氏　　名	NGUYEN Thi Thuy Tien
授与した学位	博　士
専攻分野の名称	学　術
学位授与番号	博甲第　　　５８４７　　　号
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学位授与の要件	環境生命科学研究科　　農生命科学専攻 （学位規則第４条第１項該当）
学位論文の題目	Analysis of the gut microbiota of Japanese Alzheimer's disease patients and characterization of their butyrate-producing bacteria （日本人アルツハイマー病患者の腸内細菌叢解析とその細菌叢から分離された酪酸産生菌の特徴）
論文審査委員	教授　　齋藤　　昇　　教授　　森田　英利　　教授　　西野　直樹 教授　　稲垣　賢二

学位論文内容の要旨

Alzheimer's disease (AD) is an age-associated disease which accounts for 60–80% of all dementia. Recently, there has been increasing evidence indicates the etiology of diseases associated with the central nervous system (CNS) has a relationship with gut microbiota, creating a complex gut-brain-axis. The bidirectional communication between the gut microbiota and the CNS plays a key role in physiological as well as mental health. This study aimed to investigate the alteration in the gut microbiota of AD patients by next-generation sequencing approach, as well as the cultivable butyrate-producing bacteria in AD patient's gut microbiota by the cultural approach. The gut microbiota of 17 Japanese who were diagnosed with AD (AD group) was compared with that of 17 Japanese healthy parsons (HC group) in term of their bacterial taxonomies, diversities and metabolic pathways which predicted from their gut microbiome. Furthermore, the gut microbiota of the Japanese AD group was also analyzed to compare with those of American AD group to have the insight of the gut microbiota of AD group in different countries. Next, butyrate-producing bacteria which were isolated from AD patient's feces were characterized their butyrate production ability, the determination of genes encoding for butyrate synthesis.

The first study is the comparison of the gut microbiota of 17 Japanese AD group with that of 17 Japanese HC. Total sequences of 181,580 reads were assigned to 2,583 OTUs (operational taxonomic units), consisted of 12 phyla, 22 classes, 33 orders, 70 families and 147 genera. The phyla of *Actinobacteria*, *Verrucomicrobia*, *Cyanobacteria*, and *TM7* contributed to the differences in phylum level between the two groups with $p < 0.05\%$. The weighted and unweighted UniFrac distances between AD and HC groups were significant differences at $p < 0.001$. The gut microbiota of 17 Japanese AD and 25 American AD group was evaluated. The total reads were 4,000,035 reads which were grouped into 14,593 OTUs, comprised 12 phyla, 26 classes, 30 orders, 79 families, and 180 genera. The phyla of *Proteobacteria* made the differences in the gut microbiota of the two groups.

The second study aimed to isolate butyrate-producing bacteria from Japanese AD group. 226 isolates were randomly picked from four AD patient's fecal samples, and assigned into 60 OTUs. Sixteen OTUs of 60 OTUs were selected on scan-searching against the public databases. Fifteen out of the 16 isolates were indeed able to produce butyrate in culture. Eight isolates possessed butyrate kinase (*buk*), while five isolates possessed butyryl-CoA: acetate CoA-transferase (*but*). The CoA-transfer-related gene was identified as butyryl-CoA:4-hydroxybutyrate CoA transferase (*4-hbt*) in four strains. Whole genome sequences of the butyrate-producing bacteria were sequenced and annotated for their features, included genes encoding for butyrate production. The presence of cultivable butyrate-producing bacteria in AD group suggested the further study of this community in AD group due to their positive effect.

論文審査結果の要旨

　本研究は，日本人の重度アルツハイマー病患者と同年齢の健常者の腸内細菌叢を比較し，日本人の重度アルツハイマー病患者と米国のアルツハイマー病患者の腸内細菌叢を比較した研究成果である。日本人アルツハイマー病患者と同年齢の健常者の腸内細菌叢のUniFrac解析の結果，両群間に有意差があり細菌叢に違いのあることが見い出され，具体的には健常者にみられない*Collinsella*属がアルツハイマー病患者で多く検出されていた。健常者でその存在が重要視されている*Faecalibacterium*属がアルツハイマー病患者で検出されておらず，腸内細菌叢の視点から病態を考える上で重要な知見である。食生活などの違いにより国別に健常者の腸内細菌叢の違うことは既に報告されている。そこで米国と我が国のアルツハイマー病患者の腸内細菌叢の比較は興味深い。日本人アルツハイマー病患者と米国アルツハイマー病患者の腸内細菌叢のUniFrac解析の結果，両群間に有意差があり細菌叢に違いのあることが見い出され，属レベルでの比較解析の結果，米国人の腸内細菌叢に特徴的な*Methanobrevibacter*属が，日本人の方が多くなり米国人の方が少ないという結果であった。

　*Faecalibacterium*属がアルツハイマー病患者の腸内細菌叢からまったく検出されなかったことから，アルツハイマー病患者の患者から酪酸産生菌の生菌分離を試みた。継代培養可能な226菌株を生菌分離し1,400 bp以上の16S rRNA遺伝子配列により60のOTUs（operational taxonomic units）を作成し，菌種を推定し酪酸産生能を有する菌種として16株を選択した。そのうちの15株が酪酸を産生し酪酸産生系に鍵となる酵素（*buk*、*but*、*4-hbt*）の存在について明らかにした。今後，酪酸産生菌を動物実験に用いる上で安全性を評価するために，これら15株についてはドラフトゲノム解析を実施した。本研究では，*in silico*による網羅的な解析から，wet実験による生菌分離に至る幅広い実験技術を駆使したものである。

　以上の研究成果は，環境生命科学の領域，特に畜産食品機能学かつ食品衛生学の分野に貢献すると考え，NGUYEN Thi Thuy Tien 氏に博士（学術）を授けるものとする。