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CHARACTERIZATION OF PROTEASES IN THE DIGESTIVE TRACT OF
ATLANTIC SALMON (SALMO SALAR) IN COMPARISON WITH
RAINBOW TROUT (SALMO GAIRDNERI)

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ABSTRACT

The proteolytic activities of the proteases extracted from the digestive tracts of Atlantic salmon and rainbow trout show similar characteristics in the optimum temperatures.

In Atlantic salmon, the optimum temperature of the stomach extract is 37.5°C, of the pyloric caeca and of the small intestinal extracts, 52.5°C. The extract from the large intestine shows two optimum temperatures at 45 and 52.5°C. A similar pattern was observed in the extracts from the digestive tract of rainbow trout except for the stomach extract, where the optimum temperature is 35°C. The proteolytic activities of the intestine are very pronounced at the conjunction between the small and large intestines.

The characteristics in electrophoretic mobility of these proteases will also be studied.

INTRODUCTION

Salmonid fishes have high protein requirement for growth. In species of salmon and trout, about 40% of crude protein level in diet is required for optimum growth rate (Cowey, 1979). The proteolytic activities in the digestive tract should be relatively high, and expected to influence on the growth rate of these fishes.

A lot of work on proteases in the digestive organs have been done in different fishes, such as rainbow trout (Kitamikado and Tachino, 1960; Kawai and Ikeda, 1973a; Rungruangsak and Utne, 1981), mackerel (Ooshiro, 1971) and carp (Kawai and Ikeda, 1972 and 1973b). The knowledge about digestive enzymes in Atlantic salmon is however limited.

In a preliminary work (Rungruangsak and Utne, 1981), the protease activities in the digestive tract of rainbow trout was studied. In this experiment, the proteases of the different digestive tissues were characterized in more detail to observe the optimum temperatures and electrophoretic mobilities. The characteristics of the peptic-like and the tryptic-like enzymes were compared between two salmonid fishes, Atlantic salmon and rainbow trout.

MATERIALS AND METHODS

Two groups of fishes, Atlantic salmon (Salmo salar) and rainbow trout (Salmo gairdneri), were used as experimental fishes in this study. Each group was cultured separately in freshwater in a round fibreglass tank (1.7 m³) at Matre Aquaculture Station. The fishes were fed a commercial dry pelleted feed (Tess laksefór, T.Skretting Ltd., Stavanger).

The fishes ranged from 62 to 192 g and 17 to 25 cm. The fish in each group were sampled twice, 4 fish each time, for duplication and averaging the results. They were killed and the digestive tracts were removed at once, emptied, defatted and cut into five sections: stomach, pyloric section and three equally-long sections of the intestines. During sampling the temperature was controlled at 4-5°C. The corresponding sections from 4 fish in each group were pooled and the protease of each section of the digestive tract was extracted and assayed for proteolytic activity by the method of Rungruangsak and Utne (1981). In order to compare the optimum temperatures of the protease activities in the digestive tract between Atlantic salmon and rainbow trout, the assayed

temperatures were varied from 20 to 60°C.

Another experiment was carried out in order to see the difference in protease activities of the intestinal sections in more detail. The intestines from 2 fish in each group were cut and divided into 4 sections: the first half and the second half of small intestines, and the first half and the second half of large intestines. The protease of each section was extracted and the enzyme activities were also determined by varying the temperature from 20 to 60°C.

RESULTS

By varying the temperature, the optimum temperatures for the determination of protease activities of the stomach extract and pyloric caeca extract of Atlantic salmon are 37.5 and 52.5°C, respectively (Fig,1). The first section of the intestine has very low protease activity while the second and the third sections have higher activities and show two optimum temperatures at 45 and 52.5°C. As shown in Fig.2, the similar pattern of the optimum temperatures was observed in the extracts from the digestive tract of rainbow trout except for the stomach extract, the optimum temperature is 35°C.

The protease from the stomach extract of both Atlantic salmon and rainbow trout showed peptic-like activity and has the optimum pH about 2. The tryptic-like enzymes in the pyloric caeca and the intestinal extracts have a broad optimum pH at about 9.

Since there was extremely low proteolytic activity in the first section of the intestine (Fig.1 and Fig.2), the first half

of the small intestine was omitted in the second experiment. It is clearly shown, especially in the intestine of Atlantic salmon, that the protease activity in the second half of the small intestine has the optimum temperature at 52.5°C while both sections of the large intestine show two optimum temperatures at 45 and 52.5°C (Fig.3).

DISCUSSION

The proteolytic activities in the digestive tracts of Atlantic salmon and rainbow trout show the optimum pH at about 2 for the peptic-like activity from the stomach extract and about 9 for the tryptic-like activities from the pyloric caeca and intestinal extracts. These optimum pHs are typical for pepsins and trypsins, respectively.

From Fig.1 and Fig.2, the optimum temperature of the peptic-like activity in Atlantic salmon (37.5°C) is somewhat higher than that in rainbow trout (35°C). The tryptic-like activities have the same pattern of the optimum temperatures, 52.5°C for pyloric caeca extract, 45 and 52.5°C for the intestinal extracts. The conditions were similar to those observed by Kitamikado and Tachino (1960), the optimum temperatures of the proteases in rainbow trout were observed to be 40°C for the extract from the stomach and 45°C for the extracts from pyloric caeca and intestine. Ooshiro (1971), found the purified proteinase from the pyloric caeca of mackerel to be most active at 43°C. The optimum temperature of the tryptic-like activity from the pyloric caeca extract was found to be higher in this experiment and the enzyme activities in the extracts of intestine exhibit two active

temperatures in both Atlantic salmon and rainbow trout. As shown in Fig.3, the protease activity of the second half of the small intestine has high optimum temperature at 52.5°C as in the pyloric caeca, only the large intestine shows two active temperatures at 45 and 52.5°C for tryptic-like activities. It is clearly shown in Atlantic salmon. The enzymes in the first half of large intestine have higher proteolytic activities than the other parts of the intestine.

It can be concluded that there must be at least two different tryptic-like enzymes in the large intestine. The levels of protease activities of the intestine are very high in the extracts from the second half of small intestine and the first half of large intestine, but very low in the other parts especially in the first section of intestine. The digestibility in the intestine of Atlantic salmon and rainbow trout must be fairly high at the conjunction between the small and large intestines.

The characteristics in electrophoretic mobility of these proteases is on study.

REFERENCES

- Cowey, C.B., 1979. Protein and amino acid requirements of finfish. In: J.E. Halver and K. Tiews (Editors), *Finfish Nutrition and Fishfeed Technology, Proceedings of a World Symposium, Hamburg 20-23 June, 1978. Vol. I, Berlin, pp 3-16.*
- Kawai, S. and Ikeda, S., 1972. Studies on digestive enzymes of fishes- II Effect of dietary change on the activities of digestive enzymes in carp intestine. *Bull. Jpn. Soc. Sci. Fish., 38:265-270.*

- Kawai,S. and Ikeda,S.,1973a. Studies on digestive enzymes of fishes-
III Development of the digestive enzymes of rainbow trout
after hatching and the effect of dietary change on the
activities of digestive enzymes in the juvenile stage.
Bull.Jpn.Soc.Sci.Fish.,39:819-823.
- Kawai,S. and Ikeda,S.,1973b. Studies on digestive enzymes of fishes-
IV Development of the digestive enzymes of carp and black sea
bream after hatching. Bull.Jpn.Soc.Sci.Fish.,39:877-881.
- Kitamikado,M. and Tachino,S.,1960. Studies on the digestive enzymes
of rainbow trout- II Proteases. Bull.Jpn.Soc.Sci.Fish.,
26:685-690.
- Ooshiro,Z.,1971. Studies on proteinase in the pyloric caeca of
fishes- II Some properties of proteinase purified from the
pyloric caeca of mackerel. Bull.Jpn.Soc.Sci.Fish.,37:145-148.
- Rungruangsak,K. and Utne,F.,1981. Effect of different acidified
wet feeds on protease activities in the digestive tract
and on growth rate of rainbow trout (Salmo gairdneri
Richardson). Aquaculture,22:67-79.

Figure 1: The protease activities at various temperatures of the different sections of the digestive tract of Atlantic salmon.

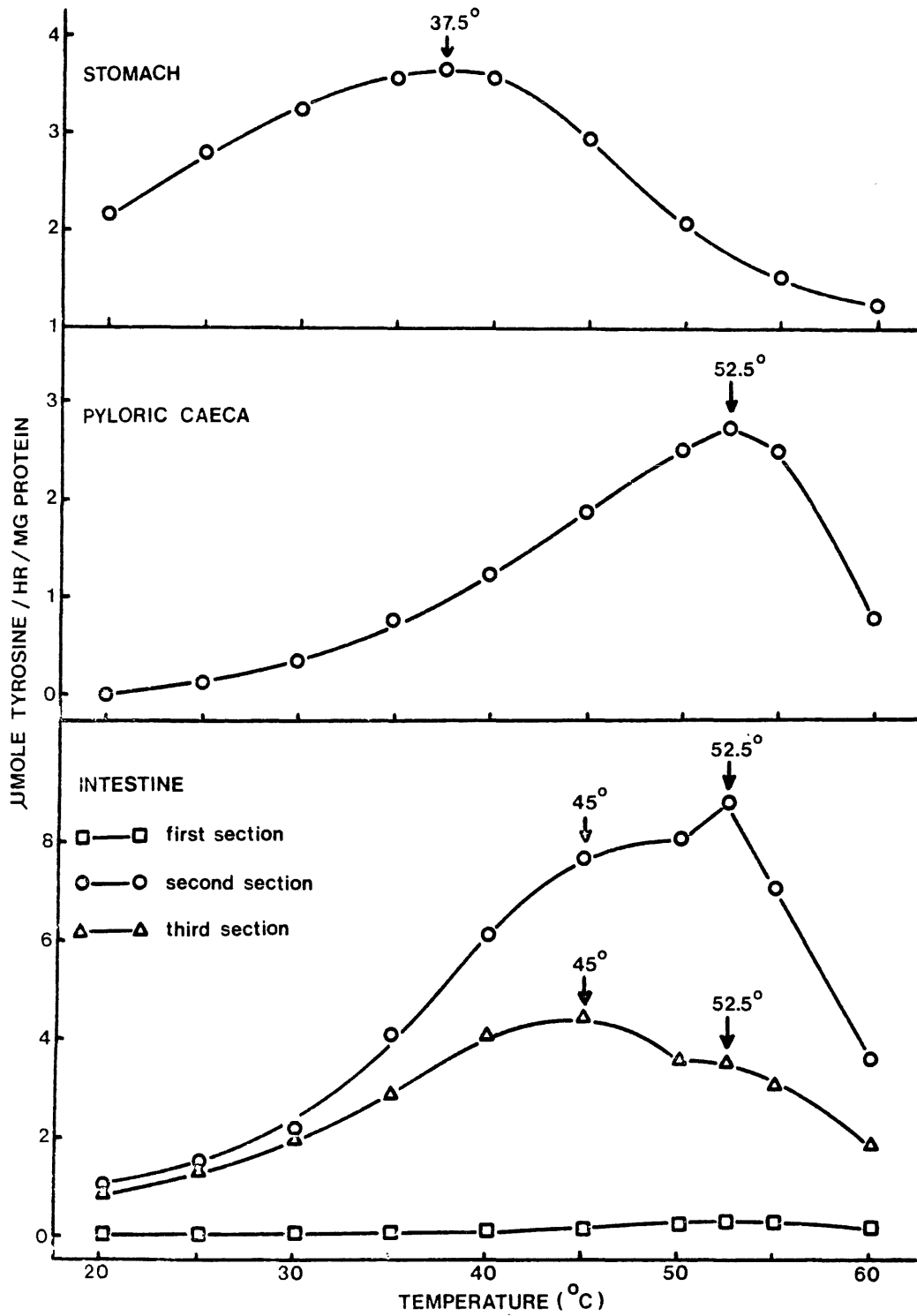


Figure 2: The protease activities at various temperatures of the different sections of the digestive tract of rainbow trout.

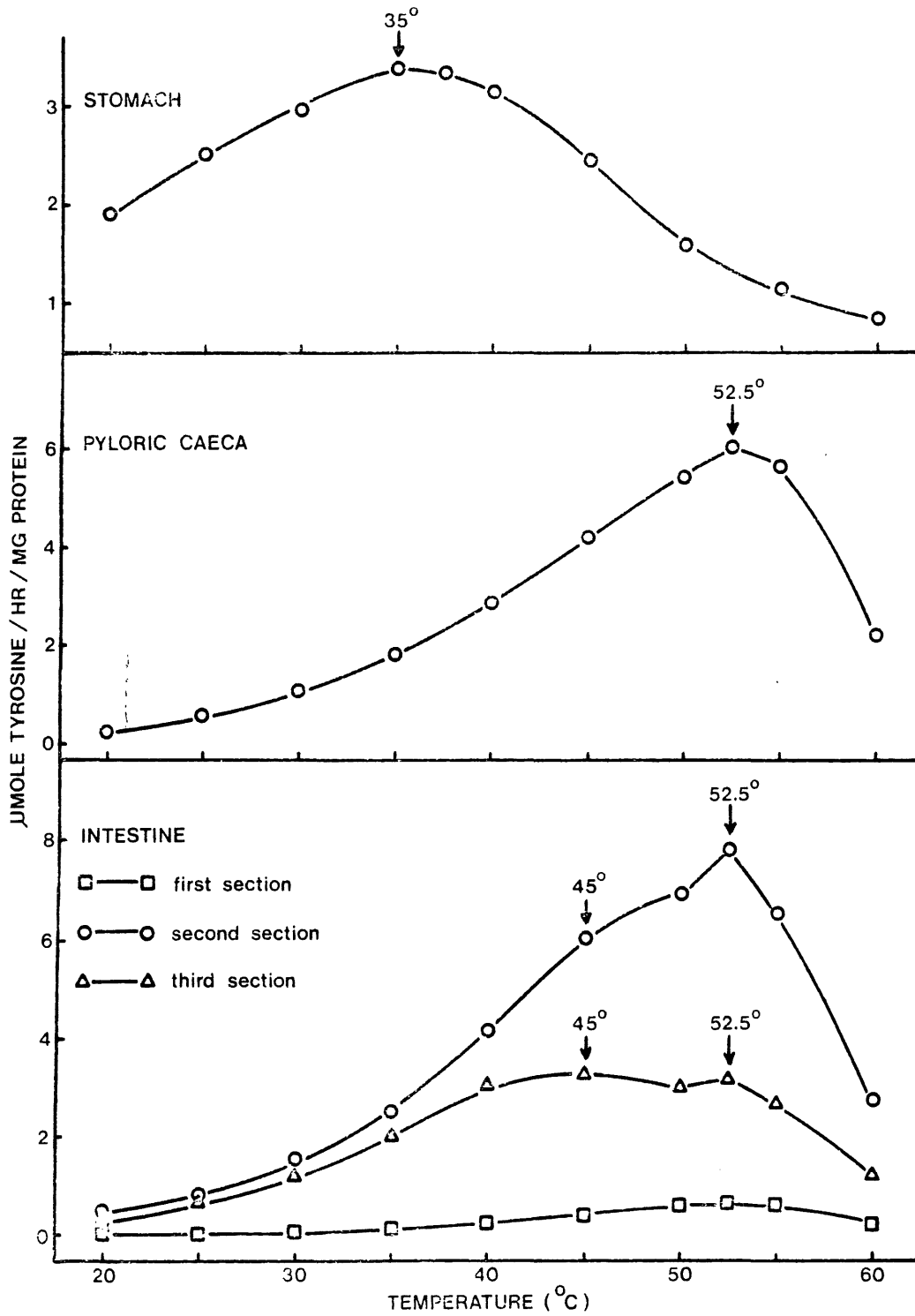


Figure 3: The protease activities at various temperatures of the different intestinal sections of rainbow trout and Atlantic salmon.

