（ 続紙 1 ）

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論文題目	Studies on Lignocellulose Decomposition and Structure of Gut Microbiota of Death Watch Beetle, *Nicobium hirtum* (Coleoptera: Anobiidae) （ケブカシバンムシのリグノセルロース分解と腸内微生物叢に関する研究）		

（論文内容の要旨）

Wood-destroying insects, mainly classified in the two major orders of Insecta, i.e., Coleoptera (beetles) and Blattodea (cockroaches and termites) which cause great economic damage to wood industries. Wood-feeding beetles are recognized as wood pests because some species can inflict severe damage to economically important forest trees or dried timbers used in buildings. There is much to learn about the mechanisms underlying lignocellulose digestion by wood-feeding insects, i.e., how they achieve efficient hydrolysis and subsequent assimilation of polysaccharides (cellulose and hemicelluloses) and their conversion to energy, and also how they circumvent recalcitrant lignin barriers to access embedded polysaccharides. These topics are of great interest not only because they will lead to better understanding of the physiology and evolution of herbivorous insects, but also to the development of biotechnological strategies for wood pest management and biochemical production methods from lignocellulosic biomass.

Among the wood-feeding beetles, anobiid beetles have a broad range of feeding substrates and are responsible for many attacks on historic wooden structures and artifacts in Japan. *Nicobium hirtum* is a serious pest of wooden furniture, cultural heritage, and architectural structures. Although many members of Anobiidae family have been observed to utilize plant/wood cell walls, little is yet known about the ability to degrade lignocellulose on *N. hirtum*. This beetle is likely to have developed a unique and diverse digestive system, often associated with intestinal microbial symbionts, which can produce the enzymes necessary to decompose lignocellulose, a recalcitrant biocomposite of cellulose, hemicelluloses, and lignin that constitutes the woody cell walls. Therefore, the aim of this thesis was to investigate in detail the properties of lignocellulose degradability by *N. hirtum* larvae.

Chapter 1 reviewed the biology of *N. hirtum*. In Chapter 2, a laboratory culture of *N. hirtum* was developed to assess the basic physiological and feeding characteristics of *N. hirtum* larvae fed different types of wood and lignocellulosic artificial diet. In-depth structural characterizations of lignocellulose in their feces found that *N. hirtum* larvae are able to deconstruct lignocellulose in their gut digestive system. The larvae can subsist on plain softwood and hardwood lignocelluloses, utilizing cellulose and hemicelluloses, especially mannans, for assimilation and energy. Such efficient polysaccharide digestion could be achieved

by overcoming lignin barriers through structural modifications of lignin polymers via reductive and/or oxidative degradation pathways.

The effects of artificial diets with different contents of starch and hardwood lignocellulose compounds were compared with the survival and growth of *N. hirtum* larvae in Chapter 3. Larvae fed diet with 80% starch content grew faster and developed larger bodies than larvae fed diets with lower starch concentrations (20% and 50%), although survival rates were significantly lower. Thus, while starch is partially important for larval growth, lignocellulose is also important for maintaining physiological activity and improving survival of *N. hirtum* larvae.

Studying the microbial communities of *N. hirtum* may provide insight into their role and capability in digesting wood components and broaden the sources of biocatalysts that may impact biotechnology. To shed light on how gut microbiota of *N. hirtum* is affected by various dietary patterns, amplicon sequencing of bacterial 16S-rRNA and fungal ITS1 were used to determine how communities vary among these populations, and along different regions of an individual insect's digestive system in Chapter 4. The feeding habits and different sources of diet may determine microorganisms' abundance, which in turn affects the cellulolytic activities of *N. hirtum's* digestive system. The amplicon sequencing data revealed that both bacterial and fungal community profiles of the *N. hirtum* larvae gut are related to the dietary variation, in particular, lignocellulose and starch ratio in the diet. Microorganisms in the host guts are nutritionally endogenous or interdependent relationships with the host and are co-evolutionary adapted. Therefore, the predominant symbionts in the gut of *N. hirtum* larvae may be highly related to host fitness. Further studies will be required to confirm the abilities of gut bacteria and fungi to metabolize wood components and defend against toxic compounds ingested by the hosts, *N. hirtum*.

In conclusion, this thesis confirmed that starch is an essential carbon source for *N. hirtum* larval growth, but lignocellulose also plays an important role as a nutrient that maintains *N. hirtum* larval physiological activity and enhances survival. The microbial community and abundance of *N. hirtum* larvae varies with the diet types, which in turn influences wood composition and carbohydrate degradation. At the same time, the study showed that it is also critical for *N. hirtum* larvae to maintain a healthy gut microbiota in the digestive tract in order to efficiently degrade lignocellulose.

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（続紙 ２ ）

（論文審査の結果の要旨）

ケブカシバンムシは日本各地の木造文化財で最も高頻度で発見される木材害虫であるが、そのリグノセルロース分解機構については未解明な点が多い。本論文はケブカシバンムシのリグノセルロース分解特性を明らかにすることを目的として、ケブカシバンムシ幼虫にデンプン／木粉含有比率の異なる飼料の投与実験を行い、リグノセルロース分解特性と腸内共生微生物の機能解明を目指したものであり、評価すべき点として以下の３点を挙げることができる。

１．幼虫の消化前後の飼料の成分比較により、幼虫がリグノセルロースを分解できることを明らかにした。すなわち、デンプン含量0.3%のアカマツ辺材飼料の場合、デンプンに加えてセルロースとマンナンを優先的に分解すること、デンプン含量50%の人工飼料を与えた場合、デンプンとマンナンをセルロースより優先的に分解すること、デンプン量の多寡にかかわらず、リグニンを部分的に変性すること、を明らかにした。このことは、ケブカシバンムシが多様な餌に順応できるポテンシャルを有する昆虫であることを示唆している。

２．幼虫の成長、生存率を検討した結果、デンプン含量の増加に伴い幼虫の体長や体重が増加するが、生存率にマイナスの影響を及ぼすことを明らかにした。これによってデンプンは幼虫の成長に重要であるが、リグノセルロースは幼虫の生理活性を維持し、生存率を高める効果があることが示唆された。

３． 幼虫の腸内微生物叢を解析した結果、腸内細菌および真菌の群集は、飼料中のデンプン／木粉含有比率により大きく異なることを明らかにした。具体的にはデンプン含量の減少（リグノセルロース含量の増加）に伴いα-ProteobacteriaやBacilliなどの特定の細菌種と、他のシバンムシ科でも見られる 真菌 *Symbiotaphrina buchneri* が優先種になることを明らかにした。

以上のように、本論文は古材の害虫として知られるケブカシバンムシについて、リグノセルロースの分解特性とその分解における腸内共生微生物の寄与について明らかにしたものであり、木材保存学、昆虫生理学、害虫管理学の発展に寄与するところが大きい。

よって、本論文は博士（農学）の学位論文として価値あるものと認める。

なお、令和 5 年 7 月 20 日、論文並びにそれに関連した分野にわたり試問した結果、博士（農学）の学位を授与される学力が十分あるものと認めた。

また、本論文は、京都大学学位規程第１４条第２項に該当するものと判断し、公表に際しては、当該論文の全文に代えてその内容を要約したものとすることを認める。

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