Note

Occurrence of *Streptomyces* Pepsin Inhibitor-insensitive Carboxyl Proteinase in Basidiomycetes*Kōhei ODA, Takao TERASHITA,
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Most carboxyl (acid) proteinases are inactivated by *Streptomyces* pepsin inhibitor (S-PI¹⁾ or Pepstatin²⁾ and active site-directed modifying reagents such as DAN and EPNP.

On the other hand, in the course of studies of fruit-body formation of Basidiomycetes,³⁾ *Lentinus edodes* TMI 563 was found to produce S-PI, and DAN-insensitive carboxyl proteinase in the culture filtrate.⁴⁾ This finding prompted us to more detailed screening for new type carboxyl proteinases in Basidiomycetes.

The Basidiomycetes used here were kindly supplied by the Institute for Fermentation, Osaka, and the Tottori Mycological Institute, Tottori. They were still cultured in 100 ml flasks containing 20 ml of potato-glucose liquid medium at 25°C for 14 to 60 days. The proteinase activity and the effect of S-PI (80 µg in the reaction mixture) were assayed at pH 2.5 by the casein-Folin method.⁵⁾ In the case of poor production, the culture filtrate was concentrated *in vacuo* at 35°C, or fractionated with ammonium sulfate.

The production of S-PI-insensitive carboxyl proteinase was investigated for 77 strains belonging to 9 families as shown in Table I. Of them, 7 strains were found to produce S-PI-insensitive carboxyl proteinases. They are *Ganoderma lucidum* IFO 4912, *Schizophyllum commune*, *Pleurotus ostreatus* IFO 7051, *Pleurotus cornucopiae*, *Flammulina velutipes* IFO 7046, *Lentinus edodes* IFO 4902 and *Lentinus edodes* TMI 563 (Table II). These carboxyl proteinases were not inactivated by 80 µg of S-PI.

It is interesting that S-PI-insensitive carboxyl pro-

teinases are found mainly in Tricholomataceae of Agaricales, which includes many edible mushrooms such as *Lentinus edodes*.

On the contrary, S-PI-sensitive carboxyl proteinases are found in Aphyllophorales. For example, *Trametes sanguinea*⁶⁾ and *Irpex lacteus*⁷⁾ produced potent carboxyl proteinases.

As S-PI-, DAN-insensitive carboxyl proteinases, carboxyl proteinases A and B of *Scytalidium lignicolum* ATCC 24568 (wood destroying-fungi)^{5,8,9)} and carboxyl proteinase A of *Aspergillus niger* var. *macrosporus*¹⁰⁾ have been reported so far.

Thus, an interesting question arises as to whether the structures of these S-PI-, DAN-insensitive enzymes are like those of usual pepstatin-sensitive carboxyl proteinases, especially in their active sites. In fact, the *Scy. lignicolum* carboxyl proteinases have been shown to be considerably different from the usual carboxyl proteinases in their behavior towards synthetic peptides.¹¹⁾

The results of this work and our previous results on *Scy. lignicolum* carboxyl proteinases¹²⁾ suggest that these S-PI-, DAN-insensitive carboxyl proteinases may be classified as a new subclass of carboxyl proteinases, the *Scy. lignicolum* type.

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* Proteinase of Basidiomycetes. Part III. See refs. 3 and 4.

Abbreviations: S-PI, *Streptomyces*-pepsin inhibitor; Pepstatin Ac, acetyl pepstatin; DAN, diazoacetyl-DL-norleucine methylester; EPNP, 1,2-epoxy-3-(p-nitrophenoxy)propane.

TABLE I. DISTRIBUTION OF S-PI-INSENSITIVE CARBOXYL PROTEINASES IN BASIDIOMYCETES

	Number of				%*
	species tested	strains tested	carboxyl proteinase producing strains	S-PI-insensitive carboxyl proteinase producing strains	
Aphylophorales					
Polyporaceae	14	14	14	1	7
Coniophoraceae	1	1	1	0	0
Agaricales					
Tricholomataceae	13	29	26	6	23
Bolbitiaceae	3	3	2	0	0
Agaricaceae	6	8	5	0	0
Strophariaceae	4	4	3	0	0
Cortinariaceae	2	2	2	0	0
Coprinaceae	14	15	11	0	0
Amanitaceae	1	1	1	0	0
Total	58	77	65	7	11

* (No. of S-PI-insensitive carboxyl proteinase producing strains/No. of carboxyl proteinase producing strains) $\times$ 100.

TABLE II. S-PI-INSENSITIVE CARBOXYL PROTEINASES OF BASIDIOMYCETES

	Family	Culture time (days)	Optimum pH	Proteinase activity (PU/ml)
<i>Ganoderma lucidum</i> IFO 4912	Polyporaceae	14	3.4	6.4
<i>Schizophyllum commune</i>	Tricholomataceae	7	3.3	2.6
<i>Pleurotus ostreatus</i> IFO 7051	Tricholomataceae	28	3.0	2.8
<i>Pleurotus cornucopiae</i>	Tricholomataceae	22	3.0	8.8
<i>Flammulina velutipes</i> IFO 7046	Tricholomataceae	34	3.2	1.9
<i>Lentinus edodes</i> TMI 563	Tricholomataceae	40	2.9	8.3
<i>Lentinus edodes</i> IFO 4902	Tricholomataceae	30	2.9	4.3