Distribution of Nuclease O Inhibitor among *Aspergillus* Species

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There are many reports on deoxyribonuclease and ribonuclease inhibitors of protein nature, such as DNase inhibitors of mammalian tissues^{1,2)} and *Actinomyces levoris*³⁾ and RNase inhibitors of mammalian tissues,^{4,5)} *Bacillus subtilis*⁶⁾ and *Aspergillus clavatus*.⁷⁾ Generally, such inhibitors are highly specific to the particular enzymes of the same strains. As for nucleases which degrade both DNA and RNA, only two instances of such inhibitors have been reported to our knowledge. The one is the nuclease O inhibitor of *Aspergillus oryzae*,^{8,9)} and the other is that of *Neurospora crassa*.¹⁰⁾ The nuclease O inhibitor of *A. oryzae*, found in the fresh mycelia of the organism, is a heat stable protein with a molecular weight about 23,000. The inhibitor is specific to nuclease O (EC 3.1.4.9),¹¹⁾ an intracellular nuclease of *A. oryzae*, and does not affect nuclease S₁, ribonuclease T₁ and ribonuclease T₂ of *A. oryzae* and pancreatic RNase. The inhibitor stoichiometrically inactivates nuclease O forming a stable complex with the nuclease. The inhibitor in the mycelia is inactivated in the course of autolysis of the organism, resulting in 3-fold activation of intracellular nuclease activity, which is accompanied by decomposition of more than 90% of cellular nucleic acids. This time, we investigated the distribution of the inhibitor of nuclease O among *Aspergillus* species, and found that the inhibitor is present in all strains of *A. oryzae* tested and some species closely related to *A. oryzae*.

Crystalline nuclease O¹²⁾ prepared from the autolyzate of *A. oryzae* IAM 2608 was used for determination of the inhibitor activity by the assay method previously reported.⁹⁾ One loopful of spores of test organism from a slant culture was inoculated into 100 ml medium (sucrose 3.5%, polypeptone 2%, KH₂PO₄ 0.5%, MgSO₄·7H₂O 0.25%, pH 5.6) in 500 ml Sakaguchi flask, and incubated at 30°C for 41 hr with reciprocal shaking at 120 strokes per minute. The mycelia were harvested by filtration through a Buchner funnel, washed twice with distilled water, and blotted by pressing them between sheets of filter paper. Two grams of mycelia were mixed with 4 g of glass powder and 20 ml of 0.05 M Tris-HCl buffer (pH 7.2), and homogenized in an ice-cold Waring blender for 5 min. The homogenate was heated at 80°C for 10 min to denature nucleases and other proteins, and centrifuged at 500 × g

for 10 min. The inhibitor activity against nuclease O in the supernatant was determined using yeast RNA and heat-denatured herring sperm DNA as substrates.

TABLE I. DISTRIBUTION OF THE INHIBITOR
OF NUCLEASE O AMONG *Aspergillus* SPECIES

Strain	Inhibitor (U/g wet mycelia)	
	RNA as substrate	DNA as substrate
<i>A. oryzae</i> IAM 2608	1.3	1.3
<i>A. oryzae</i> IAM 2604	1.3	1.3
<i>A. oryzae</i> IAM 2609	1.4	1.3
<i>A. oryzae</i> IAM 2742	1.1	1.1
<i>A. oryzae</i> IAM 2734	1.2	1.2
<i>A. oryzae</i> IAM 2630	1.2	1.1
<i>A. oryzae</i> IAM 2142	1.3	1.2
<i>A. oryzae</i> var. <i>globosus</i> IAM 2645	1.1	1.1
<i>A. oryzae</i> var. <i>microsporus</i> IAM 2728	1.1	1.1
<i>A. oryzae</i> var. <i>magnasporus</i> IAM 2719	0.4	0.6
<i>A. flavus</i> IAM 2007	1.3	1.2
<i>A. flavus</i> IAM 2184	1.5	1.4
<i>A. sojae</i> IAM 2678	0.7	0.7
<i>A. sojae</i> IAM 2634	0.9	0.8
<i>A. parasiticus</i> IAM 2150	0.4	0.5
<i>A. parasiticus</i> ATCC 15517	0.3	0.4
<i>A. tamarii</i> IAM 2138	0.4	0.6
<i>A. tamarii</i> IAM 2178	0.4	0.6
<i>A. saitoi</i> IAM 2215	0.2	0.2
<i>A. saitoi</i> var. <i>kagoshimaensis</i> IAM 2192	0.0	0.0
<i>A. niger</i> IAM 2107	0.0	0.0
<i>A. niger</i> IAM 3008	0.0	0.0
<i>A. awamori</i> IAM 2312	0.3	0.2
<i>A. awamori</i> var. <i>fumeus</i> IAM 2388	0.0	0.0
<i>A. clavatus</i> IAM 2120	0.6	1.2
<i>A. fumigatus</i> IAM 2129	0.4	0.5
<i>A. nidulans</i> IAM 2155	0.4	0.5
<i>A. japonicus</i> IAM 2083	0.5	0.5
<i>A. flavipes</i> IAM 2160	0.2	0.4
<i>A. ochraceus</i> IAM 2804	0.1	0.2
<i>A. ustus</i> IAM 2057	0.1	0.2
<i>A. versicolor</i> IAM 2080	0.0	0.3
<i>A. terreus</i> IAM 2179	0.0	0.1
<i>A. candidus</i> IAM 2015	0.0	0.1
<i>A. inuii</i> IAM 2255	0.0	0.0
<i>A. wentii</i> IAM 2133	0.0	0.0
<i>A. glaucus</i> B (A-1-6)	0.0	0.0

As shown in Table I, nearly the same level (1.1~1.4 U/g mycelia) of inhibitor was found in all the seven strains of *A. oryzae* randomly sampled from the culture

collections of our laboratory. Three strains of *A. oryzae* variety and two strains from each of *A. flavus*, *A. sojae*, *A. parasiticus* and *A. tamarii*, which are taxonomically closely related to *A. oryzae*, also contained the inhibitor. Therefore, it may be concluded that the nuclease O inhibitor is present in all species closely related to *A. oryzae*. On the other hand, little or no inhibitor activity against nuclease O was detected in black aspergilli and some other species of *Aspergilli*, although the present data do not exclude the possibility that these aspergilli may also have their own nuclease-inhibitor system. The presence or absence of such nuclease-inhibitor system and cross reaction of nuclease and inhibitor among different species of *Aspergilli* may be an interesting problem for taxonomy of *Aspergilli*.

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