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授与した学位	博 士		
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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	Bacterial and fungal microbiota involved in the anaerobic storage of forages (牧草類の嫌氣的貯蔵に関わる細菌叢と真菌叢)		
論文審査委員	教授 齋藤 昇 准教授 荒川 健佑	教授 森田 英利 准教授 鶴田 剛司	教授 西野 直樹
<b>学位論文内容の要旨</b>			
<p>Microbiota analysis using DNA information has become more sophisticated and easily accessible in the past 20 years. However, many studies have follow-upped the knowledge that the rapid growth of lactic acid bacteria after sealing determines the success or failure of silage fermentation. The fact that most microbiota analyses have focused on bacteria may be the reason. In this dissertation, three experiments were carried out to obtain integrated information on the bacterial and fungal microbiota involved in the ensiling.</p> <p>Firstly, direct-cut (DC) and wilted (WT) guinea grass silages were prepared to examine the mechanisms and control procedures of acetic acid fermentation. The silos were stored at moderate (25°C) and high (40°C) temperatures. Lactic acid was the primary fermentation product during the initial ensiling. After two months, the lactic acid content was reduced to a negligible level, and large amounts of acetic acid, butyric acid, and ethanol were produced in DC silage stored at 25°C. The lactic acid reduction and acetic acid increase were suppressed in DC silage stored at 40°C. The PCoA and network analyses showed that <i>Lactobacillus</i>, unclassified Enterobacteriaceae, <i>Wallemia</i>, <i>Saitozyma</i>, <i>Sporobolomyces</i>, and <i>Papiliotrema</i> could be involved in acetic acid fermentation. <i>Lactobacillus</i> and <i>Wallemia</i> were identified as promoting factors and unclassified Enterobacteriaceae, <i>Saitozyma</i>, <i>Sporobolomyces</i>, and <i>Papiliotrema</i> as suppressing factors.</p> <p>Secondly, guinea grass silage that exhibited lactic acid-rich fermentation was examined. Molasses were used to facilitate lactic acid production and silos were stored at 25°C and 40°C. The acetic acid content did not exceed the lactic acid even after 2 months in all silages. Regardless of the storage temperature and molasses addition, the unclassified Enterobacteriaceae, <i>Lactobacillus</i>, <i>Lactococcus</i>, <i>Enterococcus</i>, and <i>Pediococcus</i> were prevalent bacteria. <i>Wallemia</i>, <i>Saitozyma</i>, and <i>Ganoderma</i> were prevalent fungi in DC and WT silages. Network analysis indicated that the lactic acid content was positively related to the <i>Pediococcus</i>, <i>Weissella</i>, and <i>Wallemia</i> abundances, and negatively related to the unclassified Enterobacteriaceae, <i>Acinetobacter</i>, <i>Ochrobactrum</i>, <i>Enterococcus</i>, <i>Cladosporium</i>, <i>Acremonium</i>, <i>Saitozyma</i>, and <i>Moesziomyces</i> abundances. The lactic acid-rich fermentation of guinea grass silage could be obtained by the sustained activity of <i>Pediococcus</i>, <i>Lactococcus</i>, and <i>Enterococcus</i> rather than the suppression of <i>Lactobacillus</i>.</p> <p>Lastly, a practical survey of large-scale silage management was performed. Five dairy farms (A, B, C, D, and E) producing whole crop corn silage using a bunker silo were visited. Silage samples were taken from 6 locations in the upper and lower layers and near the side-wall and the center. The fermentation patterns were divided into two types: lactic acid and ethanol (B and D) and acetic acid and 1-propanol (A, C, and E) as the prevalent products. <i>Lactobacillus</i> was the predominant bacterial genus in all farms, while some silage samples showed high abundances of <i>Acetobacter</i> and <i>Aeriscardovia</i>. The fungal microbiota was dominated by <i>Candida</i> regardless of the farms and sampling locations. Network analysis indicated that the <i>Lactobacillus</i> abundance was not related to any fermentation product contents, whereas positively related to the <i>Monascus</i> abundance. <i>Aeriscardovia</i>, which belongs to Bifidobacteriaceae and may produce more acetic acid than lactic acid, can be considered a new inoculant. Unlike the findings in the guinea grass silage, the integrated bacterial and fungal microbiota data did not greatly improve the understanding of whole crop corn silage fermentation.</p> <p>This study has provided new insights into how bacterial and fungal microbiota interact in the ensiling process and the acetic acid fermentation found in tropical grass silage may be <i>Lactobacillus</i>-driven.</p>			

## 論文審査結果の要旨

牧草類, その他様々なバイオマスの調製利用において, 発酵保存(サイレージ調製)は広く普及している。食品の発酵保存と異なり, 経済性が強く問われる飼料の保存では洗浄, 低温, 加熱殺菌等の衛生管理を適用することはできない。空気侵入を防止することも不完全で, 真菌による変敗も起きやすい。また, 暖地型イネ科牧草では, 嫌気性が確保されていても酢酸主体の発酵が起こる。サイレージの発酵制御に関わる研究は50年以上の歴史があるが, これまでの研究は細菌叢に偏っていた。本研究ではアンプリコン解析, 予測メタゲノム, ネットワーク解析を行い, サイレージ調製における細菌叢と真菌叢の統合的理解を目指した。

調査対象とした牧草種は, ギニアグラス(暖地型イネ科)およびトウモロコシホールクロップ(ソルガム属)で, 実験室規模だけでなく大規模酪農家での現地調査も行った。暖地型牧草サイレージでは嫌気条件でも酢酸が主要な発酵産物となる。ネットワーク解析で酢酸量と正の関係を示したのは*Lactobacillus*, *Clostridium*, *Candida*, *Wallemia*等であり, unclassified Enterobacteriaceae, *Hannaella*, *Moesziomyces*, *Saitozyma*等は負の関係を示した。トウモロコシサイレージの実態調査では, 5軒の大規模酪農家を訪問してバンカーサイロからサンプリングを行った。微生物叢はいずれの農家でも*Lactobacillus*が優勢で, 一部のサンプルでは>50%の割合で*Acetobacter*が, 30~40%の割合で*Aeriscardovia*が見られた。これまでサイレージに*Aeriscardovia*を認めたという報告はなく, 大規模調整に有用な微生物精製となるかもしれない。一方, 真菌叢は農家および採取部位に関わらず*Candida*が優勢しており, 発酵産物と関連した違いは見られなかった。

これらの知見は, 細菌叢と真菌叢の統合的解析が発酵制御の高度化にきわめて重要であることを示している。よって, Hou Jianjian氏は博士(農学)の学位を受ける資格があると判断した。