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Title	Secondary metabolites produced by endosymbionts of shipworm woodborers (Bivalvia: Teredinidae) along the coast of Hokkaido [an abstract of dissertation and a summary of dissertation review]
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学 位 論 文 内 容 の 要 旨

博士 (環境科学)

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学位論文題名

Secondary metabolites produced by endosymbionts of shipworm woodborers (Bivalvia: Teredinidae) along the coast of Hokkaido 「北海道沿岸の木材穿孔動物フナクイムシ類 (二枚貝綱:フナクイムシ科) の共生生物によって生産される二次代謝産物」

Shipworms are a group of wood-boring and wood-feeding marine bivalve mollusks that belong to the Teredinidae family. They were collected from driftwood along the coastal areas of Hokkaido between October 2020 and November 2021. Taxonomic identification of the specimens was done up to the species level based primarily on the morphology of the pallets, identification keys in Turner (1966; 1971) and Haga (2017). It recorded a total of five species pervaded wooden debris: *Teredo navalis* Linnaeus, 1758, *Lyrodus pedicellatus* (Quatrefages, 1849), *Bankia setacea* (Tryon, 1863), *Bankia bipennata* (Turton, 1819), and *Bankia carinata* (J.E. Gray, 1827), the latter two were discovered from Hokkaido for the first time. This suggests the possibility that ocean currents and suitable environmental conditions caused the dispersal of these teredinids in driftwood along the vast coastal stretches of Hokkaido. Also, they have been most likely distributed naturally but due to rarity in occurrence, they might have been missed in the previous surveys.

On the other hand, the endosymbionts of shipworms are known to possess an arsenal of bioactive secondary metabolites ranging from antimicrobials to anticancer potentials. The collected shipworm endosymbionts along the coast of Hokkaido have been successfully cultivated and isolated a total of nine bacteria of different genera. Furthermore, the chemical profiles of endosymbionts were investigated by liquid chromatography/mass spectrometry (LC/MS). The organic extract of these endosymbionts yielded known and unknown bioactive compounds. The macrolide tartrolon D — with well-known pharmacological activities, was isolated from the lipophilic fraction in all bacteria of different genera. Tartrolon D has been reported from numerous bacteria including marine *Streptomyces* sp., terrestrial rhizobacterium *Gynuella* sp., and shipworm endosymbiont *Teredinibacter turnerae*. Additionally, lipopeptides turnercyclamycins A and B were also identified from different genera of shipworm

endosymbionts such as *Thalassospira* sp., *Alteromonas* sp., and *Marinomonas* sp. collected from different coastal sites in Hokkaido. Turnercyclamycins were only reported from *T. turnerae* shipworm endosymbiont. Other possible analogs of turnecyclamycins named turnercyclamycins E and F were detected from *Alteromonas* sp. endosymbiont isolated from shipworm *Lyrodus pedicellatus* collected in the Tomakomai area.

In terms of biological activities, tartrolon D showed bactericidal activity against pathogenic bacteria (*Bacillus subtilis* and Methicillin-resistant *Staphylococcus aureus*) and cytotoxic against MCF-7 (breast cancer) cell line with MIC of 10 µg/mL and IC₅₀ value of 11.4 µg/mL, respectively.

Alternatively, the LC-MS chemical profiles showed the prevalence of tartrolon D from the endosymbiont of shipworms collected in all study sites from the Sea of Japan to the Pacific Ocean. Turnercyclamycins A and B were detected from shipworms in three out of ten sampling sites across Hokkaido which represent cold-water and warm-water currents. As a result, compounds isolated from shipworms are most likely identical regardless of the source and its geographic location. Based on this, it suggests that antibiotic tartrolons play a role in shipworm chemical symbiosis by deterring invaders or other microorganisms that are harmful to the shipworm host.

Several published papers described the isolation of potential secondary metabolites from shipworm symbiont *T. turnerae*. However, not much has been known about other cultivated symbionts showing similar activities. In addition, among our isolated strains, we did not detect and/or identify the *T. turnerae* strain, a gamma-proteobacterium that is well-known in shipworm microbiota. Still, we found some gamma-proteobacteria members through the phylogenetic analysis based on partial 16S rRNA gene sequences, which produced known and unknown secondary metabolites with potential biological activities. Indeed, the collective results of this study provide a basis for the continued study of shipworm symbiosis which produces a multitude of diverse and significant compounds that could lead to drug discovery and the development of sustainable biotechnology. Moreover, to the best of our knowledge, *Bankia setacea* species and its symbionts were only reported from the USA. Its presence in Hokkaido waters is a new distribution record in Asia and the Pacific.