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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	Investigation of the microbiota of dairy cow milk in relation to mastitis prevention and milk quality control (乳房炎予防および乳質管理に関連した乳汁細菌叢の調査研究)		
論文審査委員	教授 齋藤 昇	教授 西野 直樹	教授 舟橋 弘晃 准教授 鶴田 剛司
<b>学位論文内容の要旨</b>			
<p>In the production of milk and dairy products, bacteria in raw milk may closely be related to health of the cows, quality of the milk, shelf life and flavor of the dairy products. In this thesis, five experiments were carried out to examine the effects of season, regions, milking methods, and cowshed environment on the microbiota of raw milk. Microbiota was assessed by Illumina MiSeq sequencing.</p> <p>In exp. 1, the microbiota, somatic cell count, and composition of milk collected from a herd managed by automatic milking systems were examined through a 1-year survey. Relative abundances of <i>Pseudomonadaceae</i>, <i>Enterobacteriaceae</i>, <i>Staphylococcaceae</i>, <i>Micrococcaceae</i>, and <i>Ruminococcaceae</i> were greater in winter (Dec. and Feb.), those of <i>Streptococcaceae</i>, <i>Microbacteriaceae</i>, and <i>Enterococcaceae</i> were greater in spring (Apr. and Jun.), and that of <i>Moraxellaceae</i> was greater in autumn (Oct.) than other seasons.</p> <p>In exp. 2, the microbiotas between manually collected and mechanically collected milk were compared. The total abundances of <i>Methylobacteriaceae</i>, <i>Sphingomonadaceae</i>, and <i>Staphylococcaceae</i> were approximately half in May-collected samples, whereas there was an overwhelming abundance of <i>Enterobacteriaceae</i> in July-collected samples. <i>Moraxellaceae</i>, <i>Pseudomonadaceae</i>, and <i>Enterococcaceae</i> were found as major families in both May- and July-collected samples in the microbiota of automatically collected milk, although their proportions were marginal in manually collected milk.</p> <p>In exp. 3, the microbiota of rumen fluid, feces, milk, water, feed, bedding, and airborne dust were examined to clarify contamination source of milk microbiota. The three most prevalent taxa (<i>Aerococcaceae</i>, <i>Staphylococcaceae</i>, and <i>Ruminococcaceae</i> at farm 1 and <i>Staphylococcaceae</i>, <i>Lactobacillaceae</i>, and <i>Ruminococcaceae</i> at farm 2) were shared between milk and airborne dust microbiota. Source Tracker indicated that milk microbiota was related with airborne dust microbiota, and hierarchical clustering and canonical analysis of principal coordinates demonstrated that the milk microbiota was associated with the bedding microbiota</p> <p>In exp. 4, a practical survey of milk composition and microbiota at dairy farms in China was performed. From five dairy farms in Heilongjiang and Shanxi provinces, China, milk samples were manually collected from five cows. Milk microbiota varied across farms and individual cows, and thus differences between regions were obscure. <i>Enterobacteriaceae</i> was a most predominant family in about one-third of the milk samples. The abundance of <i>Enterobacteriaceae</i> was positively and those of <i>Pseudomonadaceae</i> and <i>Lactobacillaceae</i> were negatively related with lactose content, and that of <i>Moraxellaceae</i> was negatively related with milk urea nitrogen; hence, appropriate management of protein-energy balance in cows can be regarded as a measure to suppress contamination.</p> <p>In exp. 5, the similarities and differences in the milk microbiota between Chinese, Vietnamese, and Japanese practices were examined. Diversity of the milk microbiota was higher in Japanese than Chinese and Vietnamese cow's milk; the number of operational taxonomy unit, Chao 1 index, and Shannon index were all greater for the milk of Japanese than those of Chinese and Vietnamese samples. Moreover, relative abundances of <i>Enterobacteriaceae</i> and <i>Propionibacteriaceae</i> were greater for Chinese and those of <i>Streptococcaceae</i> and <i>Methylobacteriaceae</i> were greater for Vietnamese milk samples.</p> <p>The fact that substantial differences were seen in the milk microbiota between manually collected and automatically collected samples should be considered for both mastitis prevention and milk quality control. Likewise, even though high abundances of typical pathogens like <i>Staphylococcaceae</i>, <i>Enterobacteriaceae</i>, and <i>Streptococcaceae</i> were shared in cows, the SCC was substantially different; hence, in addition to monitoring of the milk microbiota, cow factors associated with infection and inflammation need to be considered for better prevention and treatment. Our finding that milk urea nitrogen was related with the abundance of <i>Moraxellaceae</i> in the milk can suggest that appropriate protein-energy balance may work as a measure to suppress pathogen contaminations of the milk.</p>			

## 論文審査結果の要旨

乳房炎の予防、牛乳の保存性向上を図るうえで、乳汁の細菌叢とその変動要因を理解することは重要である。呉浩銘氏は、次世代シーケンサ（MiSeq）を用いて乳汁細菌叢を精査するとともに、季節、地域、サンプリング方法および環境細菌叢と乳汁細菌叢の関係について一連の調査研究を行った。

実験 1 では年間を通じた実態調査を行い、*Pseudomonadaceae*, *Enterobacteriaceae*, *Staphylococcaceae* 等が冬期に、*Streptococcaceae*, *Microbacteriaceae*, *Enterococcaceae* 等が春期に多いという、細菌叢の季節変動を明らかにした。これらの変動が乳成分特に尿素態窒素濃度と関係することも明らかにし、飼養技術による乳汁細菌叢制御の可能性を示した。

実験 2 では、機械的に採取した乳汁とマニュアルで採取した乳汁の細菌叢を比較した。機械的に採取した乳汁は、*Moraxellaceae*, *Pseudomonadaceae*, *Enterococcaceae* を上位菌種として含む傾向があり、乳房炎予防を目的とした細菌叢調査では、マニュアルでの乳汁採取が望ましいことを明らかにした。

実験 3 では、ルーメン液、糞便、飼料、水、牛床および空気粉塵の細菌叢と乳汁の細菌叢がどのように関係するかを調べた。空気粉塵および牛床で見られる *Aerococcaceae*, *Staphylococcaceae*, *Ruminococcaceae* 等が乳汁に共通して認められ、乳汁細菌叢の汚染源は空気粉塵および牛床であると判断した。

実験 4 および 5 では、国内外で採取した乳汁を対象として、個体、農場および地域による細菌叢の変動を調べた。中国の乳汁サンプルは *Enterobacteriaceae* と *Propionibacteriaceae* が、ベトナムの乳汁サンプルは *Streptococcaceae* と *Methylobacteriaceae* が多い傾向があったが、同一国内の地域間差は個体間および農場間差より小さいことを明らかにした。

これらの知見は、乳牛の栄養管理および牛舎の衛生管理が、乳房炎の予防および牛乳の保存性向上において非常に大きな役割を持つことを示している。実用的にも意義ある知見であり、呉浩銘氏は博士（農学）の学位を授与するに値すると判断した。