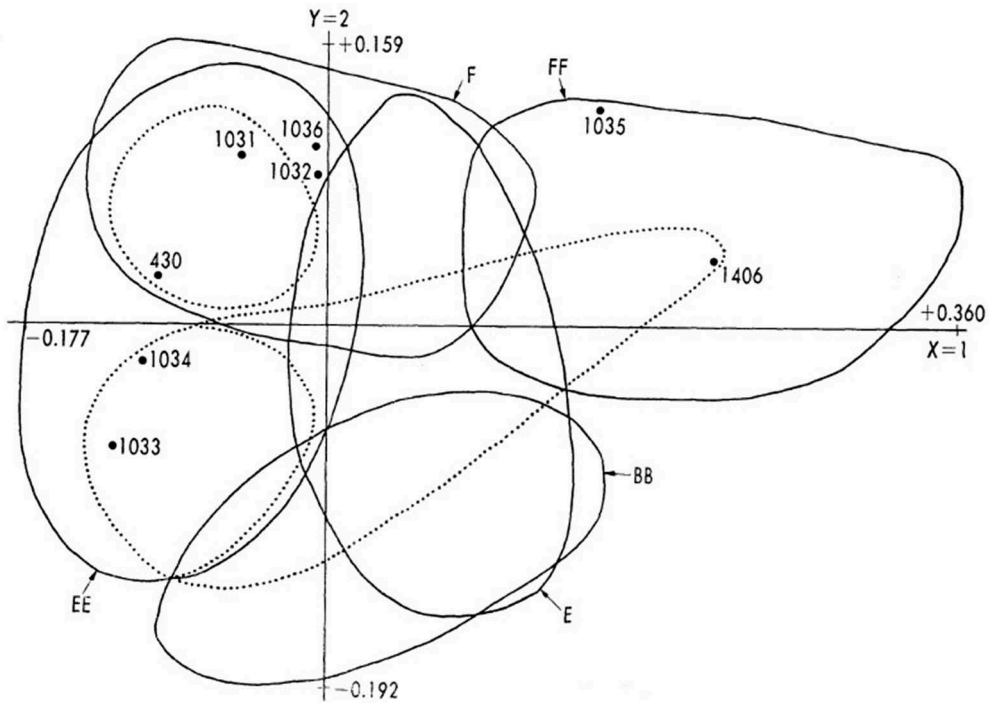


Signs for process of distribution: — reliable, ---- probable, .... theoretically reliable.

**Figure 2.** Table showing the history of the distribution of Ahlburg-Cohn's original isolate (Thom No. 113) across different culture collections in Japan, as well as in the ATCC in the United States and the CBS (now Westerdijk Institute) in the Netherlands. Reproduced from Murakami, 'Classification of Kōji Mold', 294, with permission of Terrapub.

strain, to which Wehmer had equated his own isolate as *A. flavus*, had been a strain isolated by Link from a pressed-leaf specimen in 1809. Others had subsequently attempted to isolate the same strain *Aspergillus flavus* Link again, even from the pressed-leaf specimen itself, but it was nowhere to be found. Thus the yellow-green strain that had come to be preserved and used everywhere as a standard strain for *A. flavus* was not Link's original strain, but Wehmer's. It was Wehmer's strain that was preserved as Thom No. 108 in the ATCC (and also as NRRL 482 in Peoria). Yet Wehmer's strain, Murakami argued, was not equivalent to *A. flavus* Link. As his own research showed, Thom No. 108's characteristics were more like those of *A. oryzae*, and because of this, it should not have been used as a standard strain for *A. flavus* Link. In order to follow some of these historical connections and identifications, Murakami had asked Kenneth Raper to send both Thom No. 113 and Thom No. 108 over to him in March 1966, and had corresponded by letter with Dorothy Fennell in November 1970 regarding the identity of Thom No. 108 (whereupon she had confirmed that she, too, regarded it to be *A. oryzae*, not *A. flavus*).<sup>48</sup>

Murakami used computer analysis to group the 406 different *Aspergillus* strains in his study, based on variation in multiple 'key' characters (see Figure 3).<sup>49</sup> He found that koji molds 'were proved to belong to a quite different cluster from that of *A. flavus* by computer analysis', and thus argued that *Aspergillus oryzae* was appropriate as



**Figure 3.** Graph showing the distribution of clusters of *kōji* strains analyzed with respect to 2 components, Component 1: 'Color of old cultures in Czapek agar; brown 1, brownish green to greenish brown 2, green 3', and Component 2: 'Diameter of colony: short 1, medium 2, long 3' (294). Murakami argued that in the results of component analysis, 'the strain RIB 1032 (=Thom No. 108) was always near the strain RIB 1031 (=Thom No. 113), which suggests that Thom No. 108 is not suitable for use as a standard strain of *A. flavus* Link' (297-98). Reproduced from Murakami, 'Classification of *Kōji* Mold', 299, with permission of Terrapub.

a separate group name for *kōji* molds.<sup>50</sup> Based on the results, Murakami proposed a new classification system with a new set of key characters that could clearly distinguish between the *A. oryzae* group and the *A. flavus* group.

Notable in addition was the fact that his classification scheme, unlike Raper and Fennell's, made a division between *A. sojae* (the 'soy-sauce *kōji*' microbe, as distinct from the 'sake *kōji*' microbe *A. oryzae*) – now a species within the *A. oryzae* group – and *A. parasiticus* – now a species within the group *A. flavus*, which was separate from *A. oryzae*. It did so by looking in particular at the smoothness of the conidiophore underneath the electron microscope (*A. sojae* Sakaguchi et Yamada could have smooth conidiophores, while they were rough in *A. parasiticus* Speare). This system required the recognition of *A. sojae* as a new species at the international level. In Japan itself, *A. sojae* had been established as a species as early as 1944, but within a classification scheme that had been developed specifically for Japanese *kōji-kin* only, and which could not, therefore, be used in international comparisons.<sup>51</sup> The recognition of *A. sojae* as a new species by the American Type Culture Collections in 1980 was therefore a milestone for Japanese researchers.<sup>52</sup> Overall, in the new classification system, it was easy to grasp conceptually that the *A. sojae* and *A. oryzae* species, as part of the *A. oryzae* group, never produced

aflatoxin, while *A. parasiticus* and *A. flavus*, as part of the *A. flavus* group, could produce aflatoxin.<sup>53</sup>

In the 1980s, Murakami noted more recent studies comparing nucleic DNA in yeasts and bacteria. From that perspective, he explained, some had theories that *A. oryzae*, *A. parasiticus*, and *A. sojae* were all subspecies of *A. flavus*.<sup>54</sup> For example, a 1983 paper published by D. T. Wicklow, a USDA researcher at the Northern Regional Research Center in Peoria, dealt with the yellow-green *Aspergilli*, arguing that wild strains of the *A. flavus* mold, when subcultured or domesticated in the laboratory, underwent morphological and biochemical changes that made them like *A. oryzae*. They produced fewer spores, had smoother conidiophores and conidia, and crucially did not produce aflatoxin.<sup>55</sup> Wicklow argued that *A. oryzae* (the ‘sake *kōji*’ microbe) should be understood as domesticated varieties of the wild species *A. flavus* Link, and *A. sojae* (the ‘soy-sauce *kōji*’ microbe) should be understood as domesticated varieties of the wild species *A. parasiticus* Speare. Over a period of two decades, then, Japanese mycologists had succeeded in pushing through a global consensus on a classificatory framework that could clearly distinguish between ‘domesticated’ *kōji* strains (which were unique to the Japanese brewery and safe to eat) and ‘wild’ *A. flavus* strains (which were found commonly in nature and could be very toxigenic) in the yellow-green *Aspergilli* molds.

## Conclusion

In 1960, the trajectory of aflatoxin as one of the earliest and best studied cases of a naturally occurring carcinogen in food intersected with the trajectory of an industrial microbe known in the Japanese vernacular as *kōji*. Over about two decades, the aflatoxin crisis spurred the emergence of a new evolutionary narrative of *kōji* microbes, which came hand-in-hand with the reconstruction of *kōji* classification. The knowledge infrastructure of *kōji* as it was maintained by classification systems initially functioned in relation to problems of making sake, soy sauce, and miso industrially. There was a shift in classification systems after the 1960 aflatoxin outbreak in agricultural animals. With the change in perception of *kōji* from a beneficent fungus to a hazard as well, scientists worked to rearrange classification systems to re-characterize *kōji* as domesticated and safe. As a living organism, being a manufactured product for centuries in the Japanese brewery made *kōji* safer due to the narrative of domestication, and in present food and drug regulations, *kōji* comes under the category of ‘generally recognized as safe’ (GRAS).

The crossing of the trajectories of aflatoxin and *kōji* in microbial classification highlights dimensions of the life sciences that are frequently less visible in accounts focusing on scientific discovery. It reveals the important role of industry and academic experts in defining food risk and food safety in broader ways beyond setting regulatory standards, through the hidden aspect of scientific knowledge infrastructure.<sup>56</sup> It delineates different timelines as well as actors than those operating in academic laboratories, especially the long-durée engagement of Japanese brewers with the *kōji* mold, which crucially shaped scientists’ design of new taxonomies in the twentieth century. Finally, *kōji* classification sheds light on the challenges of reconciling national and international frameworks in science due to the material basis of knowledge infrastructures, and in particular, it



demonstrates how the scientific universalization of local practices resulted in the strengthening of national identity.

## Notes

1. Kōji was designated the national fungus (*kokkin*) of Japan by the Brewing Society of Japan on 12 October 2006. A copy of the “Declaration” by the Scientific Conference of the Brewing Society of Japan may be found at: <http://www.jozo.or.jp/koujikinnituite2.pdf> (2006, revised 2013). It is important to note that although *Aspergillus oryzae* is the species that most commonly exemplifies kōji, the term *kōji* is in fact broader in meaning and includes species other than *A. oryzae*. *Kōji* is used by brewing specialists in a vernacular way to refer to Japanese brewing microbes of the *Aspergillus* genus, especially the *A. oryzae* species (which is used for making sake) but also including, for example, *A. sojae* species (for making soy sauce) which belong to the *A. oryzae* group, as well as species of the *A. luchuensis* group such as *A. luchuensis* var. *awamori* and *A. luchuensis* mut. *kawachii* (for making awamori). Moreover, the term *kōji* is used for the brewing mold preparation as a whole, in addition to particular species or varieties.
2. In current taxonomy, by contrast, *A. oryzae* and *A. flavus* are separate groups, neither of which are equivalent to the older groups. The *A. oryzae* species belongs in the *A. oryzae* group, and the *A. flavus* species belongs in the *A. flavus* group. This is the important taxonomic shift that is discussed below in the article.
3. Lee, “Mold Cultures.”
4. On coevolutionary history, see Russell, *Evolutionary History*; and Schrepfer and Scranton, *Industrializing Organisms*. For a perspective from the life sciences, see Rader, *Making Mice*; and Berry, “Plants are Technologies.”
5. For other examples of technologies of taxonomy, see Müller-Wille, “Hybrids”; and Bonneuil, “Manufacture of Species.”
6. Compare examples from the historiography of the physical sciences, in which standards express the specific agendas of a distinct set of social and economic interests; in Wise, *The Values of Precision*; Schaffer, “Manufactory of Ohms”; Alder, “Revolution to Measure”; and Slaton, *Reinforced Concrete*. Here I deal with an epistemic object, kōji, that scientists and industry specialists have frequently discussed in explicitly “techno-nationalist” terms; as defined by Edgerton, *Shock of the Old*. Similarly, historical studies have shown how scientists and engineers have created things – including living organisms – to materialize national goals and national ideologies; especially Camprubí, *Engineers*; and Saraiva, *Fascist Pigs*. On food, see Ceccarelli, Grandi, and Magagnoli, *Typicality in History*.
7. I borrow the phrase from Haraway, “Situated Knowledges.”
8. Landecker, *Culturing Life*; Rheinberger, *Epistemology of the Concrete*; and Rheinberger, *Toward a History of Epistemic Things*. The classic study of infrastructure in the history of technology is Hughes, *Networks of Power*.
9. Mueller, “Cancer in the Tropics”; Jiang, “Global Epidemiology, Local Message”; and Creager, “EAT. DIE.”
10. “Machinery of life”: thanks to Karen Rader for the turn of phrase. On the inseparability of knowledge and institutions, see Johnson, *Hitting the Brakes*; and Mody, *Instrumental Community*. In this sense, kōji classification systems served as a “boundary object” between scientific and industrial knowledge, and between national and international systems; compare Star and Griesemer, “Institutional Ecology.”
11. Jordan and Lynch, “Plasmid Prep.” For a parallel from the historiography of technology, in which the uses of a technology are not fully determined in its production, see Cowan, “Consumption Junction.”
12. Goldblatt, “Introduction”; Phillips, “Reducing Human Exposure”; Richard, “Discovery of Aflatoxins”; and Blount, “Turkey ‘X’ Disease.” For a historical account of human disease

- caused by *Aspergillus* fungi, especially aspergillosis, see Homei and Worboys, *Fungal Disease*, Chap. 5.
13. Creager, “EAT. DIE”. On the history of scientific research linking food with environmental exposure, see also Gaudillière, “DES”; and Landecker, “Food as Exposure.”
  14. Goldblatt, “Introduction,” 8–9.
  15. Diener and Davis, “Aflatoxin Formation,” 19.
  16. Dollear, “Detoxification of Aflatoxins,” 360.
  17. Kraybill and Shapiro, “Implications of Fungal Toxicity,” 421.
  18. Kraybill and Shapiro, “Implications of Fungal Toxicity,” 419. It was only later that epidemiological studies on the environmental causes of cancer were able to demonstrate conclusively that infection with the Hepatitis B virus and dietary aflatoxin exposure worked together to cause liver cancer. These studies were carried out in China, the United States, East Africa, and elsewhere from the 1970s onward; see Mueller, “Cancer in the Tropics”; and Jiang, “Global Epidemiology, Local Message.”
  19. See note 17 above.
  20. Kraybill and Shapiro, “Implications of Fungal Toxicity,” 402–3.
  21. Diener and Davis, “Aflatoxin Formation,” 14.
  22. Fuell, “Types of Mycotoxins,” 195.
  23. See e.g. Miller et al., *Naturally Occurring Carcinogens*, 169–76; 195–210. On the Ames test, see Creager, “Political Life of Mutagens.”
  24. Ui, *Industrial Pollution in Japan*.
  25. Sand, “Short History of MSG,” 45–46. MSG was in fact produced by microbes cultured on a nutrient medium that was not derived from petroleum.
  26. George, *Minamata*; Walker, *Toxic Archipelago*; and Upham, “Movements for Place.”
  27. Cwiertka, *Modern Japanese Cuisine*, 167–74.
  28. Sakaguchi, “Opening Lecture,” 8.
  29. Cwiertka, *Modern Japanese Cuisine*, 167.
  30. See note 3 above.
  31. For reviews of more recent genomic research on *A. flavus*, see Amaike and Keller, “*Aspergillus flavus*”; and Payne et al., “Whole Genome Comparison.” On the *Aspergillus* genus, see de Vries et al., “Fungal Genus *Aspergillus*.”
  32. Hesseltine et al., “Aflatoxin Formation.”
  33. *Ibid.*, 802–3.
  34. “‘Kabi’ kara hatsugan busshitsu – komugi, azukifun nado – wazuka daga eikyō shinpai” [Carcinogens from “Mold” – Wheat and Azuki Flour and So On – Minute Amounts But Fear of Effects] (*Yomiuri shinbun*, 29 March 1967); “Yūgai kokomai o yushutsu – Tōnan Ajia e 26man ton mo – hatsugan, kabi hookaburi” [Harmful Long-Storage Rice – 260,000 Tons Exported to Southeast Asia Even – Playing Dumb about Carcinogenicity and Mold] (*Yomiuri shinbun*, 8 May 1970); “Jikasei miso ni doku kabi – Kokuritsu eishi to Chibadai hakken – hatsugan busshitsu ga deru” [Poisonous Mold in Homemade Miso – Discovered by National Institute of Hygienic Health Sciences and Chiba University Institute of Food Microbiology – Carcinogens Emerged] (*Yomiuri shinbun*, 20 May 1970); “Shokuniku ni ‘kiken na kabi’ – toritsu eishiken de kenshutsu – teion hozon de fusegeru” [“Dangerous Mold” in Consumer Pork – Found by Tokyo Metropolitan Institute of Public Health – Can Be Prevented by Low-Temperature Preservation] (*Yomiuri shinbun*, 7 July 1973).
  35. O’Malley, *Philosophy of Microbiology*, Chap. 3; Murakami, “Kinkabu,” 58.
  36. Murakami, “Kinkabu,” 58. The term *kōji-kin* first appeared in a publication in 1895, written by agricultural chemists Kozai Yoshinao and Yagi Hisatarō.
  37. Murakami, “Kinkabu,” 48.
  38. Hasegawa, “Japanese Culture Collections.”
  39. The NRRL played an important role in penicillin research during World War II; see Bud, “Innovators, Deep Fermentation.”
  40. Hasegawa, “Japanese Culture Collections,” 141.

41. Ibid., 141–43.
42. Amsterdamska, “Medical and Biological Constraints”; and Lee, “Mold Cultures.”
43. Murakami, “Kinkabu,” 59.
44. Murakami, “Classification of Kōji Mold.”
45. Saitō, *Hakkō biseibukki*, 182–86.
46. Following Murakami’s work, Thom No. 113 and Thom No. 108 are now *both* classified as the species *A. oryzae* (Ahlburg) Cohn.
47. The following account draws from Murakami, “Kinkabu,” 57–60.
48. Murakami, “Classification of Kōji Mold,” 291–93.
49. The strains came from 12 different collections both in Japan and abroad, including the ATCC, NRRL, and London Tropical Products Institute. Many strains were requested from Hesseltine and Fennell at the USDA laboratory in Peoria, and from Raper at the University of Wisconsin-Madison. Murakami drew especially on the kōji collections held by the RIB, where he was based, and which consisted of more than 1200 strains gathered from inside and outside the country. The number of strains he selected for the study totaled 406 after eliminating redundancies. Murakami, “Kinkabu,” 64–65.
50. Murakami, “Classification of Kōji Mold,” 299.
51. Murakami, “Kinkabu,” 71; 61.
52. Ibid., 71.
53. Ibid., 65–71.
54. Ibid., 70.
55. Wicklow, “Adaptation.”
56. For a consideration of historical approaches to risk in industrialized societies since the late nineteenth century, see Boudia and Jas, “Risk and ‘Risk Society.’”

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