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Properties of cellulase in the stomach of Tilapia *Oreochromis niloticus*

Abstract

Cellulase isolated from the stomach of Tilapia *Oreochromis niloticus* was purified by ammonium sulfate precipitation, followed by α-cyclodextrin Sepharose 6B affinty chromatography, CM ion-exchange chromatography, polyex changer PBE94 chromato focusing and gel filtration. Cellulase was found to be a single band when examined by SDS- polyacrylamide gel electrophoresis. The purifications of cellulase was 55-fold from the crude extract. The cellulase showed the optimum activity at pH 3.0 and 45°C, and the stability at pH 3.0-5.0 and below 50°C. The molecular weight of the enzyme was 56,000 and its pI was 7.8. This enzyme was specifically found to be able to hydrolyze cellulose and agarose. The cellulase was strongly inhibited by Hg^{2+} and PCMB.

Keywords: cellulase, Tilapia ***Oreochromis niloticus,*** Purification, enzymatic properties, stomach

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Akiko Yamada Taniguchi,[1] Katsumi Takano[2]

[1]Department of Agricultural Innovation for Sustainability, Faculty of Agriculture, Tokyo University of Agriculture, Japan

[2]Department of Food Safety Research Center, Tokyo University of Agriculture, Japan

Correspondence: Akiko Yamada Taniguchi, Department of Agricultural Innovation for Sustainability, Faculty of Agriculture, Tokyo University of Agriculture, 1737 Funako, Atsugi, Kanagawa 243-0034, Japan

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Introduction

This study aims at clarifying the eating habits of Tilapia *Oreochromis niloticus* (hereinafter simply called "Tilapia"(Figure 1), which is an omnivorous fish highly suitable for fish farming, and acquiring basic findings relating to the development of fish feeds. We previously isolated the following enzymes from the major digestive organs of Tilapia and reported their features: one type each of amylase,[1] protease[2] and lipase[3] from the stomach; two types of amylase,[4] 2 types of a-glucosidase,[5] 2 types of protease,[6] one type of lipase,[7] 3 types of carboxypeptidase[8] and 2 types of aminopeptidase[9] from the intestine. Tilapia is a polyphagous fish. As compared to carnivorous fish, Tilapia is expected to have higher activity of enzymes catalyzing the degradation of non-starch polysaccharides in the digestive organs.[10] These enzymes seem to be involved in the digestion and absorption of food by this fish.

Figure 1 Tilapia *Oreochromis niloticus.*

In our study reported previously, degradation of non-strach polysaccharides in the intestine of Tilapia was examined. This study revealed the presence of enzymes catalyzing the degradation of-β-glucan, and a particularly high activity of β-galactosidase. We isolated β-galactosidase in this study[11] and it was found to degradate not only galactan but also oligosaccharides containing a β-glucoside bond. Cellulase of a higher animal is discovered only by invertebrate animals, such as a mollusk and an insect until now.[12,13] The present condition is that cellulase is not discovered from the digestive organ of fishes itself.

In the present study, I examined the degradation of non-starch polysaccharides in the stomach of Tilapia. And then we purified and characterized a cellulase from the stomach of this fish.

Material and methods

Materials

The stomach was collected from Tilapia, with a body length of about 30cm and a weight of about 1kg(Figure 2). The stomach was washed using a physiology salt solution(Figure 3). The removed stomach was stored at -80°C until use.

Figure 2 Internal organs of Tilapia *Oreochromis niloticus* used in the experiment.

Preparing crude enzyme

Using the method reported elsewhere,[1-3] 40g of the stomach was homogenized with 200mL of 0.1M McIlvaine buffer (pH 3.0). The extract was dialyzed in ammonium sulfate (60% saturation). The

sediment was collected and dissolved in the above-mentioned buffer. The solution was then dialyzed against the same buffer (pH 3.0).

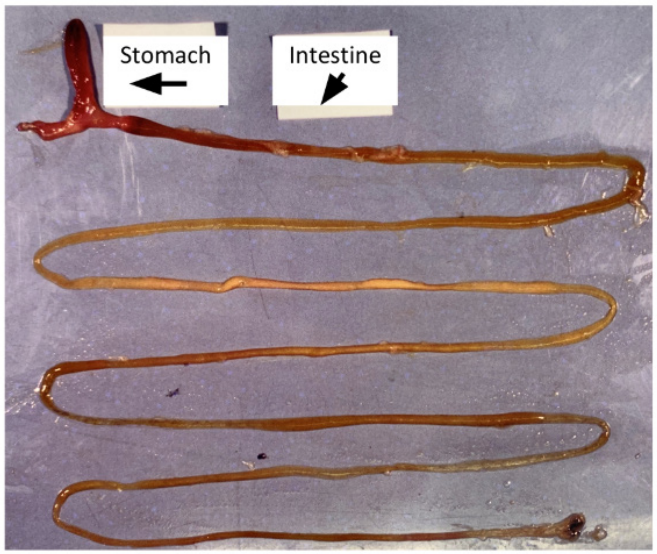

Figure 3 The stomach and intestines of Tilapia *Oreochromis niloticus* used in the experiment.

Measuring enzyme activity

Carboxymethylcellulose (CMC) served as the substrate. 0.2% CMC solution (dissolved in 0.1M Mellvaine buffer, pH 3.0), 0.5mL, was mixed with the same volume of the enzyme solution. The mixture was incubated at 40°C for lh and was then combined with 1.0mL of ethanol. The solu-bilized glucose in the supernatant was quantified by the phenol-sulfuric acid method.[14] One unit of enzyme activity was needed to free 1u mol glucose per minute at a temperature of 40°C.

Protein quantitation

Protein was quantified using modified Lowry method.[15]

α -Cyclodextrin Sepharose 6B affinity chromatography

Epoxy-activated Sepharose 6B (Pharmacia) was used to prepare the gel for chromatography. The gel, prepared by the routine method[16] and in the same way as that reported in our previous paper, was equilbrated with 20mM McIlvaine buffer containing 1mM calcium chloride (pH 3.0). The gel was then applied to the column (2×20cm), to which the crude enzyme solution was then added. Elution was performed first with the above-mentioned buffer (150mL, pH 3.0) and then with the same buffer containing 0.5% B-cyclodextrin (150mL). The eluate was divided into 5mL aliquots and subjected to ultraviolet absorptiometry of protein (280nm) and cellulase activity assay.

CM-cellulose ion exchange chromatography

The active fraction obtained by affinity chromatography was applied on a CM-cellulose column (1.5×25cm) equilibrated with 20mM Mcllvaine buffer (pH5.0). The same buffer (100mL) was first used to wash the column. A linear gradient elution using this buffer (225mL) and 0.8M sodium chloride solution (225mL) was performed thereafter.

Chromatofocusing

Polybuffer exchanger (PBE94, Pharmacia) was buffered with 25mM Tris-HCl buffer (pH 9.4).The mixture was then applied to a column (1.2×18cm). Elution was performed with 100mL of Polybuffer 96:97 (1:2, v/v) -HCl solution (pH 4.0).

Gel chromatography

Gel chromatography[17] was performed using a Sephadex G-100 column (1.5×96cm) equilibrated with 10mM McIlvaine buffer (pH 3.0). Elution was performed using the same buffer (pH 3.0).

Eluate (each 3mL) was subjected to absorptiometry at 280nm and cellulase activity assay.

Purity measurement

The purity of isolated substances was detected by SDS-polyacrylamide gel electrophoresis PAGE) using a disc gel (5×70mm).[18]

Estimation of molecular weight

Gel filtration[17] and SDS-PAGE[19] were used to estimate the molecular weights of isolated sub-stances. For gel filtration, an enzyme solution and reference proteins with a known molecular weight were applied to the Sephadex G-100 column (1.5×96cm) equilibrated with 10mM Mc-Ilvaine buffer (pH 3.0). After elution with the same buffer, molecular weight was estimated by comparing the location of elution between the test enzyme solution and the reference proteins.

For SDS-PAGE, the sample (400m g) was treated with SDS and applied to the separation gel (10% polyacrylamide gel, pH 8.3), followed by electrophoresis at a constant current (8 mA/tube).

Measurement of isoelectric point

Using the method reported elsewhere,[1] isoelectric electrophoresis was performed for 4 hours at 4°C and 300V. The device used was a Rotofore (pH3-10; Bio-Rad Laboratories, Inc.).

Results and discussion

Cellulase distribution in the stomach and intestine

The stomach and intestine, which are major digestive organs, were removed from the fish. Enzymes contained in these organs were extracted with a physiological saline. Cellulase activity in the extracts was measured. As shown in Table 1, the cellulase activity per fish was 101 units in the stomach and 184units in the intestine. The activity in the intestine was about 1.8times as high as that in the stomach. The activity per g tissue in the stomach (2.5units/g) was about 3.6 times as high as that in the intestine (0.7units/g).

Table 1 Activity of Tilapia stomach and intestine cellulases (units)

	stomach	intestine
Activity per fish	101	184
Activity per g tissue	2.5	0.7

Although herbivorous or polyphagous fish, which eat plants, is expected to have cellulase which degrades cellulose (a component of the cell wall), no reports on cellulase in the digestive organs of these kinds of fish have previously been published. The results in the present study suggest that non-starch polysaccharides first undergo rough digestion in the stomach.

Purification of enzymes

When the crude enzyme solution was applied to the a-cyclodextrin-Sepharose 6B column, cellulase activity was detected in the weakly adsorbed fraction. This fraction was subjected to purification by CM-cellulose chromatography. This resulted in elution of activity at of

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0.6M NaCl. This reaction was dialyzed, desalted and then subjected to chromatofocusing. As a result, an active fact ion was detected at pH 7.8.

A single peak of activity was detected with the Sephadex G-100 chromatography. When the purity of this fraction was tested by SDS-PAGE, a single protein band was detected, indicating that the purity level was high (Figure 4).

Figure 4 Disc-polyacrylamide gel electrophoresis of the purified cellulase from Tilapia stomach.

The purified enzyme (0.1mg) was applied to 10% polyacrylamide gel (pH 8.3). The gel was stained for protein with Coomassie brilliantblue R-250.

The results of purification mentioned above are summarized in Table 2. The activity of the purified enzyme was 55 times as high as that of the extract. The yield of the activity was 10.2%.

Properties of purified enzyme

Influence of pH

The activity of this cellulase was highest at pH3.0. When the stability of this enzyme was evaluated at varying pH (40°C, 30 min), the enzyme was stable at pH range between 3.0-5.0 (Table 3). The optimal pH of this enzyme was lower as compared to those of the enzymes derived from *Delabella* (pH 8.9)[20] and *Euhadra peliomphala* (pH7.0),[21] but it was very close to the optimal pH of the cellulases isolated from microorganisms *Stereum anguinolentum* (pH 3.7)[22] and Trichoderma viride (pH 3.0).[23]

Influence of temperature

The activity of the enzyme at various temperatures (pH 3.0) and the residual activity after incubation for 1 hour at varying temperatures (pH 3.0) were measured to determine the optimal temperature and thermal stability (Table 3). The activity of this enzyme was maximal at 45°C. It was stable at temperatures below 50°C. The optimal temperature for this enzyme was lower than those of the enzymes isolated from Trametes sanguinea(60°C)[23,24] the bacteria *Stereum sanguinolentum* (60°C),[22] and *Trichoderma viride* (60°C),[23] but was very close to those of the enzymes isolated from *Euhadra peliomphala* (40°C)[21] and *Trichoderma koningii* (45°C).[24]

Table 2 Summary of purification process of Tilapia stomach cellulase

Stages	Total Protein (mg)	Total activity (units)	Specific activity (units/mg protein)	Purification (fold)	Yield (%)
Extract	78.9	4.2	0.05	1.0	100
Crude enzyme*1	60.7	3.6	0.06	1.2	85.7
α-Cyclodetrin-sepharose 6B	41.2	2.9	0.07	1.4	69.0
CM-cellulose	0.58	0.75	1.29	25.8	17.9
PBE94 *2	0.23	0.52	2.26	45.2	12.3
Sephadex G-100	0.12	0.43	2.75	55.0	10.2

*1 Ammonium sulfate precipitation (60% saturation)

*2 Chromatofocusing on polybuffer exchange

Molecular weight and Isoelectric point

The molecular weight of this enzyme was estimated to be 56,000 by gel filtration with a Sephadex G-100 and SDS-PAGE. Because the molecular weight estimated by gel filtration was consistent with that estimated by SDS-PAGE, this enzyme was thought to be composed of a single-chain polypeptide. The molecular weight of this enzyme was equal to that of cellulase isolated from *Irpex lacteus* (56,000).[25,26] The isoelectric point for this enzyme was pI7.8. (Table 3).

Table 3 Properties of cellulase isolated from the stomach of Tilapia *Oreochromis niloticus*

Optimal pH	pH Stability	Optimal Temperature	Temperature Stability	Molecular Weight	Isoelectric Point
pH 3	pH3.0-5.0	45°C	50°C	56,000	7.8

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Influence of reagents

The test enzyme solution was combined with various reagents in a concentration of 1mM. The mixture was incubated at 40C for 30min, and the residual enzyme activity was measured. The activity was expressed in percentages relative by where the activity measured in the absence of reagents was 100%, as shown in Table 4. The enzyme was completely inhibited by $Hg^{2+}$and PCMB, and the activity was suppressed at 32% following treatment with EDTA. These results suggest that the SH-group and metals are involved in the expression of this enzyme's activity.

Table 4 Effect of various reagents on the activity of Tilapia stomach cellulase

Reagents (1mM)	Relative activity (%)
None	100
$AlCl_2$	98
$CaCl_2$	96
$MgCl_2$	85
$NiCl_2$	78
$CdCl_2$	68
$CuCl_2$	60
$PbCl_2$	59
$ZnCl_2$	58
$HgCl_2$	0
CH_2ICOOH	84
EDTA *1	32
PCMB *2	0

The enzyme was preincubated with ImM of various reagents at 40°C for 30min, and the remaining activity was deterinined.

*1 EDTA: ethylenediaminetetraacetic acid.

*2 PCMB: *p*-chloromercuribenzoic acid.

Substrate specificity

When the activity of this enzyme on non-starch polysaccharides was evaluated (Table 5), the enzyme degraded CMC, cellulose and agarose, but did not degrade galactan, gum arabic, pectin, inulin and cellobiose. Because the enzyme degraded CMC more strongly than any other substrates, we calculated relative degrading capability against each substrate by setting the capability against CMC as 100%. The relative degrading capability thus calculated was 10% against cellulose and 5% against agarose. When the products of cellulose degradation were subjected to HPLC with an amino-group bound column, oligosaccharide with the degree of polymerization being 6 (G6) was detected. Considering the result that this enzyme did not degrade cellobiose, there seems to be β-glucosidase which acts on G6.

Table 5 Digestion of non-starch polysace harides with Tilapia stomach cellulase

Substrates (0.5%)	Relative activity (%)
Galactan	0
Gum arabic	0
Pectin	0
Inulin	0
Agarose	5
Cellobiose	0
Cellulose	10
Carboxymethylcellulose	100

Conclusion

As described above, we extracted cellulase from the stomach of Tilapia *Oreochromis niloticus* and purified it by α-cyclodextrin-Sepharose 6B affinity chromatography, CM-cellulose chromatography, chromato-focusing and Sephadex G-100 chromatography. In In this way, we obtained one type of cellulase. This enzyme had maximal activity at a temperature at 45°C and a pH 3.0. Its activity was suppressed by Hg^{2+} and PCMB. The molecular weight of the enzyme was about 56,000. Its pI was 7.8. This enzyme degraded CMC, cellulose and agarose. These results suggest that Tilapia degrades the cellulose, agarose and other components of the cell wall of ingested grass with its gastric cellulase and thus causes the cells to collapse.

Tilapia are tropical fish, and this purified cellulase was extracted from the acidic stomach. This cellulase acts at an optimal temperature of 45℃ and an optimal pH of 3.0, indicating that it functions under conditions suitable for the internal environment of a Tilapia. We believe it is necessary to consider the optimal conditions for digestive enzymes when developing Tilapia feed. Furthermore, it was found that purified cellulase is inhibited by mercury. In addition to cellulase, previous reports have confirmed that amylase and protease are also inhibited by mercury. This indicates that digestive enzymes do not function in environments containing heavy metals, such as industrial water and water sources containing high concentrations of wastewater, suggesting that these environments are unsuitable for Tilapia farming.

The results also suggest that the gastric cellulase of Tilapia stimulates the digestion of starch, protein, etc. The present study partially unveiled the eating habits and mechanisms of Tilapia (a polyphagous fish) from the viewpoint of enzymes invoiced in digestion. The results from this study will be valuable as basic data for developing diets for use in cultivation of polyphagous fish.

In addition, it is considered to be a future examination whether this cellulase is the origin of

a Tilapia alimentary canal, or it is the origin of a parasite. We consider whether a cellulase gene is in the fish digestive organs want to analyze.

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Conflicts of interest

The author declares that there are no conflicts of interest.

References

1. Yamada A, Takano K, Kamoi I. Purification and properties of amylases from Tilapia stomach. *Nippon Suisan Gakkaishi*. 1996;62;269–274.
2. Yamada A, Takano K, Kamoi I. Purification and properties of protease from Tilapia stomach. *Nippon Suisan Gakkaishi*. 1993;59;1903–1908.
3. Taniguchi AY, Takano K, Kamoi I. Purification and properties of lipase from Tilapia stomach. *Nippon Suisan Gakkaishi*. 2001;67;96–101.
4. Yamada A, Takano K, Kamoi I. Purification and properties of amylase from Tilapia intestine. *Nippon Suisan Gakkaishi*. 1991;57;1903–1909.

5. Yamada A, Takano K, Kamoi I. Purification and properties of a–glucosidase from Tilapia intes–tine. *Nippon Suisan Gakkaishi*. 1992;58;2189–2195.
6. Yamada A, Takano K, Kamoi I. Purification and properties of protease from Tilapia intestine. *Nippon Suisan Gakkaishi*. 1991;57;1551–1557.
7. Taniguchi AY, Takano K, Kamoi I. Purification and properties of lipase from Tilapia intestine. *Nippon Suisan Gakkaishi*. 2001;67;78–84.
8. Taniguchi AY, Takano K. Purification and properties of carboxy–pepidase from Tilapia intestine. *Nippon Suisan Gakkaishi*. 2001; 67:1096–1102.
9. Taniguchi AY, Takano K. Purification and properties of amino–pepidase from Tilapia intestine. *Nippon Suisan Gakkaishi*. 2002. 68:382–388.
10. Takeuchi I. *Fish Physiology*. In: Y. Itazawa, Editor, *Digestion and Nutrition*. 1991:67–101.
11. Taniguchi AY, Takano K. Purification and properties of B–galactosidase from Tilapia Intestine: Digestive Enzyme of Tilapia–X. *Fisheris Science*. 2004;70:688–694.
12. Davison A, Blaxter M. Ancient origin of glycosyl hydrolase family 9 cellulase genes. *Molecular Biology and Evolution*. 2005;22:1273–1284.
13. Suzuki K, Ojima T, Nishita, K. Purification and cDNA cloning of a cellulase from abalone Haliotis discus hannai. *Eur J Biochem*. 2003;270:771–778.
14. Fukui S. Quantitation of reduced sugar with copper reagents Biochemical Experiment Methods: Quantitation of Reduced Sugar (Second Edition). *Gakkai Shuppan Center, Tokyo*. 1990;9–11.
15. Hatree EF. Determination of protein: A modification of the Lowry method that gives a linear photometric response. *Anal Biochem*. 1972;48:422–427.
16. Chihata I. Preparation of Adsorbates and Their Properties–Experiment and Application: Af fi–nity Chromatography. *Kodansha Scientific, Tokyo.* 1976;39–109.
17. Fischer L. *Gel Chromatography – Biochemistry Series: Gel Chromatography.* Tokyo Kagaku Dojin, Tokyo. 1974;59–147.
18. Nagai Y. Disc electrophoresis. *Tanpakushitsu Kakusan Koso*. 1966;11:744–749.
19. Hayashi K, Oba Y. SDS–polyacrylamide gel electrophoresis. *Tanpakushitsu Kakusan Koso*. 1972;17:304–311.
20. Okada G, Nisizawa T, Nisizawa K. Cellulases of a marine mollusc, Dolabella sp. *Biochem J*. 1966;99:214–221.
21. Okada, G. Cellulases of Euhadra peliomphala. *Bull Fac Educ Shizuoka Univ Nat Sci Ser*. 1972;23:57–61.
22. Eriksson KE, Pettersson B. Extracellular enzyme system utilized by the rot fungus Stereum sanguinolentum for the breakdown of cellulose. *Arch Biochem Biophys*. 1968;124:142–148.
23. Shoemaker SP, Brown RD. Characterization of endo–1, 4– B–D–glucanase purified from T. Viride. *Biochem Biophys Acta*. 1978;523:147–152.
24. Halliwell G, Griffin M. The nature and mode of action of the cellulolytic component C1 of Trichoderma koningii on native cellulose. *Biochem J*. 1973;135:587–590.
25. Kanda T, Wakabayashi K, Nisizawa K. Purification and properties of endocellulase of avi–celase type from Irpex lacteus (Polyporus tulipiferae). *J Biochem*. 1976;79:977–988.
26. Kanda T, Wakabayashi K, Nisizawa K. Xylanase activity of an endo–cellulase of carboxymethyl–cellulase type from Irpex lacteus (Polyporus tulipiferae). *J Biochem*. 1976;79:989–995.

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