Short Communication

FEATURES OF THE PROTEOLYTIC ENZYME SYSTEM OF HYPER-ACID-PRODUCTIVE AND NON-ACID-PRODUCTIVE FUNGI¹

KIN-ICHI MATSUSHIMA, MIKIO HAYAKAWA,² MASAKO ITO,
AND KYO SHIMADA

*Department of Agricultural Chemistry, Mie University,
Tsu 514, Japan*

(Received May 14, 1981)

A great deal of knowledge on the proteolytic enzymes of individual microorganisms has been accumulated in recent years, however, the proteolytic enzyme systems of microbial groups have been poorly characterized since they have been the subject of only a limited number of studies. YOSHIDA (1) and NEHIRA (2) reported that the proteolytic enzyme system of the black *Aspergillus* group is distinguishable from that of other *Aspergillus* species for the exclusive content of acid proteinase (EC 3.4.23), and KAWAI (3) demonstrated that, in *Basidiomycetes*, the strains of the order *Aphylophorales* produce mainly acid proteinase and those of the order *Agaricales* neutral (EC 3.4.24.4) or alkaline proteinase (3.4.2).

It seemed of interest, from the standpoint of "ecology" of microbial proteinases, to investigate the features of the proteolytic enzyme systems of various groups of microorganisms, not only from the aspect of taxonomy but also from biochemical and ecological standpoints. In previous papers (4, 5) the authors reported that the proteolytic enzyme systems of the *Penicillium* species and acid-tolerant fungi can be characterized as being rich in acid proteinase and poor in alkaline and neutral proteinases. This paper deals with the features of the proteolytic enzyme systems of hyper-acid-productive fungi and non-acid-productive fungi.

The hyper-acid-productive and non-acid-productive fungi used in this experiment were selected from 278 strains of test organisms which include 80 identified strains from the collection of this laboratory and 198 unidentified strains which were isolated by the authors from soil, manure, and other natural materials. The surface colony technique of DAVIS (6) was used: about a half loopful of spores of each fungus was transferred from the slant to the surface of glucose-peptone (5.0 and

¹ "Ecological" Study on the Proteolytic Enzymes of Microorganisms, Part III.

² Present address: Hattyo-miso Co., Okazaki, Japan.

Fig. 1. Deviation pattern of acid-productibility in 278 strains of tested fungi.

0.5%, respectively) agar plate which contained bromcresol green indicator and was adjusted to pH 4.5. After 5 days' cultivation at 28°, the width of the acid zone which was formed around the colony and changed in color from the blue-green of the original to yellow was measured. Figure 1 shows that most of the fungi tested formed an acid zone of a width ranging from 1 to 15 mm. However, some fungi formed very little (less than 1 mm), if any, acid zone, while others formed an acid zone of a width ranging from 15 to 30 mm. Both groups of fungi, *i. e.*, non-acid-productive fungi and hyper-acid-productive fungi, were used in this experiment. In the former group were *Aspergillus elegans* IFO 4048, *A. nidulans* OUT 5099, *A. sulphureus* OUT 5068, *A. sydowi* OUT 5070, *A. terreus* OUT 5075, *Penicillium namy-slowinskii* IFO 6244, *P. pallidum* IFO 5758, *P. urticae* IFO 7010, *Gliocladium roseum* AHU 9072, *Scopulariopsis brevicaulis* AHU 9289, and 17 strains of unidentified fungi which were isolated from soil by the authors. In the latter group were *P. trzebinskianum* IFO 6038, *P. multicolor* IFO 5725, *Rhizopus oryzae* IFO 5379, and 22 strains of unidentified fungi isolated by the authors from soil.

Analysis of the proteolytic enzyme systems of these fungi was carried out by incubating the fungi on wheat bran followed by extracting the enzymes from the cultures and determining the activities of proteinases according to a modification of ANSON's method (7). A 5-g amount of wheat bran was moistened with 4.5 ml of deionized water in a petri dish, sterilized, and inoculated with spores of each fungus. After cultivation for 2 to 3 days at 25°, the cultures which attained uniform sporulation were extracted with 50 ml of chilled water. The filtrates of the extracts, appropriately diluted, were used as the enzyme solution. The reaction mixture (2 ml) contained 0.1 ml of enzyme solution, 0.05 M lactate buffer at pH 3.0 (for acid proteinase) or 0.05 M phosphate buffer at pH 7.0 (for neutral and alkaline proteinases), and 0.5% of milk casein. The activity of neutral proteinase was calculated

by subtracting alkaline proteinase activity (the activity at pH 7.0 in the presence of 2×10^{-3} M EDTA) from the total activity at pH 7.0. One unit of enzyme was the amount liberating trichloroacetic acid-soluble hydrolysate corresponding to 1 μ g tyrosine per min at 40°.

Fig. 2. Composition of proteolytic enzyme systems of hyper-acid-productive fungi (left) and of non-acid-productive fungi (right).

* Those indicated by three letters are fungi from the collection of this laboratory: Ael, *Aspergillus elegans* IFO 4048; Ani, *A. nidulans* OUT 5099; Asu, *A. sulphureus* OUT 5068; Asy, *A. sydowi* OUT 5070; Ate, *A. terreus* OUT 5075; Pna, *Penicillium namyslowskii* IFO 6244; Ppa, *P. pallidum* IFO 5758; Pur, *P. urticae* IFO 7010; Ptr, *P. trzebinskianum* IFO 6038; Pml, *P. multicolor* IFO 5725; Ror, *Rhizopus oryzae* IFO 5379; Gro, *Gliocladium roseum* AHU 9072; Sbr, *Scopulariopsis brevicaulis* AHU 9289. Others are fungi isolated by the authors from soil but not yet identified.

The results of analysis of the composition of proteolytic enzyme systems of fungi belonging to each group are shown in Fig. 2. Since none of the tested organisms produced an appreciable amount of neutral proteinase, only the amounts of acid proteinase and alkaline proteinase are shown in the figure. It is seen that non-acid-productive fungi produce a very small or a negligible amount of acid proteinase as compared to the amount of alkaline proteinase which is predominantly produced. Hyper-acid-productive fungi produce acid proteinase but very little, if any, alkaline proteinase. The failure of alkaline (or neutral) proteinase in the cultures of hyper-acid-productive fungi did not seem likely to be attributable to strong acidity which leads the enzyme to inactivation, since the proteinase could not be detected even in the cultures to which calcium carbonate (20% of wheat bran) was added to neutralize acid and which gave culture filtrates of pH between 6 and 8. In conclusion, the proteolytic enzyme system of hyper-acid-productive fungi is characterized as consisting solely of acid proteinase, and that of non-acid-productive fungi as being rich in alkaline proteinase and poor in acid and neutral proteinase.

REFERENCES

- 1) F. YOSHIDA, *Nippon Nôgei Kagaku Kaishi*, **28**, 66 (1954).
- 2) T. NEHIRA and R. NOMI, *Hakko Kôgaku Zasshi*, **34**, 457 (1956).
- 3) M. KAWAI, *Nippon Nôgei Kagaku Kaishi*, **47**, 467 (1973).
- 4) K. MATSUSHIMA, K. NIHO, and K. SHIMADA, *Nippon Nôgei Kagaku Kaishi*, **54**, 871 (1980).
- 5) K. MATSUSHIMA, I. MORI, N. ITO, and K. SHIMADA, *Nippon Nôgei Kagaku Kaishi*, **54**, 875 (1980).
- 6) H. DAVIS, Master's thesis, Department of Bacteriology, University of Texas (1948), [J. W. FOSTER, *Chemical Activities of Fungi*, Academic Press, New York (1949), p. 31].
- 7) M. L. ANSON, *J. Gen. Physiol.*, **22**, 79 (1938).