論文 / 著書情報
Article / Book Information

題目(和文)	
Title(English)	Anti-inflammatory effect of gut associated Lactobacillus johnsonii
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出典(和文)	学位:博士(工学), 学位授与機関:東京工業大学, 報告番号:甲第12455号, 授与年月日:2023年3月26日, 学位の種別:課程博士, 審査員:山本 直之,越川 直彦,林 宣宏,田川 陽一,折原 芳波,門之園 哲哉
Citation(English)	Degree:Doctor (Engineering), Conferring organization: Tokyo Institute of Technology, Report number:甲第12455号, Conferred date:2023/3/26, Degree Type:Course doctor, Examiner:,,,,,
学位種別(和文)	博士論文
Category(English)	Doctoral Thesis
種別(和文)	論文要旨
Type(English)	Summary

（博士課程）
Doctoral Program

論 文 要 旨

THESIS SUMMARY

系・コース： Department of, Graduate major in	生命理工学院　系 ライフエンジニアリング　コース	申請学位（専攻分野）： Academic Degree Requested	博士 Doctor of　Engineering
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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

Commensal intestinal microbiota interacts with gut epithelial cells in the host by binding to specific host receptors. Several pattern recognition receptors on the gut that sense conserved microbial-associated molecular patterns have been reported. However, many of the gut receptor molecules involved in bacterial binding have not yet been identified.

In this study, I attempted to find novel receptor components expected on gut epithelial cell surface which likely interact with intestinal bacteria for the understanding of biological functions of commensal bacteria on the host. This included two steps of screening, firstly, *Lactobacillus johnsonii* MG with interaction with BALB/c mouse gut was isolated from intestine. Next, receptor protein was purified by affinity resin coupled MG surface layer proteins and identified as JAM-2, one of the tight junction proteins.

L. johnsonii MG incubated with H_2O_2 damaged Caco-2 cells showed the faster recovery of TEER value than that of non-treated group, suggested MG-JAM-2 binding improved barrier function of Caco-2 cells. ZO-1 protein in tight junction was increased in Caco-2 cells by MG treatment, indicated MG have the ability to enhance the barrier function of Caco-2 cells. RNA sequencing analysis of Caco-2 cells, KEGG pathway suggests that the MG binding to JAM-2 might be a signal for the up-regulation of transcriptional regulators, *JUN* and *NFKB*, then *JUN* and *NFKB* likely activate *MMP1* and *MMP15*. Above genes categorized in "Matrix", "Regulator", "Apoptosis" and "Inflammation" were mainly enhanced in Caco-2 cells.

The anti-inflammatory effect of MG on DSS treated colitis mice showed the faster recovery than that in non-treated group in body weight and colon damage. Histological analysis showed the damage of crypt, such as detachment and disappeared crypt structure by DSS treatment by DSS treatment and the recovery in MG group. Gene expression analysis of Peyer's patches showed significant downregulations of *IL-10* and *TNF-α* by DSS treatment; but upregulation of *TNF-α* by MG treatment. The expression levels of *OCLN*, *ITGA-6*, and *LAMA3* genes in the matrix and *NFKB* was significantly upregulated in the MG group compared to those in the DSS group. *ZO-1* and *JUN* showed improved trends in MG group compared to DSS group, and, immunohistochemistry indicated that ZO-1 highly expressed in MG group. The bacterial count in the MG-treated mice serum was significantly lower than that in the DSS-treated group, suggesting that oral administration of MG might improve damage to the gut barrier and reduce bacterial invasion into the serum. In the microflora analysis, MG treatment increased the abundance of *Bacteroides*, *Lachnospiraceaea,* and *Ruminococcaceae*, and decreased the abundance of *Akkermansia* in DSS-treated mice. In addition, the abundance of GAPDH-positive bacteria detected by the anti-GAPDH antibody in the control group was less than 0.5 % but was increased to 2.1 % after oral administration of 3×10^8 MG cells/day for 2 day. Intravital imaging of a ubiquitous YC3.60-expressing mouse revealed the direct interaction of GAPDH-MG in tight junctions of the intestinal tract by

induced transient Ca^{2+} signaling response in jejunal epithelium in response to injected GAPDH-positive MG, but not with GAPDH negative MG.

In additional study with MG pretreated Caco-2 cells, MG exposure could enhance the barrier function and also showed activation of the gene expressions of *NFKB*, *JUN*, the transcriptional regulators and ZO-1.

In conclusion, by the isolation of intestinal bacteria from mouse gut with interaction with epithelial cell surface of BALB/c mouse, a novel tight junction protein, JAM-2 was identified as a receptor protein for *L. johnsonii* MG binding. The expected role of the selected *L. johnsonii* MG in barrier function was confirmed in H_2O_2 damaged Caco-2 cells and DSS treated colitis mouse model. MG binding with JAM-2 in tight junction enhanced the barrier function in both model studies by induction of various tight junction genes. The present approach to isolate the intestinal bacteria to identify the receptor protein showed a great potential to find novel function of living intestinal bacteria.