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論文内容の要旨

Corynebacterium efficiens is a gram-positive non-pathogenic bacterium previously known as *Corynebacterium thermoaminogenes*. This strain has recently been shown to be a near relative of *Corynebacterium glutamicum* and *Corynebacterium callunae*, both of which are recognized as glutamic acid-producing *Corynebacterium*. The optimal temperature for glutamate production by *C. glutamicum* is around 30°C, and this microorganism can neither grow nor produce glutamate at 40°C or above. On the other hand, *C. efficiens* can grow and produce glutamate above 40°C. The glutamic-acid-producing species of corynebacteria are known to overproduce glutamic acid under a variety of conditions, such as biotin limitation, although the mechanism of this phenomenon remains unclear. Another member of this genus, *Corynebacterium diphtheriae,* is a well-known pathogen that does not produce glutamic acid. The purpose of the present study is to elucidate the mechanism underlying the thermal stability of *C. efficiens* and to investigate the evolutionary processes that are related to the glutamic-acid-overproduction mechanisms in *C. glutamicum* and *C. efficiens* through considering the genome evolution of *Corynebacterium*. In order to describe the mechanism, I conducted a comparative genomics study using a genome-wide comparison of amino-acid substitutions and metabolic pathways using whole genome sequences.

This thesis comprises five chapters. In **chapter 1**, I describe the research background on this study, placing particular emphasis on the relationship between thermostability and fermentation. I noted that the industrial fermentation process could be carried out at a higher temperature; it might be possible to reduce the electric power consumption and carbon dioxide generation.

In **chapter 2**, I describe the thermostability mechanism of *C. efficiens* revealed by the complete genome sequence comparison between *C. efficiens* and *C. glutamicum*. Differences in the growth temperature, protein stability and GC content between *C. efficiens* and *C. glutamicum* can be investigated through comparative genomics using the complete genome sequences of these bacteria. Because these two species are phylogenetically closely related, more than 1,000 orthologous genes with 60–95% amino-acid sequence identity can be compared. Taking an advantage of comparative genomic studies, I found that there was tremendous bias in amino acid substitutions in all orthologous ORFs. Analysis of the direction of the amino acid substitutions suggested that three substitutions from lysine to arginine, serine to alanine, and serine to threonine, are important for the thermostability of the *C. efficiens* proteins. On the basis of these

findings, I suggest that the accumulation of these three types of amino acid substitutions correlates with the acquisition of thermostability and is responsible for the greater GC content of *C. efficiens*.

In **chapter 3**, I make an attempt to understand the evolutionary process involved in the ability of amino acid production in *Corynebacterium*. To attain this purpose, I analyzed the differentiation of metabolic pathways based on a comparative genome analysis of high GC Gram-positive bacteria, including *Mycobacterium* and *Streptomyces*. When *Mycobacterium* and *Streptomyces* were used as outgroups, the comparative study suggested that the common ancestor of *Corynebacteria* already possessed almost all of the gene sets necessary for amino acid production. However, *C. diphtheriae* was found to have lost the genes responsible for amino acid production. Moreover, I found that the common ancestor of *C. efficiens* and *C. glutamicum* have acquired some of genes responsible for amino acid production by horizontal gene transfer. Thus, I show that the evolutionary events of gene loss and horizontal gene transfer must have been responsible for functional differentiation in amino acid biosynthesis of the three species of *Corynebacteria*.

In **chapter 4**, I discuss the evolutionary process for glutamic acid overproduction mechanism under the biotin limitation condition in *C. glutamicum*. To attain this purpose, I compared between the biotin biosynthesis related genes in high GC Gram-positive bacteria. I found that the complete biotin biosynthesis pathway was inherited in *C. diphtheriae*, while *C. glutamicum* and *C. efficiens* only possessed an incomplete pathway. Furthermore, the complete biotin biosynthesis pathway in *C. diphtheriae* suggested to be achieved by the horizontal gene transfer. I conclude that this evolutionary event may have affected metabolic regulation in corynebacteria following the loss of the glutamic acid overproduction mechanism in *C. diphtheriae*.

Finally, in **chapter 5**, I describe the summary and the conclusion of the present study. This study acquired significant knowledge of the protein thermostabilization mechanism and evolutionary process for amino acid production mechanism in *Corynebacterium* by conducting whole genome comparisons. I conclude that this study gives significant insight to the evolutionary process of bacterial diversity from view point of genome evolution.

論文の審査結果の要旨

西尾陽介氏の論文は、代表的なグルタミン酸産生菌である *Corynebacterium* 属の近縁種 *C. efficience* と *C. glutamicum* を対象として産業上有用な表現形質の差異を明らかにすることを目的として、比較ゲノム解析を行った。特に、熱安定性、アミノ酸産生能およびグルタミン酸過剰生産の機構の多様性について議論した。

C. efficience は *C. glutamicum* に比較してより高温で生育しかつグルタミン酸を産生することから、グルタミン酸生産過程においてエネルギー効率の向上と二酸化炭素の抑制に寄与することを期待できる。そこではじめに、ゲノム配列の解析結果から抽出した 1,619 のオーソロガス遺伝子を比較して、この熱安定性の機構の分析を試みた結果、アミノ酸置換の偏りの中で３種類のアミノ酸置換（Lys→Arg, Ser→Ala、Ser→Thr）が *C. efficience* において *C. glutamicum* と比較して高く、このことが、 *C. efficience* の高 GC 含量とタンパク質の安定性に寄与していることを見出した。

次に、*Corynebacterium* 属のアミノ酸産生能に関わる機構の進化過程を明にするために、前述２種に、グルタミン酸産生能がなく病原性がある *C. diphtheriae* を加え、高 GC グラム陽性菌である *Mycobacterium* と *Streptomyces* を外群とした ORF の比較解析を行い、*Corynebacterium* 属の共通祖先がアミノ酸産生に必要な遺伝子のセットをほぼ持っており、*C. diphtheriae* からはアミノ酸産生に寄与する遺伝子が失われたという結論に達した。さらに、アミノ酸産生に必要な遺伝子の一部は、*C. glutamicum* と *C. efficience* の共通祖先の時点で水平移動してきたものと推定した。

最後に、*C. glutamicum* におけるグルタミン酸の過剰生産の機構について、高 GC グラム陽性菌におけるビオチン生合成に関する遺伝子群を比較解析して、*C. diphtheriae* には完全な生合成回路が存在するが、他の２種では回路が不完全になっていることを確認して、ビオチンが制限されるとグルタミン酸の過剰生産が起きる機構を明らかにした。加えて、*C. diphtheriae* の回路は水平移動によるものと推定した。

以上、本論文は、近年の菌種のゲノム配列を元に、多数のオーソロガス遺伝子を比較することから、*C. efficience* の酵素群の熱安定性と *C. glutamicum* のグルタミン酸過剰生産の機構をゲノム構造の進化の観点から提案することに成功しており、比較ゲノム解析の産業応用への可能性を示すとともに、その学術的価値は高いと判断した。これらの研究は国立遺伝学研究所の五條堀孝教授のもとで行われたもので、西尾氏はすでに３報の論文を国際誌に発表しており、うち２報は筆頭著者として本申請論文の主な研究成果を報告している。以上を、総合的に評価して、当大学院の水準を満たしていると判断して合格とした。