

## Studies on the Fungi in Stored Rice

T.I. Mheen, H.S. Cheigh, A.N. Rangunathan\* and K.S. Majumder\*

Korea Advanced Institute of Science and Technology, Seoul, Korea

\*Central Food Technological Research Institute, Mysore, India

(Received August 12, 1982)

## 貯藏米穀中の菌類에 관한 研究

민태익, 최홍식, 에이 엔 라구나탄\*, 에스 케이 마줌다\*

韓國科學技術院 食品生物工學硏究部

\* 印度 中央食品技術硏究所

(1982년 8월 12일 수리)

### Abstract

In order to prevent the losses of the rice by fungal deterioration during storage, fungal contaminants were isolated and identified from the grain samples (Milyang Nr. 23) stored for seven months from December, 1978 to June, 1979 in silo, flat store and Tongari.

Out of thirty cultures isolated from Korean paddy and brown rice samples, twenty seven species were identified, and there are eleven species of *Aspergillus* (*A. caespitosus*, *A. candidus*, *A. chevalieri*, *A. fischeri*, *A. fumigatus*, *A. flavus*, *A. nidulans*, *A. oryzae*, *A. ruber*, *A. sydowii*, *A. versicolor*), five species of *Penicillium* (*P. atramentosum*, *P. chrysogenum*, *P. cyaneofulvum*, *P. notatum*, *P. steckii*), two species of each *Alternaria* (*Al. faeiculata*, *Al. grisea*) and *Curvalaria* (*C. interseminata*, *C. tetramea*), and one species of each *Trichothecium roseum*, *Nigrospora sphaerica*, *Rhizopus nigricans*, *Fusarium* spp., *Mucor* spp., *Helminthosporium* spp., and *Gliocladiopsis* spp.

The major types of fungi grown on the surface of paddy during storage were *A. flavus* and *A. candidus*, while *A. ruber* and *A. sydowii* appeared in brown rice samples. And also *A. candidus*, *A. versicolor* and *A. glaucus* groups were considered as major deteriorating microorganisms in stored brown and paddy rice in Korea.

### Introduction

Generally the attention in food grain storage was used to be focussed on rodent and insect damage. However, when the grain is stored in large bulks in silos, bins, ware-houses or other types of storage structures under different weather conditions, the attention has to be necessarily on microbial infection. Because the micro-ecological conditions are more conducive to microbial development than to insects.

Rice is a major crop in Korea while a little work has been reported in the area of storage losses in rice grain, specially by storage fungi. Some of the storage fungi are also known to produce mycotoxins on foodgrains.

Cho *et al* have initiated the study on the types of deterioration of polished rice, and isolated many species of fungi in the storage.<sup>1)</sup> They have concluded that the main deteriorating microorganisms during storage are few species of *A. glaucus* group. Some other work on the preservation of rice by gamma-irradiation,<sup>2-6)</sup> toxin pro-

ducing microorganisms from rice,<sup>7-11)</sup> and changes in rice quality during storage<sup>12)</sup> have been reported by earlier workers.

In connection with a systematic study on the improvement of the post-harvest technology of rice, the causative fungal contaminants in paddy rice and brown rice during storage were reported in this study.

## Materials and Methods

### Samples

Brown rice and paddy samples (variety Milyang Nr 23) which have been stored in silos, flat store and Tongari at natural condition in Asan and Seoul areas from December 1978 to June 1979 were used for the isolation of fungi in the storage. The storage condition and appearance of samples are shown in Table 1.

### Media

The media used for isolation and identification of fungi from stored rices are shown in Table 2.

Malt salt agar (I), Czapek's agar (3 and 20% sucrose, Ila and IIb), and YM agar (III) were used for isolation of

storage fungi, and Czapek's agar (IIa and IIb), Czapek's yeast extract agar (IV), malt extract agar (V), and 25% glycerol nitrate agar (VI) were used for identification of fungi.

### Isolation of storage fungi

After surface sterilization of grains, using perforated stainless steel cups according to the procedure as in Fig. 1, each of the rice and paddy grains were plated on agar plate. Colonies growing on each grain surface at room temperature (18-28°C) and high temperature (45-50°C) were transferred to the same agar slants and stored in refrigerator for further studies.

### Identification of fungi

Identification of the isolate was carried out according to Raper and Fennell<sup>13)</sup> for *Aspergillus* species, Raper and Thom,<sup>14)</sup> and Pitt<sup>15)</sup> for *Penicillium* species, and Gilman<sup>16)</sup> for soil fungi.

One loopful of two week's old culture was transferred to the sterilized semisolid suspension consisting of 0.2% agar, then 0.05% Tween 80 was added and mixed well. This spore suspension was inoculated to each of the

**Table 1. Storage Conditions of Brown and Paddy Rice Samples**

Rice samples	Storage structure*	Storage period	Appearance	Moisture content (%)	Temperature (°C) when sampled
Brown rice (A)	Silo Nr 1	7 months	Good	16.5	19.2
Brown rice (B)	Flat store	7 months	Good	16.5	19.2
Paddy (C)	Silo Nr 3	7 months	Good	16.5	18.0
Paddy (D)	Tongari A	7 months	Medium infected	16.4	20.5
Paddy (E)***	Tongari B (tin plate wrapped)	7 months	Heavily infected	16.5 (18.9)***	20.5 (37.5)***

\*Silo Nr 1 : Steel silo with 8.1m (diameter), 6.3m (height) and 2.4m (grain height).

Flat store Nr 5 : Concrete type flat store with 7.2m x 7.2m (square), 6.6m (height) and 2.95m (grain height).

Silo Nr 3 : Steel silo same as silo Nr 1 (3.0m in grain height).

Tongari A : Korean traditional straw bin for paddy storage with 1.2m (diameter), 1.3m (height) and 1.0m (grain height).

Tongari B : Same as Tongari A, which was warped with tin plate (about 50cm height from earth) on the wall.

\*\*Samples were collected at heavily infected part of paddy in Tongari B

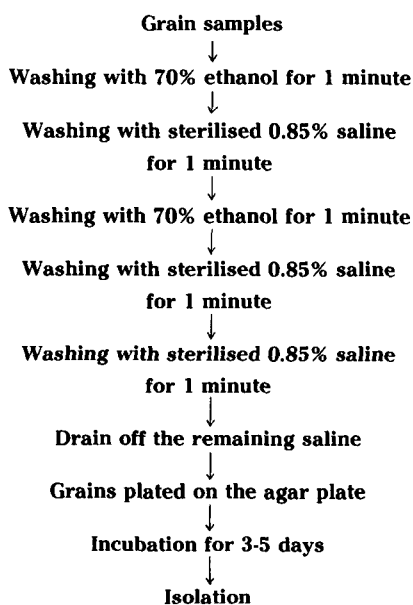
\*\*\*The values in parenthesis indicated the moisture content and temperature at heavily infected part of paddy in Tongari B.

**Table 2. Media Used for Isolation and Identification of Fungi**

Ingredients	Media (g/l)						
	I	IIa	IIb	III	IV	V	VI
Glucose	—	—	—	10	—	—	—
Sucrose	—	30	200	—	30	—	—
Glycerin	—	—	—	—	—	—	250
Malt extract	20	—	—	3	—	20	—
Yeast extract	—	—	—	3	5	—	—
Peptone	—	—	—	5	1	—	—
Sodium chloride	75	—	—	—	—	—	—
Sodium nitrate	—	3	3	—	3	—	2.2500
Dipotassium phosphate	—	1.00	1.00	—	1.00	—	0.7500
Potassium chloride	—	0.50	0.50	—	0.50	—	0.3750
Magnesium sulphate	—	0.50	0.50	—	0.50	—	0.3750
Feric sulphate	—	0.01	0.01	—	0.01	—	0.0075
Agar	20	15	15	15	15	15	15
Distilled water (lit)	1	1	1	1	1	1	1

testing agar medium and incubated at different temperatures, *viz.* room temperature (18-28°C), 37°C and 5°C.

After 7 and 10 days of incubation, morphological and growth characteristics of *Aspergillus*, *Penicillium* and other soil fungi were examined.



**Fig. 1. Procedure of Isolation of Internal Storage Fungi.**

## Results and Discussion

### Fungal flora of rice grains

The fungi isolated from Korean brown rice and paddy samples stored for 7 months in silo, flat store, and Tongari are shown in Table 3.

Out of the total fungi isolated 27 species were identified. The identified fungi are eleven species of *Aspergillus* (*A. caespitosus*, *A. candidus*, *A. chevalieri*, *A. fischeri*, *A. fumigatus*, *A. flavus*, *A. nidulans*, *A. oryzae*, *A. ruber*, *A. sydowii*, *A. versicolor*), five species of *Penicillium* (*P. atramentosum*, *P. chrysogenum*, *P. cyaneofurum*, *P. notatum*, *P. steckii*), two species of each *Alternaria* (*Al. faeniculata*, *Al. grisea*) and *Curvalaria* (*C. interseminata*, *C. tetramea*). *Trichothecium roseum*, *Nigrospora sphaerica*, *Rhizopus nigricans*, *Fusarium* spp., *Mucor* spp., *Helminthosporium* spp., and *Gliocladiopsis* spp. are also found.

Comparing the brown rice and paddy, paddy had more field fungi such as *Alternaria*, *Nigrospora*, *Cur-*

**Table 3. Types of Storage Fungi from Korean Brown Rice and Paddy**

Rice samples	Storage fungi	Media					
		I	IIa	IIb	III	I*	
		**					
<b>Brown rice</b> (Silo)	<i>A. caespitosus</i>	3/30	—	—	—	—	
	<i>A. fumigatus</i>	—	3/30	—	—	—	
	<i>A. ruber</i>	1/30	—	—	—	—	
	<i>A. versicolor</i>	1/30	—	—	—	—	
Brown rice (flat store)	<i>A. caespitosus</i>	1/30	—	—	—	—	
	<i>A. nidulans</i>	—	—	—	—	1/30	
	<i>A. sydowii</i>	1/30	—	—	—	—	
	<i>A. versicolor</i>	—	—	1/30	—	—	
Paddy (Silo)	<i>A. chevalieri</i>	7/25	—	—	—	—	
	<i>A. fumigatus</i>	—	1/15	2/15	—	—	
	<i>P. chrysogenum</i>	1/25	—	—	—	—	
	<i>Nigrospora sphaerica</i>	10/25	—	5/15	3/15	—	
	<i>Curvalaria interseminata</i>	—	1/15	—	—	—	
	<i>Curvalaria tetramea</i>	2/25	—	—	—	—	
	<i>Alternaria grisea</i>	1/25	—	—	—	—	
	<i>Fusarium</i> spp.	—	10/15	3/15	2/15	—	
	<i>Mucor</i> spp.	—	—	2/15	—	—	
	Paddy (Tongari A)	<i>A. oryzae</i>	4/25	—	—	—	—
<i>A. versicolor</i>		3/25	—	—	—	—	
<i>A. fischeri</i>		—	—	12/15	—	9/20	
<i>P. cyaneofurvum</i>		10/25	—	—	—	—	
<i>P. notatum</i>		—	—	2/15	—	—	
<i>P. steckii</i>		—	1/15	—	—	—	
<i>Mucor</i> spp.		13/25	11/15	1/15	15/15	11/20	
<i>Fusarium</i> spp.		8/25	—	—	5/15	—	
Paddy (Tongari B)		<i>A. candidus</i>	7/25	—	—	—	—
		<i>A. flavus</i>	2/25	1/15	—	—	—
	<i>A. fumigatus</i>	—	2/25	—	—	—	
	<i>A. versicolor</i>	1/25	—	—	—	—	
	<i>A. ruber</i>	1/25	—	—	—	—	
	<i>P. atramentosum</i>	—	—	3/15	—	—	
	<i>P. notatum</i>	—	1/15	—	—	—	
	<i>Trichothecium roseum</i>	—	—	1/15	—	—	
	<i>Curvalaria teramea</i>	2/25	—	—	—	—	
	<i>Nigrospora sphaerica</i>	—	1/15	—	—	—	
	<i>Rhizopus nigricans</i>	1/25	—	—	—	—	
	<i>Alternaria faeniculata</i>	—	—	—	2/25	—	
	<i>Fusarium</i> spp.	—	8/15	1/15	—	—	
	<i>Helminthosporium</i> spp.	1/25	1/15	—	—	—	
	<i>Mucor</i> spp.	—	—	—	—	4/20	
<i>Gliocladiopsis</i> spp.	—	—	1/15	—	—		

\*45-50°C incubation

\*\*Nr of infected grains/Nr of grains plated

*valaria*, *Fusarium*, and *Mucor* spp. Among infected paddy, the microflora were more variable in Tongari B wrapped with tin plate than those stored in silo, flat store and Tongari A.

Among the media used, malt salt agar (Table 2, I) was the best for the isolation of storage fungi. YM agar (III) was not good for the isolation of storage fungi such as *Aspergillus* and *Penicillium*, other field fungi were growing very rapidly on this medium than *Aspergilli* or *Penicillia*.

Table 4 shows the storage fungi of the brown rice and paddy. The majority of fungi from paddy were *A. flavus* and *A. candidus*, but *A. glaucus* group was predominant in brown rice.

**Table 4. Types of Surface Fungi Growing from Korean Brown Rice and Paddy**

Rice samples	Surface fungi	Percent species. Isolated out of grains
Paddy	<i>A. flavus</i>	80.0
	<i>A. candidus</i>	33.3
	<i>A. versicolor</i>	10.0
	<i>A. steckii</i>	6.7
Brown rice	<i>A. ruber</i>	73.3
	<i>A. sydowii</i>	13.3
	<i>A. versicolor</i>	6.7
	<i>P. chrysogenum</i>	6.7
	<i>Nigrospora sphaerica</i>	3.0
	<i>Rhizopus nigricans</i>	3.0

Note: Medium I used, and 30 grains plated without surface sterilization.

The occurrence of species of *Actinomucor*, *Mucor*, *Syncephalastrum*, *Rhizopus*, *Alternaria*, *Curvalaria*, *Helminthosporium*, *Cladosporium*, *Fusarium*, *Aspergillus* and *Penicillium* in rice have been reported by earlier workers,<sup>1, 17-21</sup> Reported microflora among the genus *Aspergillus* and *Penicillium* were as follow: 22 species of *Aspergillus* (*A. awamori*, *A. amstelami*, *A. candidus*, *A. carneus*, *A. cervinus*, *A. chevalier*, *A. flavus*, *A. flavipes*, *A. fumigatus*, *A. glaucus*, *A. montevidensis*, *A. niger*, *A. nidulans*, *A. oryzae*, *A. ochraceus*, *A. parasiticus*, *A. penicilloids*, *A. ruber*, *A. sydowii*, *A. terreus*, *A. ustus*, *A. versicolor*) and 13 species of *Penicillium* (*P. citrinum*, *P. chrysogenum*, *P. commune*, *P. cyclopium*, *P. frequentans*, *P. implicatum*, *P. islandicum*, *P. lanosum*, *P.*

*mickzenskii*, *P. notatum*, *P. oxalicum*, *P. rugulosum*, *P. simplicissimum*).

On the other hand Cho *et al* identified 14 species of *Aspergillus*, 9 species of *Penicillium*, 4 species of *Bacillus*, 3 species of *Brevibacterium*, and one species of *Pseudomonas* and *Kurtia* from the 27 specimens of deteriorated stored polished rice in Korea and also they reported that the main deteriorating microorganisms during storage were those few species of *A. glaucus* group.<sup>1)</sup>

In this study, *A. caespitosus*, *P. cyaneofurum*, *P. steckii*, *P. atramentosum*, and the species of *Trichothecium*, *Nigrospora* and *Gliocladiopsis* were isolated from Korean brown rice and paddy samples for the first time. However, *A. niger* and *P. islandicum* those were reported in previous research were not found. And also *A. candidus*, *A. versicolor* and *A. glaucus* groups were considered as a major deteriorating microorganisms in stored brown rice and paddy in Korea.

## 要 約

米穀貯藏中 菌類에 의한 米穀의 損失을 防止 하기 위하여 1978년 12月 부터 1979年 6月까지 7個月間 싸일로, 平倉庫 및 통가리에 貯藏한 米穀試料(밀양 23号)에서 菌類를 分離, 同定하였다.

韓國産 벼 및 玄米에서 分離된 30種의 菌類中, *Aspergillus*屬 11種(*A. caespitosus*, *A. candidus*, *A. chevalieri*, *A. fischeri*, *A. fumigatus*, *A. flavus*, *A. nidulans*, *A. oryzae*, *A. ruber*, *A. sydowii*, *A. versicolor*), *Penicillium*屬 5種(*P. atramentosum*, *P. chrysogenum*, *P. cyaneofulvum*, *P. notatum*, *P. steckii*), *Alternaria*屬 2種(*Al. faesiculata*, *Al. grisea*), *Curvalaria*屬 2種(*C. interseminata*, *C. tetramea*), *Trichothecium roseum*, *Nigrospora sphaerica*, *Rhizopus nigricans*, *Fusarium* spp., *Mucor* spp., *Helminthosporium* spp. 및 *Gliocladiopsis* spp. 各 1種, 總 27種이 同定, 確認되었다.

韓國産 벼에서 分離된 表面菌類는 *A. flavus* 와 *A. candidus*가 大部分이었으며, 玄米에서는 *A. sydowii*가 많이 出現하였다. 또한 貯藏中 變質米에는 *A. candidus*, *A. versicolor* 및 *A. glaucus* group들이 주로 많았다.

## References

- 1) Cho, D.H., J.K. Chun and Y.B. Kim: *J. Korean Agr. Chem. Soc.*, **15(3)**, 193-198 (1972).
- 2) Kim, H.S. and Y.R. Choi: *J. Korea Assoc. Food Sci.*, **1(1)**, 51-61 (1969).
- 3) Kim, H.S., Y.R. Choi, S.K. Kim and I.J. Harn: *J. Korea Assoc. Food Sci.*, **2(1)**, 104-112 (1970).
- 4) Kim, H.S., Y.R. Choi, S.K. Kim and I.J. Harn: *J. Korea Assoc. Food Sci.*, **2(1)**, 113-120 (1970).
- 5) Kim, H.S., S.K. Kim and I.J. Harn : *Korean J. Food Sci. Technol.*, **3(1)**, 15-18 (1971).
- 6) Kim, H.S. and S.K. Kim: *J. Food Sci. Technol.*, **3(1)**, 19-24 (1971).
- 7) Lee, K.Y. and S.R. Lee: *Korean J. Food Sci. Technol.*, **7(1)**, 7-10 (1974).
- 8) Lee, K.Y., Y.B. Kim and S.R. Lee: *Korean J. Food Sci. Technol.*, **7(1)**, 7-10 (1975).
- 9) Choi, E.H., H.L. Kim and S.R. Lee: *Korean J. Food Sci. Technol.*, **7(3)**, 148-153 (1975).
- 10) Choi, E.H. and S.R. Lee: *Korean J. Food Sci. Technol.*, **7(3)**, 145-158 (1975).
- 11) Kim, Y.H. and S.R. Lee: *Korean J. Food Sci. Technol.*, **10(1)**, 46-51 (1978).
- 12) Hwangbo, J.S. and S.R. Lee: *Korean J. Food Sci. Technol.*, **8(2)**, 74-79 (1976).
- 13) Raper, K.B. and D.I. Fennell: "*The genus Aspergillus*". The Williams and Wilkins Company (1965).
- 14) Raper, K.D. and C. Thom: "*A manual of the Penicillia*", The Williams and Wilkins Company (1965).
- 15) Pitt, J.J.: *Mycologia*, **65**, 1135-1157 (1973).
- 16) Gilman, J.C.: "*A manual of soil fungi*", Oxford and IBH Publishing Company (1959).
- 17) Majumder, S.K. et al: *Final technical report, Project No. A7-MQ-12* (1966-1972). CFTRI, Mysore, India (1974).
- 18) Ghosh, J.J.: *Science and Culture*, **17**, 523-533 (1951).
- 19) Iizuka, H.: *J. Gen. Appl. Microbiol.*, **3(2)**, 146-161 (1957).
- 20) Christensen C.N. and H.H. Kaufmann: *Ann. Rev. Phytopathol.*, **3**, 69-84 (1965).
- 21) Hasany S.M., M. Yousuf and S.S. Husain: *Pakistan J. Sci. Ind. Res.*, **11**, 288-293 (1968).