

Abstract of Thesis/Dissertation

Applicant

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GANZORIGTitle : Exploration of bioactive milk compounds from wild and domestic animals(野生動物と家畜の乳に由来する生理活性物質の探索)

Abstract

This study was aiming to explore functional components from raw and fermented milks of wild and domestic animals by means of profiling milk oligosaccharides in addax colostrum, profiling peptides in Mongolian fermented camel's and mare's milks, and further screening antimicrobial activities of low molecular fractions of the fermented milks.

Mammalian milk and colostrum usually contains milk oligosaccharides along with the predominant lactose. Although milk oligosaccharides have been characterized in a various species of Bovidae including cow, sheep and goat, in contrast those of the addax, an Antelopinae species of the Bovidae, have not as yet been clarified. Therefore, to explore the biofunctional compounds, several acidic oligosaccharides were purified from a sample of addax colostrum and characterized by ¹H-NMR spectroscopy. Those of oligosaccharides have been found in the addax colostrum as follows: Neu5Ac(α2-8)Neu5Ac(α2-3)Gal(β1-4)Glc (disialyllactose), Neu5Gc(α2-8)Neu5Gc(α2-3)Gal(β1-4)Glc (diglycolyllactose), Neu5Ac(α2-3)Gal(β1-4)Glc (3'-sialyllactose), Neu5Ac(α2-6)Gal(β1-4)GlcNAc (6'-sialyl-N-acetyllactosamine), Neu5Gc(α2-3)Gal(β1-4)Glc (3'-glycolyllactose), Neu5Gc(α2-6)Gal(β1-4)Glc (6'-sialyllactose), and Neu5Gc(α2-6)Gal(β1-4)GlcNAc (6'-glycolyllactosamine). In addition, an oligosaccharide nucleotide Neu5Gc(α2-6)Gal(β1-4)GlcNAcα1-UDP (uridine 5'-diphospho-N-acetyllactosamine) was characterized. Molecular species of a variety of acidic oligosaccharides found in milk and colostrum of these Bovidae were compared. The ratio of Neu5Ac/Neu5Gc in sialyl oligosaccharides had previously been reported that differs depending on species such as 97:3 in the cow, 37:64 in the goat and 6:94 in sheep. This ratio was rather equal in this addax colostrum sample. The oligosaccharides containing Neu5Ac/Gc(α2-3) predominate over those containing Neu5Ac/Gc(α2-6) in the addax colostrum; this was similar to cows as well as sheep, but different from goats. Moreover, a nucleotide linked to sialyl N-acetyllactosamine, namely as Neu5Gc(α2-6)Gal(β1-

4)GlcNAc α 1-UDP, was identified in the addax colostrum while it has previously been detected only in ovine colostrum. These oligosaccharides may have several biological functions. For instance, act as prebiotics that stimulate the growth of bifidobacteria in the infant colon, as decoy receptors that inhibit the attachment of pathogenic microorganisms to the colonic epithelium, and as modulating factors for the maturation of colon epithelial cells.

Fermented camel's and mare's milk products are regarded as effective nutrient and health beneficial sources and widely consumed in Mongolia. Among their components, peptides are one of the possible candidates to exhibit beneficial activities on human health. Milk proteins are degraded by proteolytic bacterial enzymes into numerous bioactive peptides during the fermentation process, leading enhanced functionality of the fermented milks. In this study, fermented camel's and mare's milk samples were collected from nomadic herders whose mainly produce locally in southern of Mongolia. The whey was separated from fermented milks and further serially ultrafiltrated by cellulose membrane with NMWL 3 and 10 kDa. Then antimicrobial activities of ultrafiltrates were evaluated by using disc diffusion assay. As a result, lower than 3 kDa and 3-10 kDa molecular weight fractions extracted from both of fermented camel's and mare's milk shows inhibition activity against Gram-negative bacteria, *S. Typhimurium* and *S. sonnei*. Then those of <3kDa ultrafiltrates with anitmicrobial activity had been fractionated by RP-HPLC and eluted 23 fractions from both of fermented milks. According to a result of the first trial, no antibacterial activity found from 23 fractions of RP-HPLC. It is thought that peptide concentration in the RP-HPLC fraction was not enough to inhibit the growth of pathogenic bacteria.

To determine the amino acid sequences of 23 peptide fractions, MALDI-TOF MS/MS analysis were conducted. In total, 11 and 24 peptides were identified from fermented camel's and mare's milk, respectively. Peptide components in the fermented camel's milk were mixtures of β -CN, α_{s1} -CN, κ -CN and lactophorin whereas β -CN fragments were dominant in the fermented mare's milk. Muhialdin *et al.*, (2018) has recently been reported that peptide mixture of α_{s1} -, α_{s2} -, β -CN and lactophorin, isolated from fermented camel's milk shows antibacterial activity against *E. coli*, *S. aureus*, *S. faecalis* and *S. dysenteria*. The peptide mixture found in my study, α_{s1} -CN f16-22 [RPKYPLR] and β -CN f194-199 [VPYPQR], f210-218 [QEPVPDPVR] f221-226 [HPVPQP] from fermented camel's milk, were partially matched with findings of above study. Therefore, it is hypothesised that they may have antibacterial activity.

Finally, it was concluded that wild animals' milks and fermented milks of livestock were recognized as good resources of novel bioactive substances including carbohydrates and peptides.