Note

Vitamin B_{12}-Producing Ability of the Intestinal Microflora of Rainbow Trout (*Oncorhynchus mykiss*)

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Received October 1, 1990

In previous papers,[1,2] we reported that tilapia (*Oreochromis niloticus*) and carp (*Cyprinus carpio*), which do not require dietary vitamin B_{12}, harbor vitamin B_{12}-producing obligate anaerobes (especially *Bacteroides* type A) predominantly, while channel catfish (*Ictalurus punctatus*) and Japanese eel (*Anguilla japonica*), which require the vitamin in their diets, harbor them poorly. It, however, remains unclarified whether other fish species living in different environments are also supplied with enough vitamin B_{12} by obligate anaerobes. Therefore this study was undertaken to examine a cold-water fish, rainbow trout (*Oncorhynchus mykiss*), which do not require dietary vitamin B_{12}.[3]

The contents of the intestines were aseptically removed from five cultured specimens of rainbow trout (76–88 g body weight), diluted serially and plated onto Trypticase soy blood agar (BBL), PEA blood agar (BBL), MacConkey agar (Eiken), EG blood agar (Nissui), FM-CW blood agar (Eiken), AS agar,[4] and BS agar.[4] The first three media were incubated aerobically, and the last four media were incubated anaerobically, both groups at 20°C for 10 days. Anaerobic cultivation was done by the GasPak anaerobic system (BBL).

After incubation, about 20 colonies were isolated at random from each plate and identified as described previously.[4] On suitably diluted samples, colonies of each bacterial component were counted and expressed as the number of colony forming units (CFU) per gram. The maximum count of each bacterial component through the seven media was regarded as the estimated viable count of that component.

Selected strains from each bacterial component were incubated in GAM broth (Nissui) at 20°C for 5 days under anaerobic conditions. The culture was then assayed microbiologically for vitamin B_{12} (as cyanocobalamin) using *Lactobacillus leichmannii* IAM 12066 by the procedure of Sato,[5] and the amount of vitamin B_{12} produced or consumed was calculated from the difference between the inoculated and uninoculated broths.

A total of 11 bacterial genera were isolated from the trout's intestine, and *Aeromonas*, Enterobacteriaceae, and *Pseudomonas* predominated in all five specimens (Fig. 1). Eighty-three strains of the genus *Aeromonas* were divided into *A. hydrophila* (56 strains) and other *Aeromonas* (27), which could not grow at 37°C and produced brown water-soluble pigment. Obligate anaerobes, including *Bacteroides* type A, other Bacteroidaceae and *Clostridium*, were few in number ($\leq 8.7 \times 10^4$ CFU/g) with an occurrence of 60–100%.

Of 105 strains examined, 13 strains consumed vitamin B_{12} and the remaining 92 strains conversely produced it. Especially, 33 strains, consisting of *Bacteroides* type A (9 strains), *Clostridium* (8), *Aeromonas* (including *A. hydrophila*) (6), Bacteroidaceae (4), *Moraxella* (2), coryneforms (2), Enterobacteriaceae (1), and *Acinetobacter* (1) produced vitamin B_{12} at levels greater than 1 ng/ml, as shown in Fig. 1.

Lovell and Limsuwan[6] reported that the apparent rate of intestinal bacterial synthesis of vitamin B_{12} in tilapia fed a vitamin B_{12}-deficient diet (at least 11.2 ng per gram of body weight per day) was eight times that found in channel catfish fed the same diet (about 1.4 ng/g/day). In our previous study,[1,2] we found that the difference of the

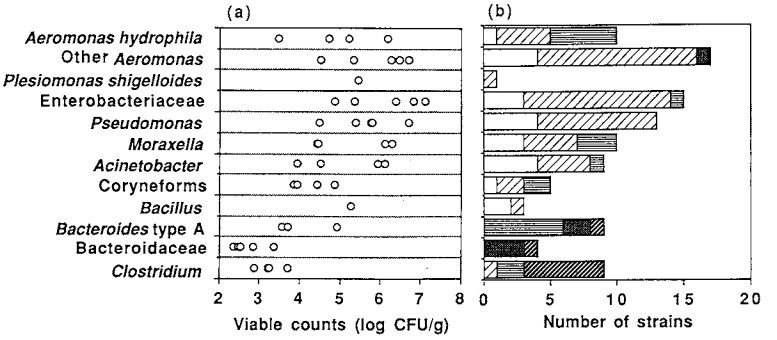

Fig. 1. The Intestinal Microflora of Five Specimens of Rainbow Trout (a) and the Vitamin B_{12}-Producing Ability of Each Bacterial Component (b).

□, <0.1 ng/ml; ▨, 0.1–1.0; ▤, 1.1–5.0; ▩, 5.1–10.0; ▨, >10.0.

vitamin B_{12}-producing rate in the intestinal tract between tilapia and channel catfish was attributed to the fact that the former harbors obligate anaerobes abundantly but the latter poorly. This result strongly suggests that the rainbow trout also uses vitamin B_{12} that is mainly produced by obligate anaerobes, especially *Bacteroides* type A. This study, however, indicates that 6–100% strains of seven bacterial components, including aerobes and anaerobes, can produce much of this substrance but the aerobes/facultative anaerobes were much greater in number than the obligate anaerobes in the intestine of rainbow trout (Fig. 1). These results strongly suggest that the amounts of vitamin B_{12} required by rainbow trout are mainly supplied by *Aeromonas* (including *A. hydrophila*), Enterobacteriaceae, *Moraxella*, and *Acinetobacter*, along with *Bacteroides* type A, Bacteroidaceae, and *Clostridium*.

Acknowledgments. We thank the Institute of Applied Microbiology (University of Tokyo) for supplying the *Lactobacillus leichmannii* IAM12066 strain. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan.

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