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学位論文題目 Role of gut bacteria in domestication of mice

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Summary of Doctoral Thesis

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Title : Role of gut bacteria in domestication of mice

Domestication significantly influences animal behaviour, often guided by human selection for traits that are advantageous for cohabitation or productivity. Traditionally, the focus has been on how genetic factors contribute to changes in behaviour through selective breeding. My research, however, diverts from this path to explore a relatively understudied area—the role of gut microbiota in influencing animal behaviour, specifically tameness, in mice.

This study pivots on the hypothesis that the gut microbiome, a complex community of microorganisms residing in the digestive tracts of animals, plays a critical role in the modulation of behaviour. To test this hypothesis, I conducted an extensive analysis of the gut microbiota from four groups of mice: two selectively bred for tameness and two control group, comprising 80 mice (20 in each group). The method of choice was shotgun metagenomic analysis, a powerful tool that provides insights into both the taxonomic diversity and functional capacity of microbial communities.

Interestingly, my findings indicated that selective breeding for tameness did not lead to a significant alteration in either the taxonomic or functional diversity of the gut microbiota as a whole. However, there was a significant increase in the abundance of a particular bacterium, *Limosilactobacillus reuteri*, in the tameness-selected mice. This change was accompanied by notable increases in the levels of pyruvate and oxytocin in their plasma, suggesting a possible biochemical pathway mediated by microbial activity influencing behaviour.

To further explore this connection, I conducted additional experiments where non-selected mice were given pyruvate. This intervention aimed to determine if increasing circulating pyruvate levels could enhance tameness behaviour. Surprisingly, administering pyruvate countered the reduction in tameness typically caused by repeated injection stress. The results were promising, showing a significant improvement in stress-induced behavioural changes.

Building upon these findings, I isolated specific strains of *L. reuteri* capable of secreting extracellular pyruvate. Administering one of these strains to non-tame mice via their drinking water led to significant behavioural changes, notably an increase in tameness and a corresponding rise in serum oxytocin levels. These results underscore a potentially novel microbial-endocrine pathway in which gut bacteria could influence host behaviour through metabolic and hormonal changes.

In addition to these interventions, I performed microbial exchange experiments

by cross-fostering between control and tame mice. The results did not show any behavioural changes, suggesting that while the gut microbiome influences behaviour, genetic factors may exert a stronger influence on tameness. Nevertheless, the alteration in *L. reuteri* abundance suggests that microbial factors do have a role, albeit secondary to genetic factors.

To complement the behavioural studies, I utilized the metagenomic data to compile a comprehensive catalogue of the mouse gut microbiota. This cataloguing effort resulted in the assembly of 374 high-quality metagenome-assembled genomes (MAGs) spanning 11 phyla, significantly enhancing our understanding of this microbial community. Notably, this collection includes 27 novel bacterial MAGs previously unreported in the mouse gut, which highlights the extensive, yet largely unexplored microbial diversity in this model organism.

The implications of this research are manifold. First, it illuminates the significant, though perhaps underappreciated, role of microbiota in the process of domestication. By demonstrating that microbial populations can influence host behaviour, it suggests new mechanisms by which domestication—and the behavioural modifications it entails—might occur beyond direct genetic manipulation.

Furthermore, the study opens new perspectives on the interplay between host genetics, behaviour, and the gut microbiota. Understanding how microbial communities influence host behaviour extends our comprehension of domestication from a purely genetic view to a more holistic, integrative perspective. This shift has profound implications for evolutionary biology, animal science, and potentially, the development of microbial-based therapies for managing animal behaviour.

Moreover, these insights into the microbiome's role in behaviour modification could lead to practical applications in animal husbandry and wildlife management, where behaviour control is crucial. The potential for developing probiotic treatments to enhance desirable traits or diminish undesirable behaviours in domesticated animals could revolutionize traditional practices.

In conclusion, my research contributes to the broader understanding of the complex interactions that define the relationship between animals and their microbial residents. By focusing on the gut microbiome's role in behaviour, particularly tameness, this work sheds light on novel pathways through which domestication can influence animal behaviour, thereby enriching our understanding of this fundamental biological and cultural process. This research not only bridges gaps in our scientific knowledge but also opens doors to innovative approaches for influencing animal behaviour through microbial manipulation.

Results of the Doctoral Thesis Defense

博士論文審査結果

Name in Full

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T i t l e

論文題目 Role of gut bacteria in domestication of mice

家畜化は、人類に有益な形質を選抜することによって動物の行動を大きく変化させる。なかでも、従順性の上昇は顕著な例である。出願者は、所属研究室で確立された従順性に対して人為選抜をかけたマウス系統と選抜をかけなかったコントロールのマウス系統を用いて、これらの間の腸内細菌叢の違い、および、腸内細菌が従順性に与える影響を研究した。

まず、40匹の従順化マウスと40匹のコントロールマウスから採取した糞便サンプルについてショットガンメタゲノム解析を用いて調べた結果、従順性が増す方向に選抜をかけた系統で、腸内細菌叢の分類学的多様性や機能的多様性が大きく変化することはないものの、*Limosilactobacillus reuteri*の相対量が増加していることを見出した。ついで、従順化系統で上昇のみられた遺伝子ファミリーを解析した結果、アミノ酸代謝や脂肪酸代謝等に関わる複数の酵素遺伝子の存在量が増加していることを見出された。そこで、血液のメタボローム解析を実施し、従順化系統では血液中のピルビン酸濃度が高いことを見出した。そこで、*L. reuteri*が分泌するピルビン酸が従順性に関与している可能性を検証するために、従順化マウスから、ピルビン酸を多く分泌する*L. reuteri*系統とあまり分泌しない*L. reuteri*系統を単離し、これらの*L. reuteri*の2系統およびピルビン酸を多く分泌する近縁種*Lactobacillus helveticus*をコントロールマウスにそれぞれ投与して従順性への影響を調べた。その結果、ピルビン酸分泌量の多い少ないに関わらず*L. reuteri*系統は従順性を上昇させ、*L. helveticus*は従順性を上昇させなかったことから、*L. reuteri*由来のピルビン酸が原因となって従順性が誘導されているという仮説は支持されなかった。

ついで、別のグループの先行研究によって*L. reuteri*は哺乳類腸管でのオキシトシン分泌を誘発することが示されていたことから、出願者は、従順化において*L. reuteri*が血中オキシトシン濃度を上昇させて行動を変化させているという仮説を立てた。そして、実験によって、*L. reuteri*を投与したマウスでは血中オキシトシン濃度が上昇していること、従順化マウス系統の方がコントロール系統よりも血中オキシトシン濃度が高いことを見出した。これらの結果は出願者の仮説と合致するものであった。

本研究は、家畜化過程において、腸内細菌叢の変化が動物の行動を変化させたという仮説について検証した独自性の高い研究であり、審査委員会は、本論文は学位の授与に値すると判断した。