研究課題名

和文：マダイ（*Pagrus major*）肝臓プロテオームにおける抗生物質の影響評価

英文：Hazard assessment of antibiotics in the hepatic proteome of red seabreams (*Pagrus major*)

共同研究者名

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Abstract

養殖業で使用される抗生物質の約 80%は活性を維持したまま環境中に放出される。オキシテトラサイクリン（OTC）は、最も消費量の多い抗生物質の一つであることから、OTC の標的魚・非標的魚への影響が懸念されている。本研究計画では、マダイの幼魚を対象に、OTC の短期・長期・投与後の影響をプロテオームレベルで調査した。その結果、OTC は全ての期間で代謝系へ影響し、短期投与では免疫系、長期投与では神経系に影響を与えることが明らかになった。本研究は OTC の短期・長期・投与後の影響をプロテオームレベルで評価した初めての研究である。本研究の結果は、多くの非標的魚における抗生物質の潜在的なリスクを示唆した。この研究結果は現在、Aquatic toxicology へ投稿準備中である。

Introduction

Oxytetracycline (OTC) is one of the most consumed antibiotics. Due to its broad-spectrum and low-cost, OTC has been widely used to both healthy and diseased animals in aquaculture Approximately 80% of antimicrobials used in aquaculture enter the environment with their activity intact [1], raising concerns about the effects of OTC on target and non-target fish.

Investigations of adverse effects of OTC have been performed mainly in freshwater fish. Short-term OTC administration induced oxidative stress and altered metabolic status in zebrafish[2] and silver catfish[3]. Long-term OTC administration induced oxidative stress[4,5],

reduced growth performance[6,7], immune modulation [4,8] and changed energy allocation[6,9] in zebrafish and Nile tilapia. Julinta et al. (2019) demonstrate that reduced growth and elevated stress biomarker levels were continued on day 42 post-OTC-dosing[10].

Despite these pioneering researches on fish, the adverse outcome pathways (AOPs) that provided insights into the molecular mechanisms underlying the phenotypic effects of OTC on marine fish is still poorly understood because these previous studies were mainly focused on freshwater fish and performed by histopathological and biochemistry analysis. Furthermore, only few studies have systematically demonstrated the effects of short, long, post-OTC administration, hence time series variation of OTC side-effects is largely unknown.

In this study, to reveal the molecular mechanisms underlying the short-, long-, post-OTC effects at the translational level in marine fish, we characterized the biological and biochemical effects of OTC and investigated the global changes in abundances of proteins in the liver of juvenile red seabreams.

Materials and Methods

[Experimental design]

The detail of the OTC administration schedule and seawater for rearing are described in Obayashi et al., 2020[11].

[Identification of differentially abundance proteins (DAPs)]

To identify the differential abundance proteins (DAPs), the empirical Bayes method was used and $p<0.05$ considered as significant.

Result and Discussion

[Pathway enrichment analysis]

Pathway enrichment analysis was carried out through the Comparative Toxicogenomics Database (CTD; http://ctdbase.org/) to investigate the biological functions of DAPs. For this analysis, the accession number of DAPs was converted to zebrafish ortholog gene symbols and used.

The top fifteen significant pathways of each group were listed in Fig.1. Eight of the forty-three listed pathways have linked with metabolism, including carbon metabolism, histidine metabolism, metabolic pathways, metabolism, metabolism of amino acids and derivatives, metabolism of proteins, pyruvate metabolism, and seven amino acid metabolisms (Fig. 1). Metabolic related pathways were significantly enriched in all timepoints except high-OTC exposure group in the long-term OTC administration (Fig. 1), suggesting metabolic disruption is one of the main outcomes of OTC-administration. This observation consisting with many other fish, such as zebrafish[2], Nile tilapia[7], and silver catfish[3].

Interestingly, innate immune system pathway-related genes were enriched in the low-OTC exposure group in the short-term OTC administration (Fig. 1). The abundance of proteins involving in innate immunity and neutrophil degranulation (i.e., hspa8, lta4, and tkfc) was significantly changed in the high-OTC group in the short-term OTC administration, although the immune pathway was not significantly enriched in this group. Similarly, rab10, an innate immune system-related gene, was changed the abundance by OTC administration in the long-term OTC administration low and high group, but the immune pathway was not significantly enriched at the timepoint. These results suggest that immune system disruption was the early response for OTC exposure. Consisting of this, OTC interferes with immune mechanisms in guiled seabream after 7 days and 14 days OTC treatment but not at 21 days.

Enrichment analysis also revealed that the OTC effects on the nervous system. The genes related to Alzheimer's disease were significantly enriched in the long-term low OTC administration group (Fig. 1). This result suggests that long-term OTC administration might induce nervous system disruption. Neuro system disruption by long-term OTC administration was also observed in zebrafish[12] and rainbow trout[13].

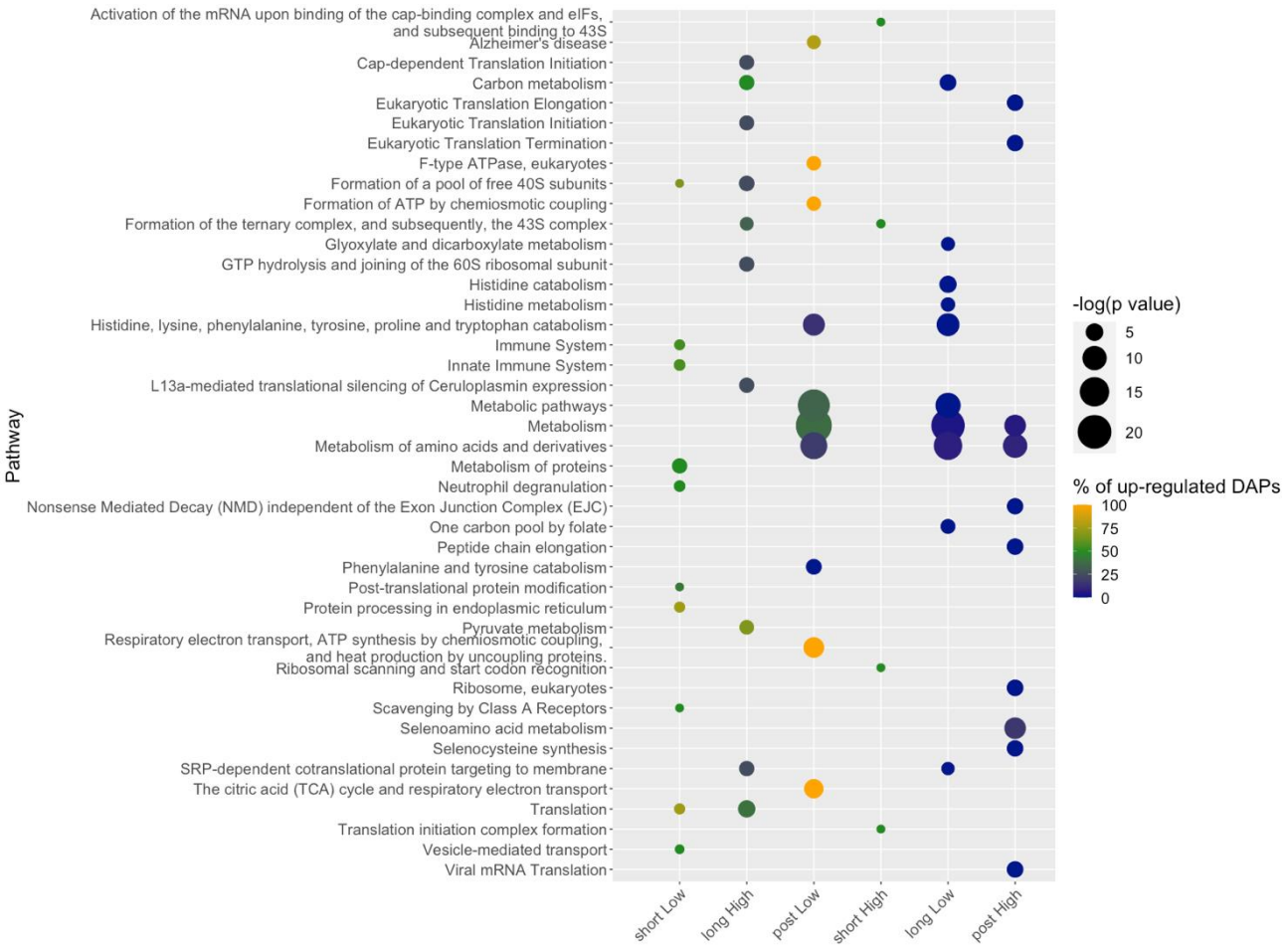

Fig.1 The top 15 pathways enriched in each treatment group. Color shows percent of up-regulated DAPs by OTC. The size of radius of circle demonstrates *p* value of enrichment, bigger means more significant.

Conclusion

We investigated the effects of short-, long-term, and post-OTC administration in young red seabream at the proteome level. Our bioinformatics approach demonstrated that OTC can interfere with the metabolic system in all the term, immune systems in the short-term, and nervous system in long-term administration. To our knowledge, this is the first study to assess the short-, long-term to post-OTC administration effects at the proteome level in marine fish. The results reveal the potential risk of antibiotics in many non-target fish.

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