Mycotoxin producing ability of *Aspergillus tamarii*

Tetsuhisa GOTO^{*1}, Yoko ITO^{*2}, Stephen W. PETERSON^{*3}
and Donald T. WICKLOW^{*3}

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後藤 哲久^{*1}, 伊藤 陽子^{*2}, ステフェン・W・ピーターソン^{*3}, ドナルド・T・
ウィックロー^{*3}: アスペルギルス・タマリのマイコトキシン産生能

Summary

Aspergillus tamarii is known to produce mycotoxins such as cyclopiazonic acid (CPA) and kojic acid (KA). Recently we isolated several cultures of *A. tamarii* from Japanese tea fields and found one of the isolates "No. 19", and single-spore cultures (NRRL 25517, 25518, 25519) derived from this isolate, produced substantial quantities of aflatoxin B₁ and B₂. Here we report the results of a limited survey of 15 *A. tamarii* strains isolated from Japanese tea fields, one strain from silk worm excrement and 29 strains received from culture collections. Most *A. tamarii* isolates produced CPA and KA but only the strains derived from isolate "No. 19" and strain NRRL 443 produced aflatoxins B₁ and B₂. Each aflatoxin-producing strain of *A. tamarii* showed yellow-green colors in young colonies and did not darken in age, suggestive of the "bronze series". The relation between the levels of CPA or KA production by the individual strains was very weak.

Key words: *Aspergillus tamarii*, アスペルギルス・タマリ; Aflatoxin, アフラトキシン;
Cyclopiazonic acid, サイクロピアゾン酸; Kojic acid, こうじ酸

Aspergillus tamarii Kita, a species classified in the *A. flavus* group (subgenus *Circumdati* section *Flavi*)¹⁾, is often isolated from soil and agricultural commodities²⁾. This fungus reportedly produces mycotoxins such as cyclopiazonic acid (CPA) and kojic acid (KA), but not aflatoxins^{3,4)}. Among *A. flavus* group, *A. flavus*, *A. parasiticus* and *A. nomius* produce aflatoxins, *A. flavus*, *A. oryzae* and *A. tamarii* produce CPA and most of the *A. flavus* group including *A. sojae* produce KA^{3,4)}.

We recently isolated several aflatoxigenic *A. flavus* group strains from Japanese tea field soils and found one of the isolates produced substantial quantities of aflatoxins B₁ (AFB₁) and B₂ (AFB₂)^{5,6)}. Examination of cultures representing single-spore isolates of subcultures of isolate No. 19^{5,6)} (NRRL 25517, NRRL 25518, NRRL 25519) suggested taxonomic placement in the "bronze series" of Thom and Raper (1945)⁷⁾. This prompted us to examine other *A. tamarii*

^{*1} National Food Research Institute, Ministry of Agriculture, Forestry and Fisheries (Tsukuba 305, Japan)
農林水産省食品総合研究所 (〒305 茨城県つくば市観音台2-1-2)

^{*2} National Research Institute for Vegetables, Ornamental Plants and Tea, Ministry of Agriculture, Forestry and Fisheries (Kanaya, Shizuoka 428, Japan)
農林水産省野菜・茶業試験場 (〒428 静岡県榛原郡金谷町金谷2769)

^{*3} National Center for Agricultural Utilization Research, ARS/USDA (Peoria, IL 61604, USA)
農務省農業利用研究所 (61604 米国イリノイ州ペオリア市)

strains for their aflatoxins, CPA and KA productivity.

Materials and Methods

Fungal strains The 45 cultures of *A. tamarii* that were examined in this study included 11 isolates (15 strains) from Japanese tea field soils, one isolate from silk worm excrement, and cultures obtained from culture collections including: ARS Culture Collection (National Center for Agricultural Utilization Research, USA) (22 strains); IAM Culture Collection (Univ. of Tokyo) (5 strains); NFRI Culture Collection (Ministry of Agriculture, Forestry and Fisheries, Japan) (2 strains).

Analysis of aflatoxin Fungi were inoculated in 10 ml of glucose-yeast extract liquid medium in 18×180 mm test tube and incubated for 1 week at 25°C in the dark. Five grams of culture medium was placed in a 18×180 mm test tube and mixed with 300 mg of KCl and five ml of methanol. Aflatoxins were extracted twice with three ml of chloroform. The chloroform extracts were combined and evaporated to dryness under N₂ gas stream. The residue was dissolved in benzene-acetonitrile (98+2, v/v) then analyzed by TLC^{6,8)}.

Analysis of cyclopiazonic acid Fungi were inoculated in 10 ml of modified Czapek-Dox liquid medium in 18×180 ml test tube and incubated for 10 days at 30°C in the dark. One ml of culture medium was absorbed to one gram of equal weight mixture of Celite 545 and anhyd. Na₂SO₄ in 13×100 mm test tube. Cyclopiazonic acid was extracted in one ml of ethylacetate and analyzed by HPLC^{9,10)}.

Analysis of kojic acid Fungi were inoculated in 10 ml of glucose-peptone medium¹¹⁾ in 18×130 mm test tube and incubated for 2 weeks at 30°C in the dark. The culture medium was directly injected, in some case diluted with mobile phase, into HPLC column. Kojic acid was detected at the absorbance of 270 nm¹²⁾.

Results and Discussion

As shown in Table 1, most *A. tamarii* strains produced KA and CPA. Three strains did not produce detectable amounts of KA and seven strains did not produce detectable amounts of CPA. Four strains produced aflatoxins, including NRRL 443 sent to Charles Thom in 1923 by Da Fonseca, Brazil as *Aspergillus spadix* Amons (Thom No. 277-4707.757) and three strains derived from subcultures of isolate No. 19⁵⁾ from tea field soil in Japan.

The quantities of mycotoxins produced varied among strains. The highest KA producer made nearly 3.5% (w/v) of KA in the liquid medium. For CPA some strains produced more than 13 ppm. Two out of three non KA producers did not produce detectable amounts of CPA while a third non KA producer formed relatively small quantities of CPA. Strong CPA producers tended to produce more KA, however the association between the production of these two mycotoxins was weak (Fig. 1).

Aflatoxigenic strains produced AFB₁ and AFB₂ but did not produce detectable amounts of AFGs. These strains made more than one (>1) ppm of CPA and quantities of KA (1.9%). Colonies of these aflatoxigenic strains initially showed some yellow-green colors becoming bronze colored in age. The colonies did not continue to darken becoming dark brown as

Table 1 Mycotoxin productivity of *A. tamarii*.

Strain	CPA (ppm)	AFB ₁ (ppm)	AFB ₂ (ppm)	KA (%)
87K5723 (NRRL 25586)	0.19	N.D.	N.D.	0.84
93MZ2A (NRRL 25400)* ¹	7.8	N.D.	N.D.	0.95
93MZ2C (NRRL 25401)* ¹	3.9	N.D.	N.D.	2.70
93MZ2D (NRRL 25517)* ¹	1.7	30	0.1	1.92
93MZ2E (NRRL 25518)* ¹	2.5	30	0.2	2.38
93MZ2F (NRRL 25519)* ¹	1.8	30	0.2	2.01
93KO6 (NRRL 25399)	0.17	N.D.	N.D.	2.43
94SZ1-1 (NRRL 25402)	1.7	N.D.	N.D.	2.28
94KO1 (NRRL 25403)	0.22	N.D.	N.D.	1.04
94KO2 (NRRL 25404)	N.D.* ²	N.D.	N.D.	0.13
95KO1 (NRRL 25565)	0.69	N.D.	N.D.	0.53
95KO7 (NRRL 25570)	0.36	N.D.	N.D.	0.05
96MZ1 (NRRL 25578)	5.0	N.D.* ³	N.D.* ³	0.89
96MZ2 (NRRL 25579)	13.0	N.D.	N.D.	1.21
96MZ3 (NRRL 25580)	2.5	N.D.	N.D.	0.46
96MZ5 (NRRL 25626)	2.4	N.D.	N.D.	0.87
IAM 2138	N.D.	N.D.	N.D.	1.56
IAM 2499	3.0	N.D.	N.D.	1.56
IAM 2502	N.D.	N.D.	N.D.	1.64
IAM 13907	13.9	N.D.	N.D.	3.12
IAM 13908	6.4	N.D.	N.D.	2.63
NFRI 1172	N.D.	N.D.	N.D.	1.43
NFRI 1296	6.4	N.D.	N.D.	1.79
NRRL 427	3.5	N.D.	N.D.	0.22
NRRL 428	1.8	N.D.	N.D.	2.23
NRRL 429	0.8	N.D.	N.D.	0.99
NRRL 430	3.6	N.D.	N.D.	3.28
NRRL 431	0.9	N.D.	N.D.	2.65
NRRL 432	1.1	N.D.	N.D.	2.43
NRRL 433	1.5	N.D.	N.D.	0.05
NRRL 434	0.41	N.D.	N.D.	N.D.* ⁴
NRRL 435	2.7	N.D.	N.D.	1.77
NRRL 436	6.6	N.D.	N.D.	3.05
NRRL 437	0.5	N.D.	N.D.	0.42
NRRL 438	3.0	N.D.	N.D.	0.49
NRRL 439	6.8	N.D.	N.D.	0.18
NRRL 440	N.D.	N.D.	N.D.	N.D.
NRRL 441	0.4	N.D.	N.D.	0.27
NRRL 443	3.1	3.8	0.04	3.46
NRRL 559	0.1	N.D.	N.D.	0.41
NRRL 572	N.D.	N.D.	N.D.	0.04
NRRL 1252	5.1	N.D.	N.D.	1.18
NRRL 1654	0.5	N.D.	N.D.	1.13
NRRL 4960	N.D.	N.D.	N.D.	N.D.
NRRL 13139	11.4	N.D.	N.D.	1.57

*¹: derived from isolate "No. 19" (Ref. 5).*²: Not Detected, Detection limit for cyclopiazonic acid was 0.01 ppm.*³: Detection limits for aflatoxins were 0.5 ppb.*⁴: Detection limit for kojic acid was 0.01%.

Fig. 1 Relation the level of cyclopiazonic acid and kojic acid production.

described for *A. tamarii*¹¹⁾ and therefore are suggestive of strains included in the “bronze series” of *A. tamarii*⁷⁾.

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