The Science of Koji Mold

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and
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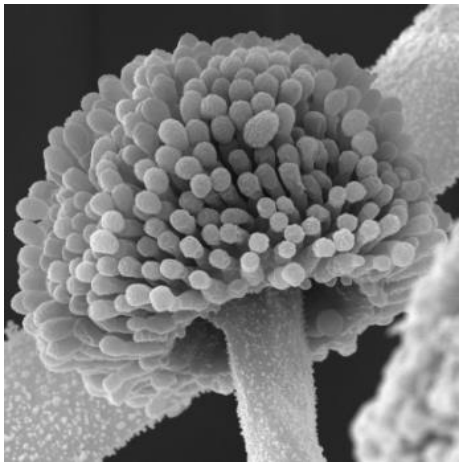
Katsuya Gomi

21.09.2025 The power of fermentation (Bern, Switzerland)

Koji mold (*koji-kin*): industrially-important fungus in Japanese fermentation industries

一ノ蔵
Ichinokura
Koji preparation



「萩丸 Hagimaru」
Original sake of Tohoku University

米麹
rice koji

キッコーマン 御用蔵 Kikkoman *Goyogura*
http://washoku-lab.net/archives/16362

What is koji mold?

✍Koji mold (*Koji-kin*;麹菌 in Japanese)

The filamentous fungi employed in Japanese traditional fermentation industries as follows;

(1) *Aspergillus oryzae*

(2) *Aspergillus sojae* and albino mutants of *Aspergillus oryzae*

These belong to *Aspergillus* section *Flavi*, and are so-called yellow-green koji mold after their color of conidia.

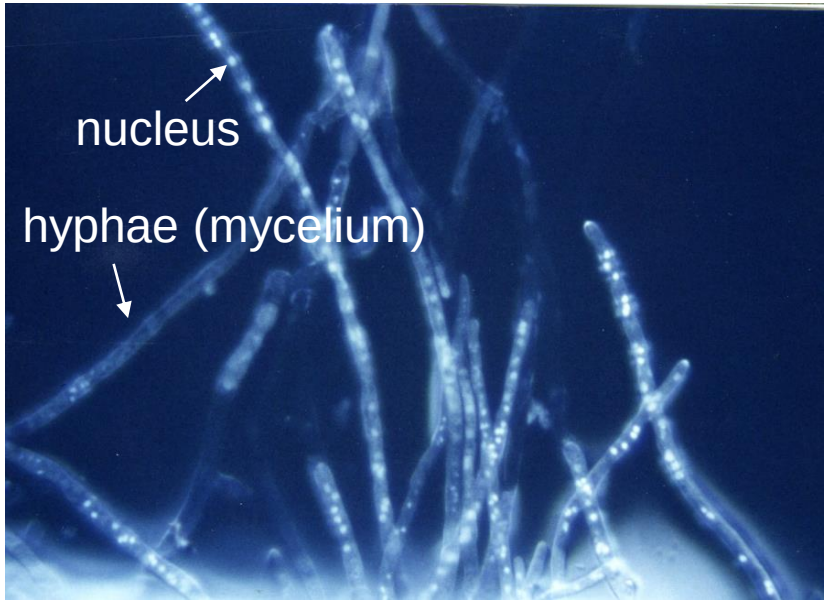
(3) *Aspergillus luchuensis* and its albino mutant, *Aspergillus luchuensis* mut. *kawachii*

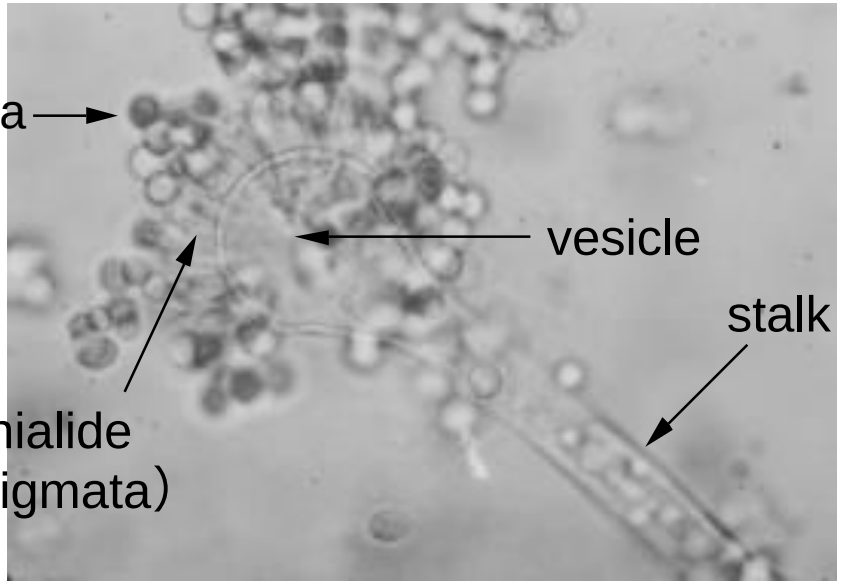
These belong to *Aspergillus* section *Nigri*, and so-called black koji mold after their color of conidia.

All three filamentous fungi are designated as “national fungi of Japan”.

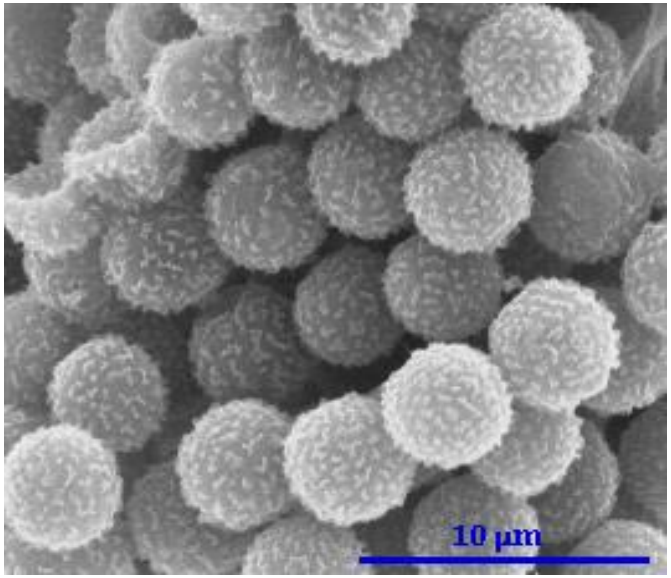
Observing koji mold under a microscope.....

Optical microscope images of koji mold

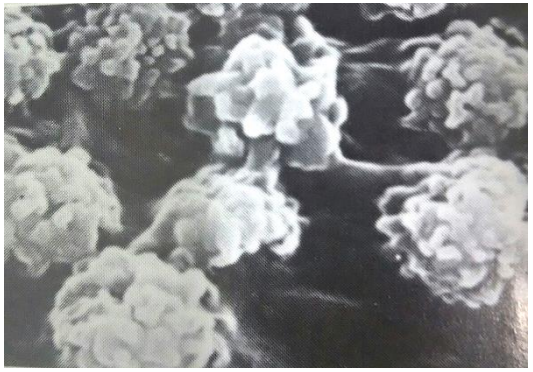




Scanning electron microscope images of conidia

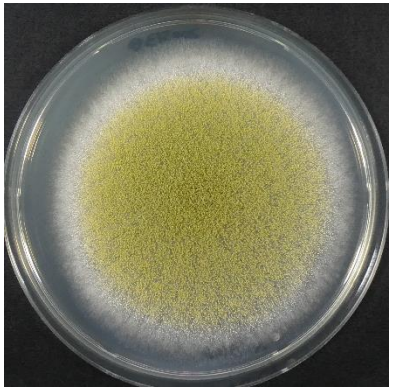


Aspergillus oryzae

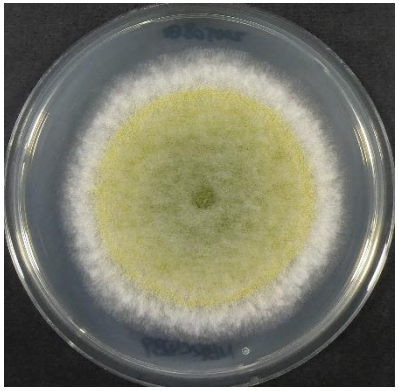


Aspergillus sojae

Culturing koji mold that can only be observed under a microscope…



Aspergillus oryzae
（For sake）

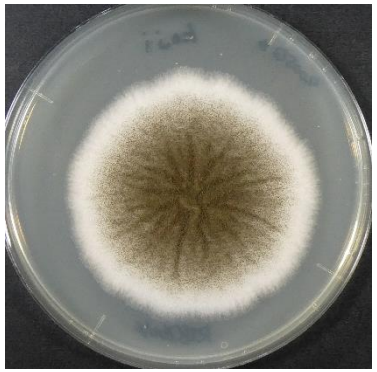


Aspergillus sojae
（For soy sauce）

Yellow-green koji mold→Essential for sake, soy sauce, miso, mirin, amazake etc.

Trivia

It was previously called *Aspergillus awamori*, but has been renamed back to its original name when isolated in 1901.



Aspergillus luchuensis
（For awamori and shochu）

Aspergillus luchuensis mut. *kawachii*
（For shochu）

Black koji mold→Essential for shochu and awamori

Tane-koji (koji starter; conidia of koji mold)

Aspergillus oryzae
(standard type)

Albino mutant of *Aspergillus oryzae*
(For miso and amazake)

Aspergillus luchuensis
(For awamori and shochu)

Aspergillus luchuensis mut. *kawachii*
(For shochu)

Yamashita, *J. Fungi,* **7**, 569 (2021)

Important roles of koji mold in fermentation

1) Decomposition of raw materials

① Decomposition of starch ← Amylolytic enzyme production

α-amylase, glucoamylase, α-glucosidase

Enzymes essential for production of sake, shochu, and awamori made from rice, barley, and sweet potatoes

② Decomposition of proteins ← Proteolylic enzyme production

Acid protease, acid carboxypeptidase

Involved in forming umami in sake making

Neutral and alkaline proteases, aminopeptidase

Enzymes essential for production of soy sauce and miso made from soybean and wheat

③ Decomposition of cellulose and hemicellulose ← Cellulolytic and xylanolytic enzyme production

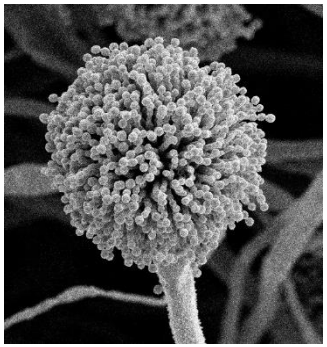
Important for shochu and soy sauce made from sweet potatoes, barley, soybean, and wheat

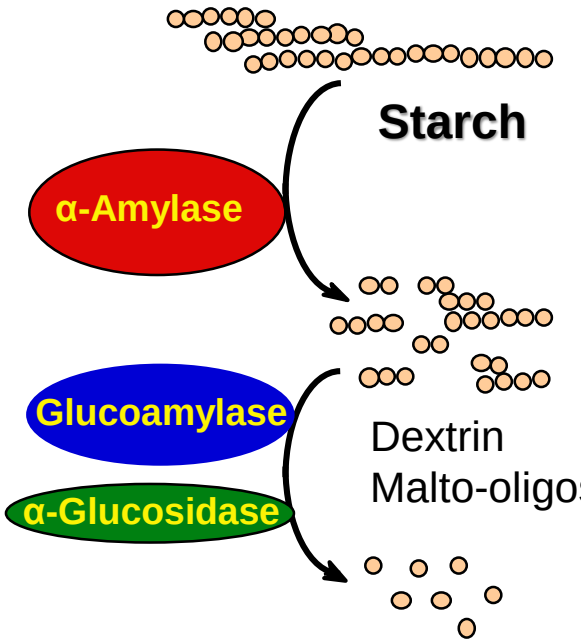
2) Acidification of moromi mash

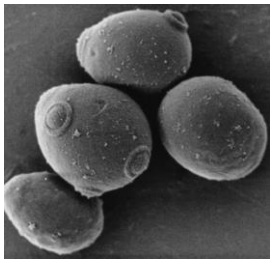
Citric acid production → Prevention of detrimental microbial contamination by acidifying moromi mash in shochu and awamori making

Important role of amylolytic enzymes produced by koji mold in sake fermentation

Koji mold: *Aspergillus oryzae*







Yeast
Saccharomyces cerevisiae

「萩丸Hagimaru」
Original sake of Tohoku University

Role of koji mold in soy sauce (shoyu) making

Protein in soybean and wheat
(polymer of 20 species of amino acids)

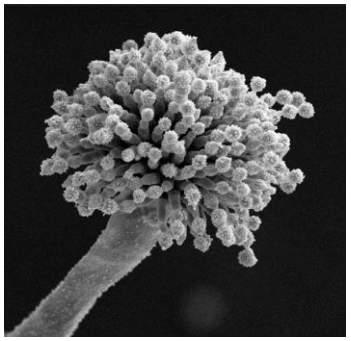
Hydrolysis

Proteolytic enzymes
proteases
peptidases

Amino acids, peptides

Umami

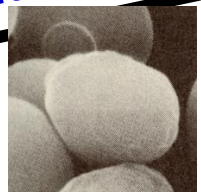
Koji mold: *Aspergillus sojae*



Shoyu koji

Glucose

Ethanol fermentation

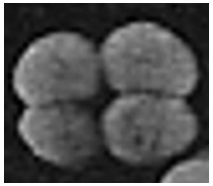


Ethanol
+Carbon dioxide

Shoyu yeast: *Zygosaccharomyces rouxii*

Lactic acid fermentation

Lactic acid



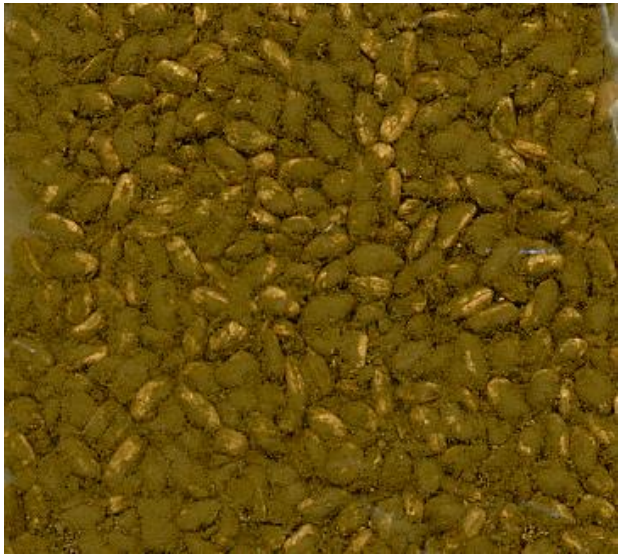
Tetragenococcus halophilus

Flavor

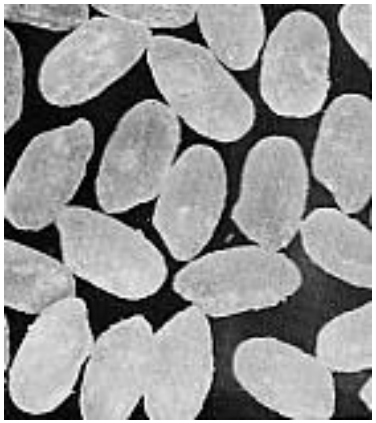
Amylolytic enzymes

Starch

Growth of koji mold on steamed rice

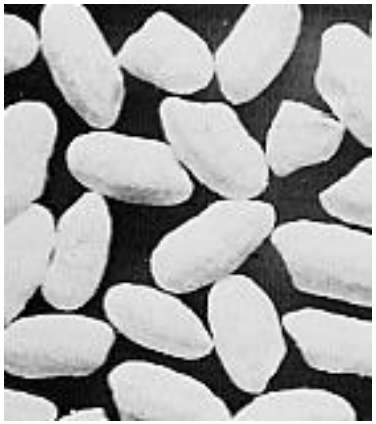


Tane-koji (conidia of koji mold) starter for koji preparation



12 hr

24 hr

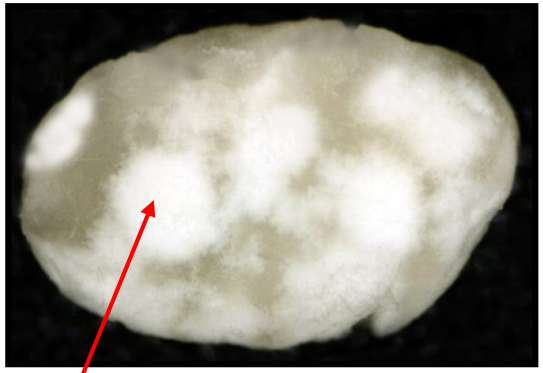


46 hr
（Finishing）

Murakami ed. “Koji Science”
（Brew. Soc. Jpn）

Representative types of rice koji

Surface



Haze (mycelium)

Cross section

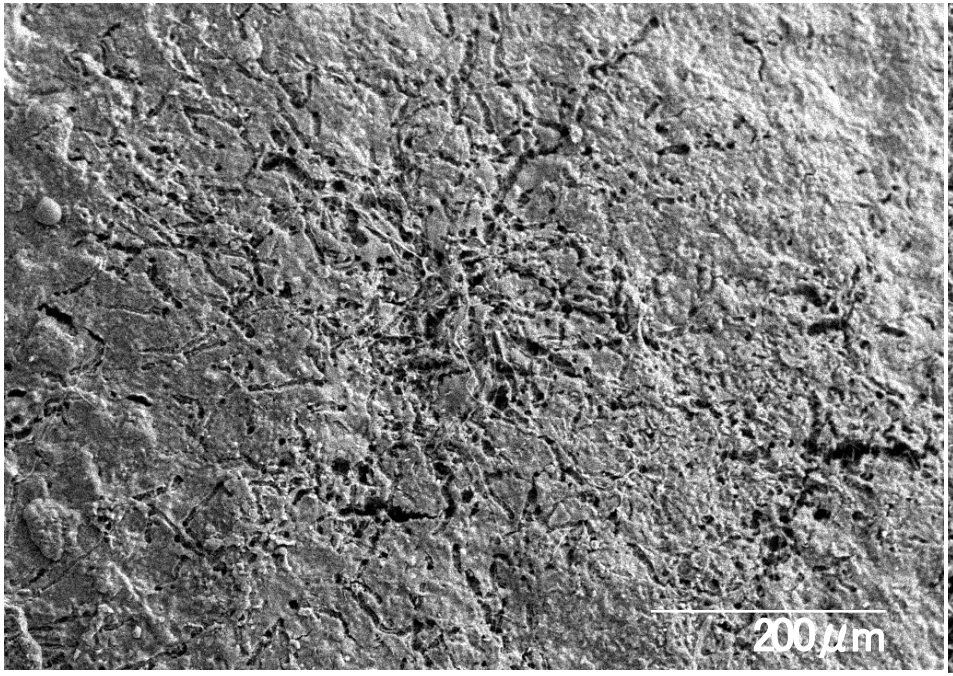
Souhaze *Tsukihaze* *Nurihaze*

Higher enzyme activity

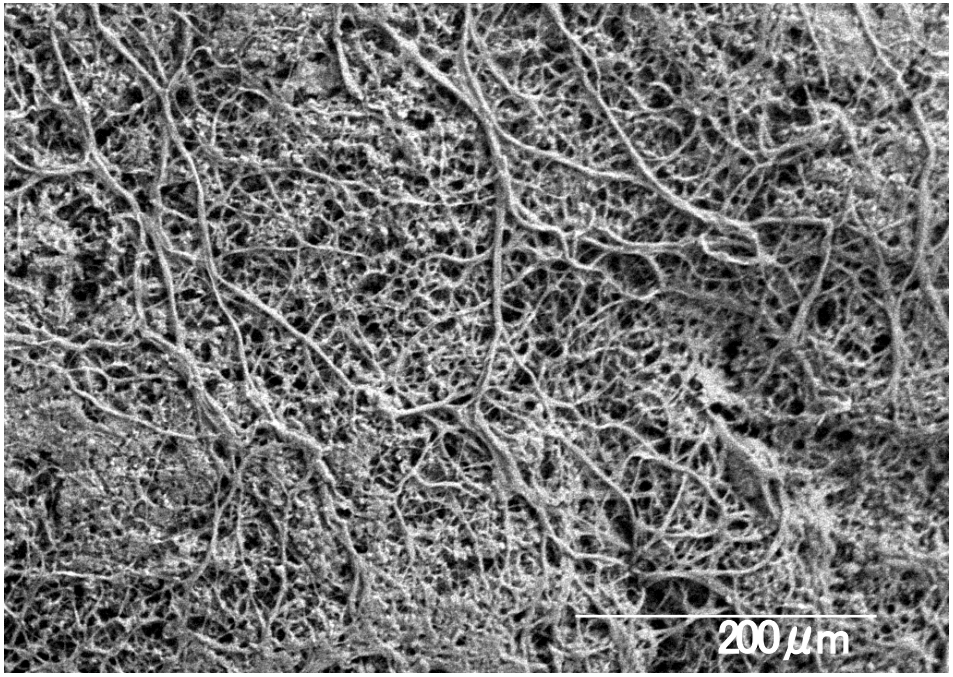
Lower enzyme activity

Yoshii & Aramaki, *J. Brew. Soc. Jpn.*, **96**, 806–813 (2001)

Growth of koji mold on steamed rice



21 hr after inoculation

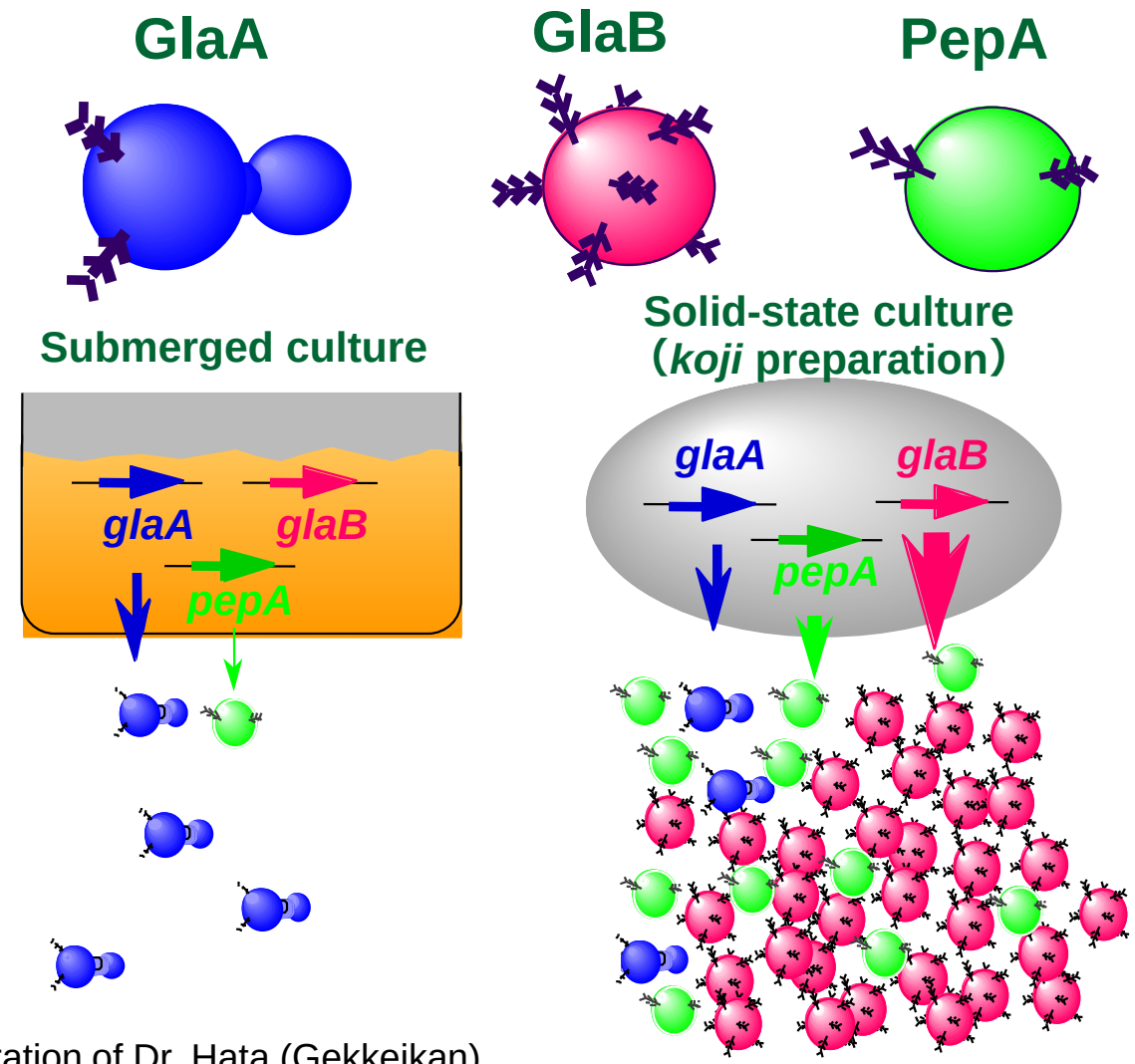


34 hr after inoculation

Yoshii & Aramaki, *J. Brew. Soc. Jpn.,* **96**, 806–813 (2001)

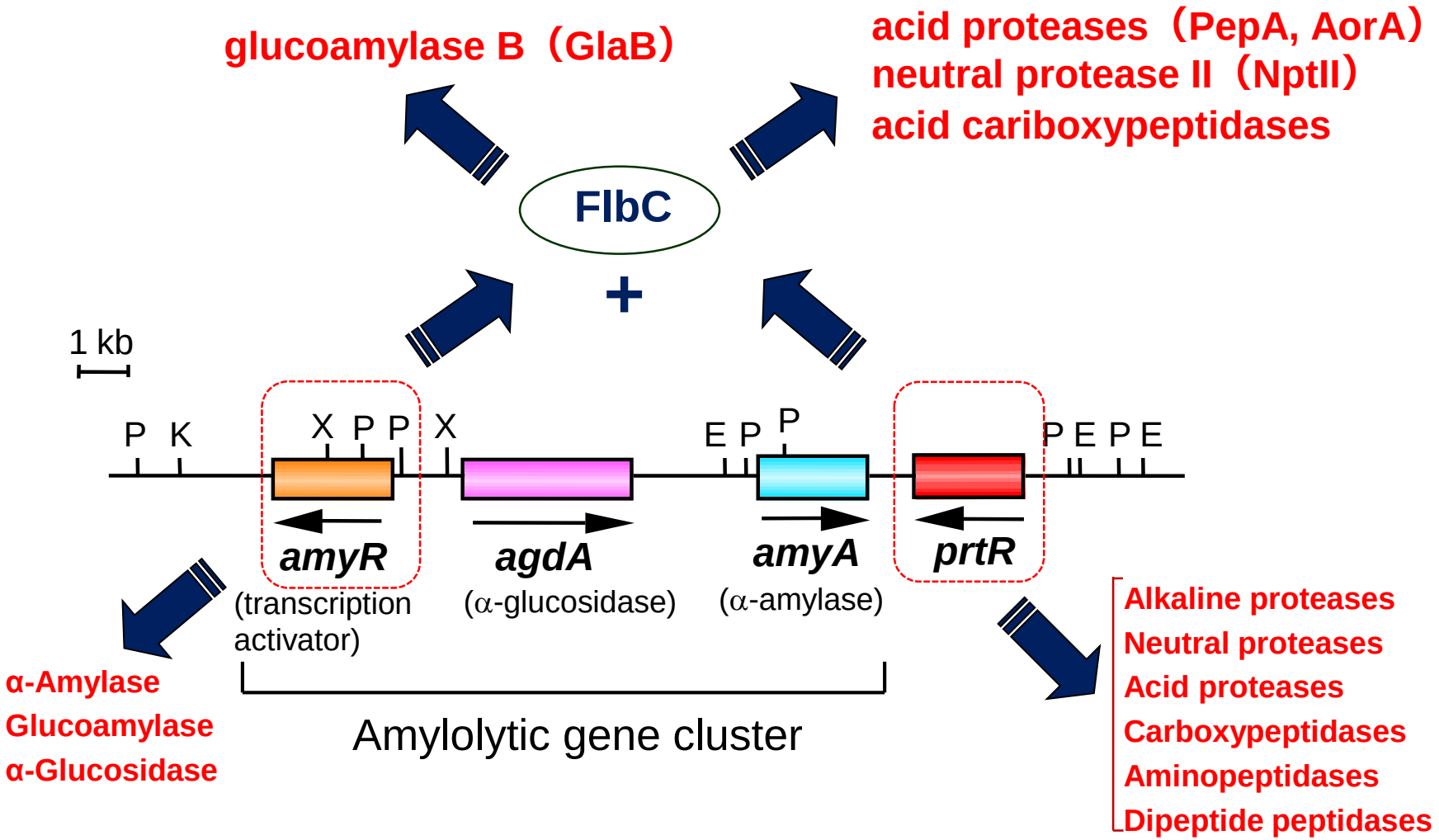
Why we employ the solid-state culture (koji)?

Ans: Glucoamylase and protease genes are specifically expressed in solid-state culture



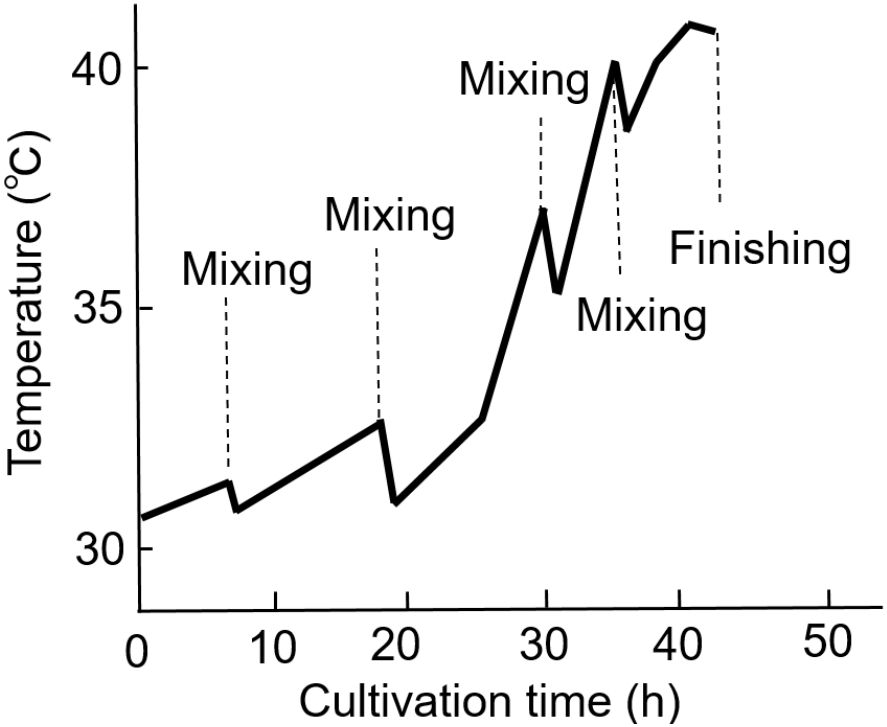
modified the illustration of Dr. Hata (Gekkeikan)

FlbC regulates glucoamylase and protease genes specifically expressed in solid-state culture

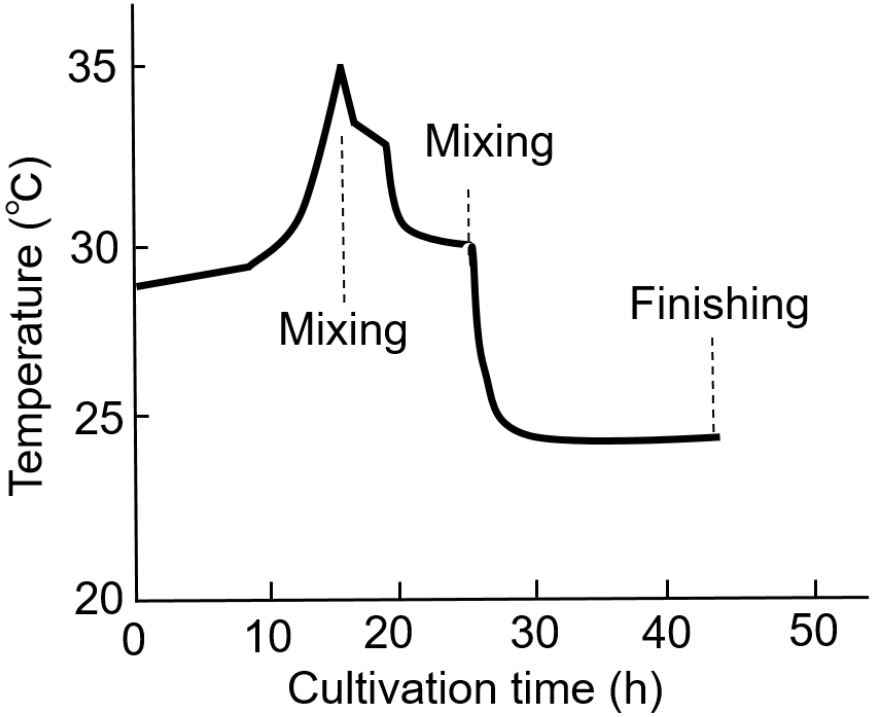


Temperature control in koji preparation process

Koji for sake



Koji for soy sauce



Optimal temperature suitable for the production of amylolytic (35-40°C) and proteolytic (25-30°C) enzymes is different each other.

In rice koji for sake, the temperature is kept high to produce high levels of amylases and low levels of proteases, whereas for soy sauce koji, the opposite temperature profile is employed.

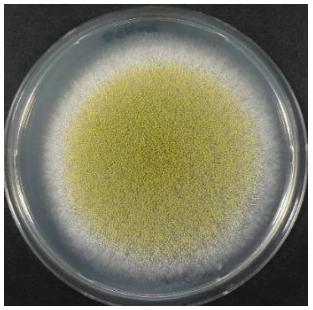
Evil twin of *Aspergillus oryzae* = *Aspergillus flavus*

Aspergillus flavus

- infects on cereals such as nuts, corn, rice and so on.
- produces potent carcinogenic compound, aflatoxin (*A. fla*vus + toxin).

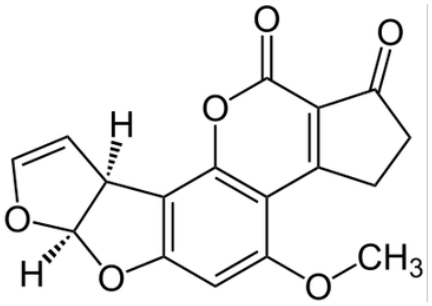
  In 1960, aflatoxin was found through the incident involving mass death of turkeys in the UK due to contamination of peanut meal feed by *A. flavus*.

- quite difficult to distinguish from *A. oryzae* based on morphological characteristics, although diameter of conidia: *A. oryzae* > 5∼6 μm, *A. flavus* < 4∼5 μm.
- homology of both genomes is 99.5%.



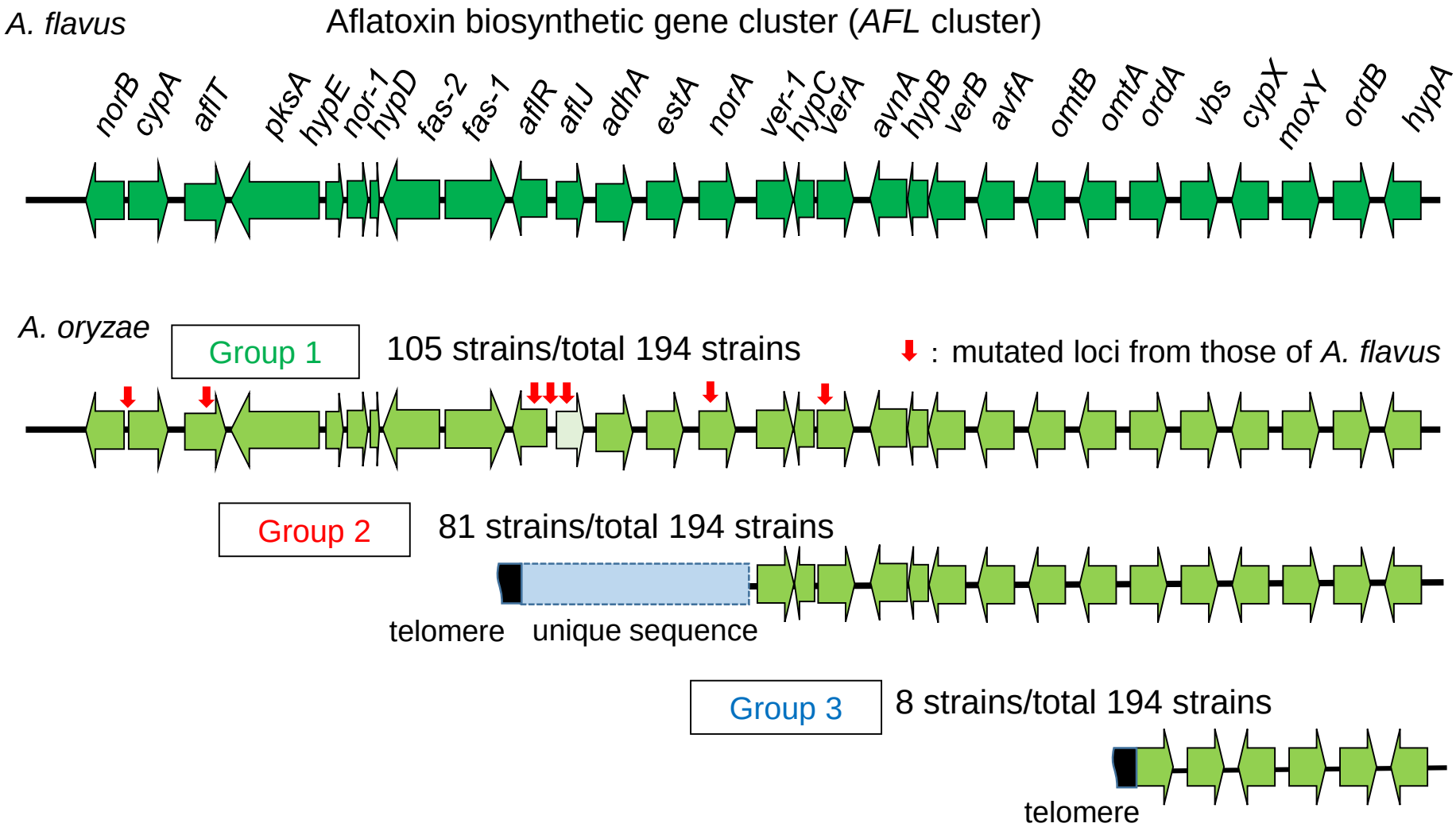
A. oryzae

A. flavus



aflatoxin B_1

Non-aflatoxigenic *A. oryzae* revealed by genome analysis

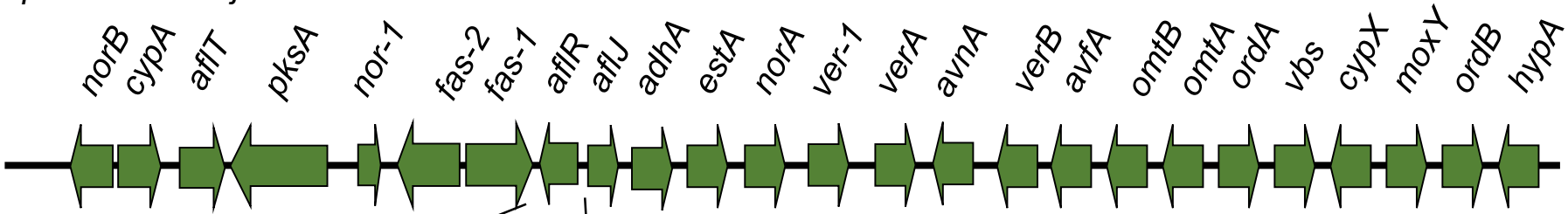


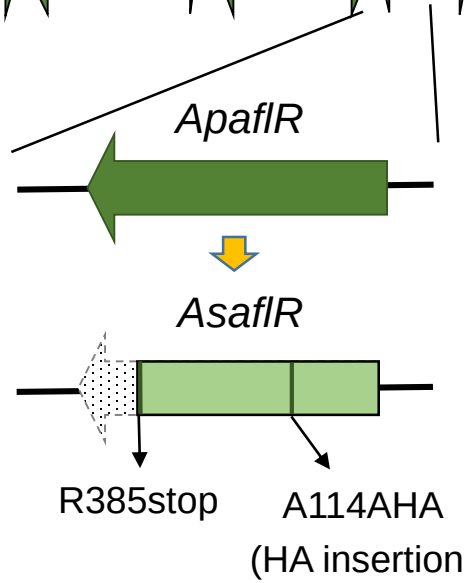
Group 2 and 3 cannot produce aflatoxin due to the deletion of most genes in the *AFL* cluster.

Group 1 cannot produce aflatoxin due to the loss of function caused by mutations in *aflJ*.

Non-aflatoxigenic *A. sojae* revealed by genome analysis

A. parasiticus/sojae





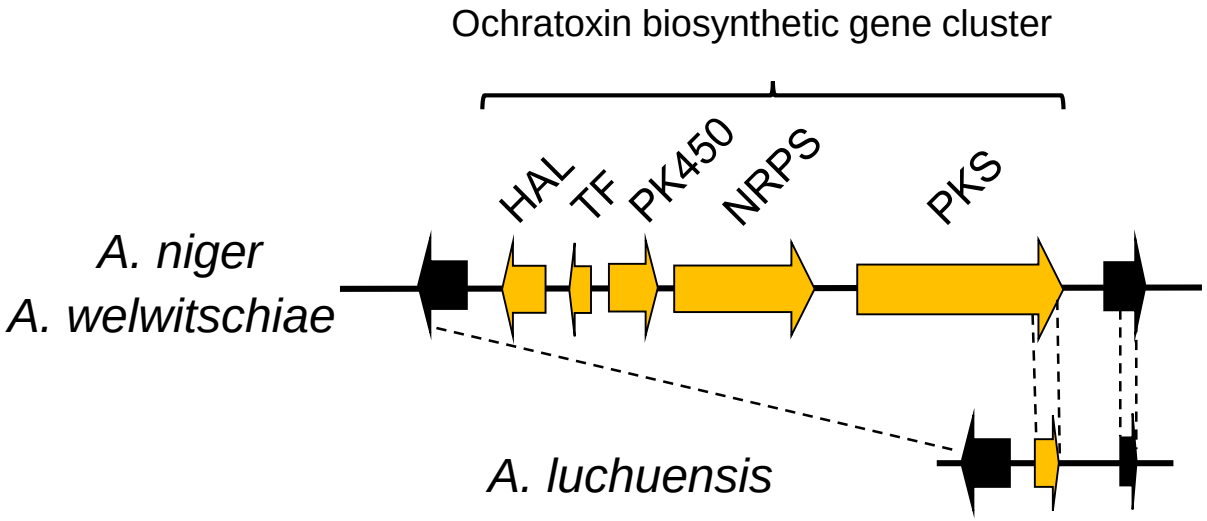
Strains	YES media(ppb)				Koji (ppb)			
	B_1	B_2	G_1	G_2	B_1	B_2	G_1	G_2
A. sojae								
ATCC 201946	–	–	–	–	–	–	–	–
ATCC 201938	–	–	–	–	–	–	–	–
ATCC 201940	–	–	–	–	–	–	–	–
ATCC 201939	–	–	–	–	–	–	–	–
ATCC 201941	–	–	–	–	–	–	–	–
ATCC 201942	–	–	–	–	–	–	–	–
ATCC 201943	–	–	–	–	–	–	–	–
ATCC 201944	–	–	–	–	–	–	–	–
ATCC 201945	–	–	–	–	–	–	–	–
strain 477	–	–	–	–	–	–	–	–
A. parasiticus								
IFO 4082	24.1	2.5	314.7	22.5	190.2	6.6	2.3	27.5
IFO 30179	352.9	29.6	1380	139.4	24.0	0.3	13.9	1.8

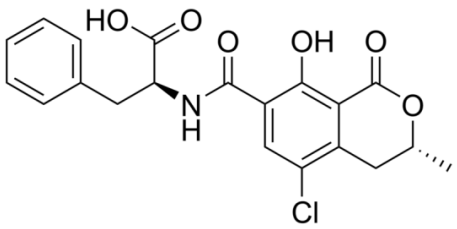
–, not detected.

A. sojae cannot produce aflatoxin due to the deletion of C-terminal region of *aflR.*

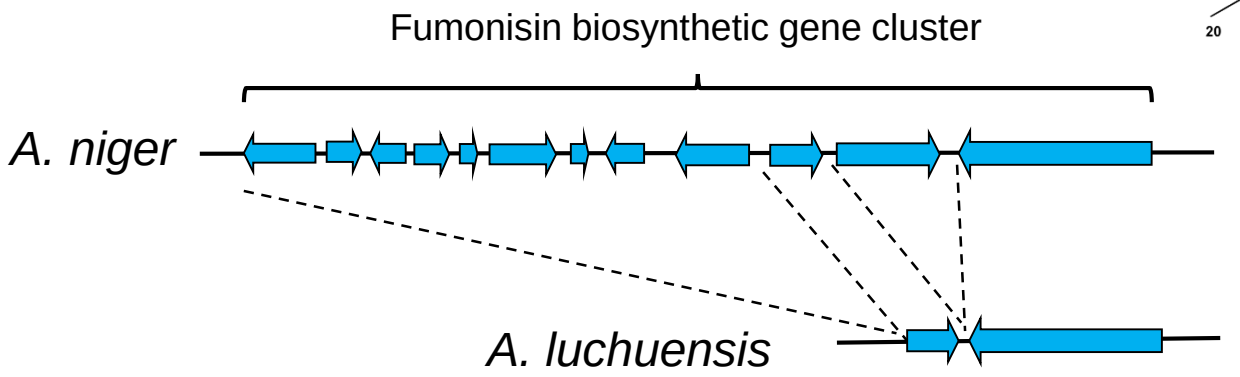
Matsushima, *J. Brew. Soc. Jpn.,* **97**, 559–566 (2002)

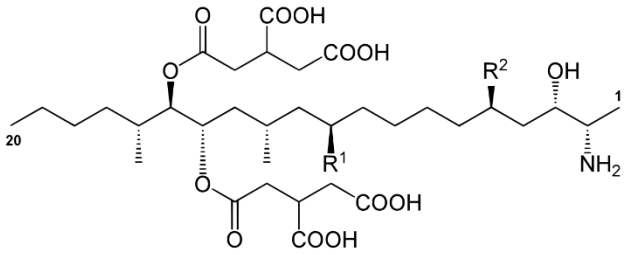
A. luchuensis does not produce mycotoxins





A. luchuensis does not produce ochratoxin due to the deletion of all the biosynthetic gene cluster.





A. luchuensis does not produce fumonisin due to the deletion of most of the biosynthetic gene cluster.

Not to say “Take home message”, but…..

Isolating and using koji mold strains from the natural environment should be done with caution.

Just because the *A. oryzae* strains preserved by koji starter manufacturers do not produce aflatoxins, it does not necessarily mean that strains living in natural environments do not produce aflatoxins.

✔ It cannot be ruled out that a new strain considered to be *A. oryzae* isolated from the natural environment may produce mycotoxins such as aflatoxins.

✔Since it is not easy to distinguish between *A. oryzae* and *A. flavus* based on morphological characteristics, there is a risk of misidentification.

✔Due to recent global warming, the proportion of *A. flavus* isolates has also been increasing even in temperate regions including Japan.

It is desirable to select and use *A. oryzae* strains suitable for the purpose from the many strains stored in koji starter manufacturers.

However, if isolating and using strains from the natural environment that are considered to be *A. oryzae*, it is required to confirm that they are indeed *A. oryzae* through genomic analysis and to thoroughly verify that they do not produce mycotoxins, including aflatoxins.

Thank you for your attention!