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Environmental Factors influencing the Production of Fusarenon-X, a Cytotoxic Mycotoxin of *Fusarium nivale* Fn 2B

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In order to obtain a large amount of fusarenon-X, a cytotoxic mycotoxin of *Fusarium nivale* Fn 2B, environmental factors influencing the production of the toxin were investigated with the following results. 1) With the stationary culture of *F. nivale* on Czapek medium, the supplemented peptone or yeast extract increased the lethal toxicity of culture filtrate to mice. 2) Peptone-supplemented Czapek medium (PSC medium) provided all necessary ingredients for the high level production of fusarenon-X. 3) With PSC medium, the maximal yield of fusarenon-X was observed 6—8 days of cultivation at 27°, and the yield decreased at lower temperature. 4) Rice, barley, wheast and corn supported the production of the toxin, and the highest toxicity was observed with rice grains.

Fusarium nivale Fn 2B isolated from actually damaged wheat[2] produces a cytotoxic fusarenon-X (3,7,15-trihydroxyscirp-4-acetoxy-9-en-8-one) in peptone-supplemented Czapek medium.[3,4] Toxicological investigation revealed that the toxin is highly irritant to skin[5] and induces radiomimetic injury characterized with cellular degradation and karyorrhexis of bone marrow, thymus, intestinal mucosa, ovary and testis.[4] Biochemical examination proved that this mycotoxin inhibited DNA and protein synthesis in Ehrlich ascites tumor[4] and rabbit reticulocytes.[6]

In the present paper, with an aim to obtain an enough amount of the toxin for toxicological examination, the authors investigated environmental factors influencing a toxicity of the fungus as well as formation of the toxin, revealing the following characteristics of the fungus thereupon.

Experimental

Methods

Fungus——*F. nivale* Fn 2B[2] was used throughout the experiment. The culture was inoculated on potato-dextrose agar, and incubated at 27° for 2 weeks. In some experiments, it was cultivated on sterile polished rice grains, and the moldy rice was used directly as an inoculum. Repeated experiments indicated that the latter method was favorable for a maintainance of toxicity of the fungus.

Culture——In case of liquid culture, a Roux type flask containing 200 ml of a liquid medium was sterilized for 20 min, and inoculated with the spore or the grains of the moldy rice. At least two flasks were run to obtain each result. After a stationary cultivation, a mycelium was washed with water and dried at 60° overnight to measure the dry weight, and a culture medium was filtrated over filter paper to give culture

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2) H. Tsunoda, N. Toyazaki, S. Morooka, N. Nakano, H. Yoshiyama, K. Ohkubo and M. Isoda, *Proc. Food Res. Inst.*, **23**, 89 (1968).
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filtrate. In case of a solid culture, a 500 ml-Erlenmyer flask, containing 200 g of polished rice grains which were previously immersed in water for 30 min, was autoclaved for 20 min, and the fungus was cultivated for desired intervals at different temperature. After the cultivation, the moldy rice grains were dried at 60° overnight, and powdered.

Preparation of the Crude Toxin——Preparation of a crude toxin from culture filtrate or moldy rice grains was conducted by the method summarised below; In case of a liquid culture, the culture filtrate was mixed with 1% (w/v) of active charcoal, and stood with occasional shaking in a cold room. After overnight, the charcoal filtrated was immersed in 10 volumes (v/w) of methanol for one day. Evaporation of the yellowed methanol solution gave brown materials from which hot-methanol insoluble materials were removed. By the addition of 5 volumes of chloroform to the methanol solution, a yellow precipitate was eliminated and the methanol-chloroform soluble materials remained were referred to as "crude toxin". In case of a solid culture, the moldy grains powdered were immeresed in 3 volumes of *n*-hexane overnight to eliminate a lipoid material, and the residue was mixed with 3 volumes of methanol. After standing overnight, the deep reddish methanol solution was evaporated to dryness, and the resulting brown material was suspended in 100 volumes of water to mix with 1% (w/v) of active charcoal. The following procedure was the same as in the case of the liquid culture described above.

Toxicity Test——Male mice of ddS strain, weighing 20—25 g, were administered intraperitoneally with the culture filtrate, the crude toxin or the fractionated material, and the mortality was determined 7 days after administration. Usually 3—5 mice were used for a each test sample.

Chemicals——Diacetoxyscirpenol (4,15-diacetoxyscirp-9-en-3-ol) and nivalenol (3,4,7,15-tetrahydroxy-scirp-9-en-8-one) were kindly provided by Dr. Taescheler (Sandoz LTD., Basel) or Dr. Tatsuno (the Institute of Physical and Chemical Research, Saitama), respectively.

Results

Thin-Layer Chromatography of Scirpen Toxins

Rf values of fusarenon-X, nivalenol and diacetoxyscirpenol in different sorts of developer were listed in Table I. In all developers, three toxins separated each other onplate of Kieselgel G.

Table I. *Rf* Values of the Scirpen Toxins in Silicagel Chromatography

Developers	Fusarenon-X	Nivalenol	Diacetoxyscirpenol
Chloroform–Methanol (5:1)	0.89	0.44	0.95
Chloroform–Methanol (97:3)	0.19	0.05	0.55
Ethylacetate–Toluene (3:1)	0.36	0.09	
Ethylacetate–*n*-Hexane (3:1)	0.37	0.17	0.63

Thin-layer chromatography was carried out with Kieselgel G and developers listed in the Table.

Among four systems, chloroform: methanol (5:1) and ethylacetate: *n*-hexane (3:1) were generally used for the detection of toxins in the following experiments. Spot test with H_2SO_4 showed time dependent change of color as follows: fusarenon-X; purple to brown, nivalenol; pink to brown, diacetoxyscirpenol; brown. In the case of the last toxin, the heated spot gave a skylightblue under an ultraviolet lamp after spraying of H_2SO_4.

Lethal Toxicity of Culture Filtrate in Different Medium

Fusarium nivale Fn 2B was cultured at 27° for 14 days in liquid media with different kinds of complex nitrogen sources and different pH. A lethal toxicity of cultured broth and yield of fungal mycelia was also measured. As shown in Table II, 0.5 ml/10 g of Czapek, Raulin–Thom and Sabouraud media did not kill the mice, while, when 10 g/liter of peptone or yeast extract was supplemented to Czapek medium, the yield of mycelium was increased and the same dose of the broth was lethal to mice. Pathological examinations revealed that, in the fatal cases with the peptone or yeast extract–supplemented Czapek medium, necrosis of the ileum, karyorrhexis of the thymus, bone marrow and spleen were noted.

These findings indicated that Czapek medium supplemented with peptone or yeast extract favored the production of toxic metabolites which cause affection to rapidly dividing

Table II. Effect of Nitrogenous Extracts and Culture Media on the Toxicity of the Fungus

Culture medium	Initial pH	Final pH	Mycelium (g/200 ml)	Toxicity to mice[a] (dead/used)
Czapek	7.0	5.9	0.11	0/4
Czapek plus peptone[b]	7.0	8.0	1.71	4/4
Czapek plus malt ext.[b]	7.0	6.2	0.26	0/4
Czapek plus yeast ext.[b]	7.0	8.5	1.58	4/4
Raulin–Thom	3.9	7.4	2.80	0/4
Sabouraud	7.0	4.8	1.94	0/4

F. nivale Fn 2B was cultured in Roux-type flask containing 200 ml of the culture medium at 27° for 14 days.
Each value is an average of two flasks.
a) 0.5 ml/10 g was injected intraperitoneally
b) 10 g/liter was supplemented

cells of tissues. Therefore, in the following experiment, the authors used peptone (10 g/liter)–supplemented Czapek (PSC) medium as a standard medium for cultivation of the fungus.

Effects of Carbon and Nitrogen Sources

PSC medium contains sucrose and $NaNO_3$ as a carbon source and a nitrogen source, respectively. In following experiments, the authors replaced these components with other type of compound. Table III showed the effect of varied form of carbon compound on the

Table III. Effect of Carbon Sources

Carbon sources	Mycelium (g/200 ml)	Crude toxin (mg/200 ml)	Toxicity to mice[a] (dead/used) Culture broth at 6 (days)	9 (days)	12 (days)	14 (days)	Crude toxin
Sucrose (control)	2.35	49	2/2	3/3	0/3	0/3	3/3
Glucose	2.02	335	0/2	0/3	0/3	0/3	1/3
Fructose	2.27	49	0/2	0/3	0/3	0/3	0/3
Sorbitol	1.05	72	0/2	0/3	0/3	0/3	2/3
Mannitol	1.02	85	0/2	0/3	0/3	0/3	0/3
Galactose	2.13	83	0/2	0/3	0/3	0/3	3/3
Xylose	1.80	151	0/2	0/3	0/3	0/3	3/3
Maltose	2.37	56	0/2	1/3	0/3	0/3	1/3
Lactose	1.20	172	0/2	0/3	3/3	0/3	1/3
Starch	1.75	66	0/2	0/3	3/3	0/3	2/3
Glycerol	1.00	58	0/2	0/3	0/3	0/3	3/3

F. nivale Fn 2B was cultured on PSC medium at 27° for 14 days. Sucrose in the medium was replaced by carbon compounds listed in the Table.
a) 0.5 ml of the culture filtrate or 5 mg/10 g of the crude toxin was administered intraperitoneally.

toxicity of culture filtrate and the yield of crude toxin. All the carbon compounds except mannitol and fructose supported both growth of the fungus and production of the crude toxin. Toxicity test conducted at the 6th, 9th, 12th and 14th day of the cultivation resulted that in the case of sucrose the lethal effect appeared in the early stage (6 to 9th day) of the culture. Whereas, in cases of lactose and starch it appeared in the later stage (12 to 14th day). When the concentration of sucrose in the medium was changed ranging from 15 g/liter to 60 g/liter, as shown in Table IV, maximal yields of the mycelium and crude toxin were attained at 30 g/liter of sucrose, and no lethality was observed when sucrose was ommitted from the medium. Noticeable finding was that the final pH of PSC medium declined in parallel to the content

Table IV. Effect of the Concentration of Sucrose

Sucrose (g/liter)	pH	Mycelium (g/200 ml)	Crude toxin (mg/200 ml)	Toxicity[a] (dead/used)		TLC[b]	
				Culture filtrate	Crude toxin	F-X	N
0	8.4	1.50	99	0/3	0/3	−	−
15	7.5	1.52	100	0/3	3/3	+	+
30	6.7	2.00	185	2/3	3/3	++	+
45	6.5	1.63	172	2/3	3/3	+++	−
60	6.1	1.83	129	2/3	3/3	+++	−

The fungus was cultured at 27° for 10 days in PSC medium with varied concentration of sucrose.
a) 0.5 ml/10 g of the culture filtrate or 5 mg/10 g of the crude toxin was injected to mice
b) F-X: fusarenon-X, N:nivalenol

of sucrose added, and that nivalenol was not detected in the crude toxin when the final pH was below 6.5.

Effect of varied form of nitrogen sources was examined, as shown in Table V. All the nitrogen compounds supported the fungal growth and the production of toxic metabolites.

Table V. Effects of Nitrogens

Nitrogen	Mycelium (g/200 ml)	Crude toxin (mg/200 ml)	Toxicity to mice[a] (dead/used)			
			Culture broth at			Crude toxin
			6th	9th (day)	14th	
$NaNO_3$ (control)	1.93	70	2/2	2/2	2/2	3/3
NH_4Cl	2.03	59	1/2	2/2	2/2	3/3
NH_4NO_3	1.27	68	0/2	0/2	1/2	2/3
$(NH_4)_2CO_3$	1.47	73	0/2	1/2	1/2	3/3
$(NH_4)_2SO_4$	2.00	55	0/2	2/2	0/2	2/3
$(NH_2)_2CO$	2.03	107	1/2	0/2	0/2	3/3

F. nivale Fn 2B was cultured at 27° for 14 days on PSC medium in which $NaNO_3$ was replaced by varied forms of nitrogens listed in the Table.
a) 0.5 ml/10 g of the culture filtrate or 2.5 mg/10 g of the crude toxin was administered intraperitoneally.

In the case of $(NH_2)_2CO$, the growth rate of the fungus and the produced amount of crude toxin were higher than the others.

Table VI. Effect of Metal Ions

Metal ions (10 mg/liter)	Final pH	Mycelium (g/200 ml)	Crude toxin (mg/200 ml)	Toxicity of crude toxin[a] (dead/used)
—	6.5	2.08	115	3/3
$MnSO_4$	6.6	2.38	164	2/3
$CuSO_4$	7.5	2.34	75	3/3
$ZnSO_4$	7.2	2.07	93	3/3
$Fe_2(SO_4)_3$	6.8	2.38	76	2/3
$(NH_4)Mo_7O_{24}$	6.7	2.45	108	3/3

F. nivale Fn 2B was cultured on PSC medium at 27° for 10 days. 10 mg/liter of metal compound was supplemented.
a) one mg/10 g of the crude toxin was injected to mice intraperitoneally.

Effect of Metal Ions

Effect of supplemented metal ions (10 mg/liter) on the toxicity of the fungus was examined. As shown in Table VI, in no case did any of the additives increased significantly the yields of mycelium and the toxicity of the crude toxin, except for $MnSO_4$, which increased the crude toxin and formation of red pigment. However, when 1 mg/10 g of the crude toxin was administered to mice, the control crude toxin killed mice 2 days after injection whereas the crude toxin isolated from metal ions-supplemented medium killed some of mice one day after injection. These observation suggested that the addition of metal ions seemed to increase the lethal toxicity of the crude toxin.

Effects of Organic Acid and Amino Acids

Tables VII and VIII showed the effect of supplemented organic or amino acids on toxicity of the fungus. Among organic acids tested, fumaric acid, malonic acid, *n*-butyric acid and

Table VII. Effect of Organic Acids

Organic acids	Final pH	Mycelium (g/200 ml)	Crude toxin (mg/200 ml)	Toxicity of crude toxin[a] (dead/used)
—	6.8	2.03	79	3/3
Succinic acid	7.5	2.40	75	2/3
Fumaric acid	7.3	2.22	122	3/3
Citric acid	7.2	2.17	147	0/3
Malonic acid	7.4	2.33	111	3/3
Acetic acid	7.5	2.08	80	3/3
n-Butyric acid	7.5	2.50	109	3/3

The fungus was cultured in PSC medium in the presence of 0.01M of organic acid at 27° for 10 days.
All the medium was adjusted to pH 7.0.
a) one mg/10 g of the crude toxin was injected to mice.

Table VIII. Effect of Amino Acids

Amino acids	Final pH	Mycelium (g/200 ml)	Crude toxin (mg/200 ml)	Toxicity to mice[a] (dead/used)	
				Culture filtrate	Crude toxin
—	7.1	2.37	125	2/2	3/3
Aspartic acid	7.2	2.27	146	2/2	1/3
Glutamic acid	7.3	2.33	145	1/2	3/3
Glycine	7.6	2.13	91	0/2	3/3
Leucine	7.1	2.07	90	2/2	3/3
Phenylalanine	7.3	2.25	132	0/2	3/3
Tryptophan	6.3	1.45	186	1/2	3/3
Histidine	6.5	1.83	164	2/2	3/3
Alanine	7.2	2.17	119	1/2	3/3
Methionine	7.5	1.77	241	1/2	3/3

The fungus was cultured at 27° for 10 days in PSC medium to which 1 g/liter amino acid was added.
a) 0.5 ml/10 g of the culture filtrate or 1 mg/10 g of the crude toxin was injected intraperitoneally.

citric acid promoted the yield of the crude toxin, but in the case of citric acid, the crude toxin was not lethal to the mice at the intraperitoneal dose of 1 mg/10 g. Noticeable finding was that malonic acid, an inhibitor of succinate dehydrogenase, did not restrict toxin-producing system of the fungus. Among amino acids tested, phenylalanine, tryptophan and methionine promoted the formation of the crude toxin without influencing the mycelial growth.

Effect of Temperature on Toxicity of the Fungus

In the next experiments, the authors investigated the fungal growth, pH and the toxicity of the culture broth at different temperature of cultivation.

When the fungus was cultured at 10°, 20° or 27° for varied time of incubation, the pH of the medium changed depending upon time of cultivation, as shown in Fig. 1. In all cases,

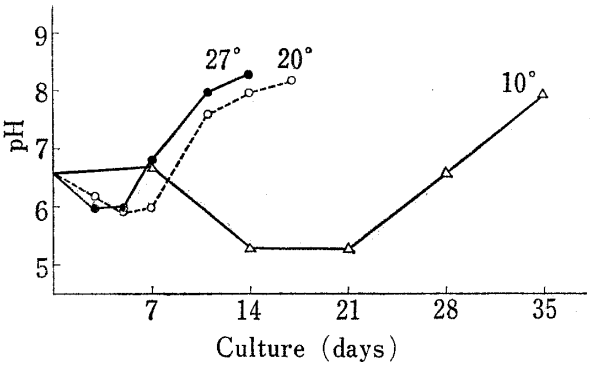

Fig. 1. Change of pH of Cultured Medium at Different Temperature

F. nivale Fn 2B

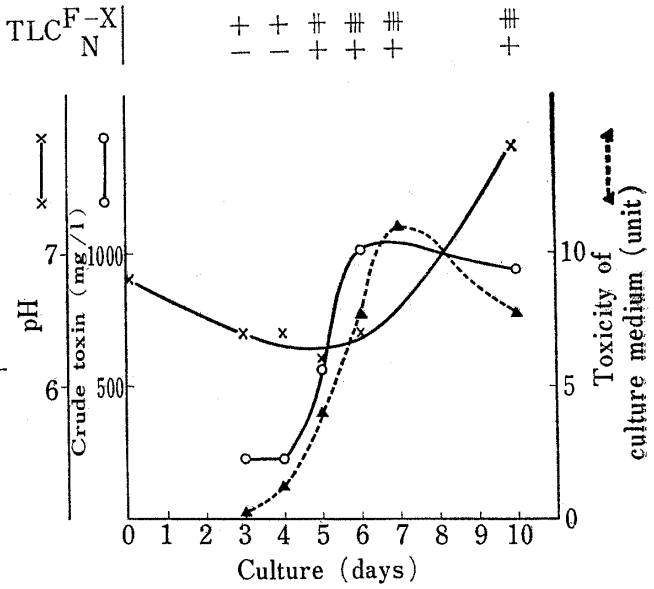

Fig. 2. Relation among pH, Yield of Crude Toxin and Toxicity of Culture Broth

the initial pH declined at first to around 6 and reversed to over 8 after 10 days at 27°, 19 days at 20° and 35 days at 10°, as illustrated in Table IX. Quantitative determination revealed

Table IX. Effect of Temperature on the Toxicity of the Fungus

Temperature (°C)	Culture (days)	Final pH	Mycelium (g/200 ml)	Crude toxin (mg/200 ml)	Toxicity of crude toxin (dead/used) 1	2.5 (mg/10 g)	5
10	35	8.0	2.72	77	—	0/3	3/3
20	17	8.2	2.27	83	3/3	—	3/3
27	14	8.3	1.93	115	3/3	—	3/3

The fungus was cultivated in PSC medium at different temperature until the pH of culture medium reached to around 8.

that the yield and the toxicity of the crude toxin was higher at 27° than 10° or 20°, whereas the yield of mycelium contradicted with the temperature. Table X showed the relation among lethal toxicity of culture filtrate, temperature and time of culture. In case of 10°, 0.5 ml/10 g of the medium did not kill mice even when the fungus was continued to culture for 35 days, and in case of 20°, 0.12 ml/10 g was lethal to mice after 11—14 days of the cultivation. While, in case of 27°, 0.06 ml/10 g killed mice after 7 days and 0.25 ml/10 g was also lethal after 5 days. These results strongly suggested that the fungus produced toxic metabolites at a rather high temperature of cultivation.

As previously reported,[4] with PSC medium *F. nivale* Fn 2B produces two mycotoxins, fusarenon-X and nivalenol, and the yield of the former toxin was higher than that of the

Table X. Effect of Time and Temperature on Toxicity of the Culture Medium

Temperature (°C)	Dose[a] (ml/10 g)	Toxicity (dead/used) 3 (days)	5	7	11	14	17	21	28	35
10	0.5	—	—	0/3	—	0/3	—	0/3	0/3	0/3
20	0.12	—	—	0/3	1/3	2/3	0/3			
	0.25	—	—	3/3	2/3	2/3	3/3			
	0.5	0/2	0/3	3/3	3/3	—	—			
27	0.06	—	—	2/3	1/3	0/3				
	0.12	—	0/3	3/3	3/3	3/3				
	0.25	—	3/3	3/3	3/3	3/3				
	0.5	0/2	3/3	—	—	—				

a) Culture filtrate was administered intraperitoneally to mice

latter. In this connection, the authors investigated the order of formation of these two toxins at different time of the cultivation. As illustrated in Table XI and Fig. 2, the yields of the

Table XI. Time Course of the Production of the Crude Toxin and Fusarenon-X in Peptone–supplemented Czapek Medium

Time (days)	Final pH	Mycelium (g/liter)	Crude toxin (mg/liter)	Toxicity (dead/used) 0.13 (ml/10g)	0.25	0.5	2.5	TLC[a] F–X	N
3	6.4	2.6	204	—	—	0/3	3/3	+	—
4	6.4	5.9	234	—	0/3	1/3	—	+	—
5	6.2	6.1	554	1/3	2/2	3/3	—	++	+
6	6.4	8.7	1010	3/3	2/2	3/3	—	+++	+
10	7.8	9.4	940	3/3	2/2	3/3	—	+++	+

The fungus was cultured at 27° in PSC medium.
a) F–X: fusarenon–X N: nivalenol

mycelium and the crude toxin and also the toxicity of culture medium gradually increased in paralell to time of culture and they reached to the maximum after 6 days of culture. Qualitative analysis of the crude toxin with thin layer chromatography indicated that fusarenon-X was detected 3 days after culture and nivalenol was detected 5 days after culture, and furthermore, the amount of fusarenon-X was relatively larger than nivalenol. These results support that fusarenon-X is produced prior to nivalenol and is a major toxic product of the fungus.

Table XII. Time Course of Production of the Crude Toxin on Rice

Time (weeks)	Crude toxin (mg/600 g)	Toxicity (dead/used) 0.1 (mg/10 g)	0.5	1.0	5.0	TLC[a] F–X	N
1	180	0/2	2/2	3/3	3/3	+	±
2	380	0/2	0/2	2/3	3/3	+	+
3	410	0/2	2/2	3/3	3/3	+	+
4	810	0/2	1/2	2/3	3/3	+	+

The fungus was cultured on polished rice grains at 27°. Three flasks each containing 200 g of rice were used in each experiment.
a) F–X: fusarenon–X N: nivalenol

Production of Toxic Metabolites on Cereal Grains

When the fungus was cultur . on polished rice grains at 27°, the yield of crude toxin increased in paralell to the time o' cultivation, and after 4 weeks around 1 g of the crude toxin with lethal toxicity of 1 mg/10 g was obtained from 1 kg of rice grain. Thin-layer chromatography showed the presence of fusarenon-X and nivalenol in all stages of cultivation examined, as shown in Table XII. When the temperature of cultivation was changed ranging from 10° to 37°, as illustrated in Table XIII-A, the highest yield of the crude toxin was attained when the fungus was cultured at 27° for 4 weeks, and at 20° it decreased to about one third of the maximal yield.

Table XIII. Effects of Temperature and Duration of Cultivation on the Production of Toxic Materials of the Fungus cultivated on Polished Rice Grains

A) Yield of the crude toxin

Temperature (°C)	Time (weeks) 1	2	3	4
		Crude toxin (g/600 g)		
10		0.04		0.17
20		0.16		0.24
24		0.16		
27	0.18	0.38	0.41	0.81
37		0.13		

B) Toxicity of the crude toxin

Temperature (°C)	Time (weeks)	Toxicity to mice (dead/used) 0.1	1.0	5.0	10
			(mg/10 g)		
10	2		—	—	
	4		0/2	2/2	
20	2		0/2	2/2	2/2
	4		1/2	2/2	2/2
24	2		0/2	2/2	2/2
27	1	0/2	2/2	3/3	
	2	0/2	0/2	3/3	
	3	0/2	2/2	3/3	
	4	0/2	1/2	3/3	
37	2		0/2	2/2	2/2

An Erlenmyer flask containing 200 g polished rice grains was inoculated with *F.nivale* Fn 2B, and the fungus was cultured at different temperature for desired intervals.

Comparative toxicities among the crude toxins were summarized in Table XIII-B. With 5 mg/10 g the mortality of mice was nearly the same in all cases, but with 1 mg/10 g a fatal affection was noted in the crude toxin obtained at 20° or 27°.

Judging from the yield and toxicity of the crude toxin, it is very likely that the fungus favors 20—27° for the production of toxic metabolites on rice grains, same as in the case of liquid culture described in the above.

The production of toxic metabolites was dependent largely upon substraction used, as shown in Table XIV. The yield of the crude toxin was in the order of barley>corn>wheat>rice. However, the toxicity of the isolated crude toxin was just the reverse. Regardless of the source of cereals, fusarenon-X and nivalenol were detected on a silicagel plate.

Table XIV. Toxicity of the Fungus in Different Cereal Grains

Cereals	Crude toxin (mg/200 g)	Toxicity[a] (dead/used) 5	10	20 (mg/10 g)	40	50	TLC[b] F–X	N
Rice	104	1/2	2/2				+	+
Barley	531	0/2	0/2	0/2		2/2	+	±
Wheat	280	0/2	0/2		2/2		+	±
Corn	327	0/2	0/2		1/2		+	±

F. nivale Fn 2B was cultured on cereal grains listed in the Table at 27° for 3 weeks. The moldy grains was extracted with *n*-hexane followed by methanol. Methanol-soluble materials were dissolved in chloroform, and the methanol-chloroform soluble fraction was referred to as "crude toxin," as described in the method.

a) The crude toxin was administered intraperitoneally to mice

b) F–X: fusarenon–X N: nivalenol

Discussion

Czapek medium supplemented with 10 g/liter of peptone (PSC medium) apparently provided all necessary ingredients for the production of mycelium and fusarenon-X. None of the varied sort of ingredients such as metal ions, organic acids or amino acids stimulated remarkably the production of toxic metabolites when they were added to the PSC medium.

The PSC medium is easy to prepare and not so expensive. For these reasons, PSC medium appears to be suitable for both production of fusarenon-X and for screening fungi which are suspected to produce fusarenon-X.

Production of fusarenon-X depends upon time and temperature of cultivation. With PSC medium, the maximal yield was observed at 27° and 6—8 days, and at lower temperature the fungus gave only a larger amount of the mycelium without accompanying the production of fusarenon-X. With rice grains, production of toxic metabolites was also higher at 24—27° than below 20°. In this connection, Smally, *et al.*[7] pointed out that production of diacetoxyscirpenol, a skin-irritant and cytotoxic mycotoxin of *Fusarium tricinctum*, favors lower temperature, in contradiction to the case of fusarenon-X.

Noticeable finding is that, fusarenon-X is a major toxic metabolite of *F.nivale* and nivalenol is a minor, and that the latter toxin is presumed to be derived from the former toxin. Because, in PSC medium, fusarenon-X is produced prior to nivalenol and it's yield is highly remarkable than nivalenol.[3,4] Furthermore, no nivalenol was detected in the culture filtrate with low value of pH. Chemically speaking, nivalenol lacks one acetyl group in structure of fusarenon-X,[8] therefore, a chemical or enzymatic deacetylation reaction is presumably proceeded during fungal cultivation.

All the cereal grains tested are proved to support the formation of fusarenon-X and nivalenol, and rice grains give the crude toxin with the highest toxicity to mice, in agreement with the finding of Tsunoda, *et al.*,[2] that in feeding experiments with cereal grains molded with *F. nivale* a lethal affection to rats was in the order of rice>oats>corn>barley. Relatively large amounts of the crude toxin obtained with corn or barley indicated that a large amount of a methanol and chloroform-extractable material was contained in these cereals.

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