Culture supernatant produced by Lactiplantibacillus plantarum strain BF1-13 isolated from deep seawater of Izu-Akazawa protects the intestinal epithelial barrier from the dysfunction caused by hydrogen peroxide

[課程博士・論文博士共通]

博士学位論文内容要旨
Abstract

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論文題目 Title	Culture supernatant produced by *Lactiplantibacillus plantarum* strain BF1-13 isolated from deep seawater of Izu-Akazawa protects the intestinal epithelial barrier from the dysfunction caused by hydrogen peroxide		

Introduction: It is well-known that the intestinal epithelial barrier as an important biological membrane defending against the extracorporeal environment takes responsibility to mediate water transport, nutrient absorption, and drug metabolism. The intestinal epithelial barrier function which was mainly regulated by tight junctions (TJs) was reported to be decreased by oxidative stress (OS) induced by reactive oxygen species (ROS) such as hydrogen peroxide (H2O2). Recently, it has been proved that the transportation of H2O2 across the cell membrane by utilizing peroxiporins like aquaporin-3 (AQP3) was superior to it by simple diffusion. Lactic acid bacteria (LAB) which have a long history of a wide range of applications also take a large community in gut microbiotas. Due to its ability to colonize the intestine after passing through the gastrointestinal tract, where they are capable to play their important role as probiotics, LAB along with their fermented products are among hot spots in the food industry for centuries. Lactic acid (LA) produced by LAB also has a long history with various applications not only in the food industry but also in others such as cosmetics, and pharmaceuticals. Although some species of Lactobacillus mostly isolated from the probiotics or the fermentative products have been proved to inhibit or release the OS induced by H2O2, there is no report on the strains isolated from the marine environment. This study aims to investigate the effect of the culture supernatant (CS) of Lactiplantibacillus plantarum strain BF1-13 isolated from deep seawater (DSW) on the intestinal epithelial barrier integrity against degradation by H2O2 treatment. It also aims to research on the effective compound included in the CS and the underlying mechanisms with reveling the potential relationship between the barrier function of tight junctions (TJs) and the H2O2 intracellular invasion facilitated by AQP3.

Materials & Methods: L. plantarum strain BF1-13 isolated from the bag filter (Figure 1) which was used for the filtration of DSW (depth at 800m) in the pumping station in Izu-Akazawa in Shizuoka Prefecture was cultivated. Then, it was compared with L. plantarum strain H-6 collected from seaweed and terrestrially derived L. plantarum strain JCM11125 [1] of NaCl tolerance, pH tolerance and range of growth temperature during 2 weeks incubation. Both of two isolates and the standard strain were incubated in MRS liquid medium (pH 6.5) at 37℃ for 12 h. The CSs of each strain were sterilized separately by a filter of 0.2 μm pore size after the centrifuge on 13,200×g at 4℃ for 5 min. Also, cell-growth ability and lactic acid (LA) production of each strain in MRS medium were investigated during the incubation for 12 h. The strain BF1-13 was chosen as the most potential one among all the strains. The supplementation effect of the CS of strain BF1-13 on the barrier function of the intestinal epithelial model treated with H2O2 was compared with that of strain JCM11125 by evaluating the transepithelial electrical resistance (TEER) using Caco-2 cells. The effects of the CSs on the expressions of TJs-related proteins claudin-4 (CLDN4) and occludin (OCLN) were investigated by immunofluorescence staining and by quantitative RT-PCR. The supplementation effect of an equal amount of authentic LA contained in the CS of strain BF1-13 was compared to the CS itself by TEER assay, immunofluorescence staining, and quantitative RT-PCR to testify if LA is the effective compound to protect the barrier function.
The effect of the CSs of two strains and the equal amount of LA contained in the CS of strain BF1-13 on the expression of AQP3 were determined by immunofluorescence staining and quantitative RT-PCR to investigate the relationship between the barrier function of TJs and the H2O2 intracellular invasion facilitated by AQP3.

Results: It was suggested that the 5% (v/v) CS of strain BF1-13 alleviated the intestinal epithelial dysfunction caused by H2O2 treatment in Caco-2 cells, according to the TEER assay. The 5% (v/v) CSs of both two strains

enhanced the expression of CLDN-4 observed by immunofluorescence microscopy. As a result, only the CS of strain BF1-13 enhanced CLDN-4 expression at a transcription level by TEER assay, an equal amount of authentic LA (1.32mM) produced from homofermentative strain BF1-13 was proved to have the same protection of the intestinal epithelial barrier function. However, the protective effect of LA decreased by higher concentration more than 1.32mM. LA also showed a similar enhancement on CLDN-4 expression. Except for the enhancement on the expression of CLDN-4 at a transcription level, LA also induced the expression of OCLN. Furthermore, it was suggested that the 5% (v/v) CSs of both two strains suppressed the expression of AQP3. However, LA showed no suppression of AQP3 expression.

Conclusion & Discussion: L. plantarum strain BF1-13 isolated from DSW in Izu-Akazawa was suggested to be fast on cell-growth with the highest LA production compared to other strains in this study. According to this, strain BF1-13 was suggested to have the possibility of wide application in the LAB market. Also, the supplementation effect of the CS of strain BF1-13 clarified in this study will provide the possibility of the application in the maintenance of human health against intestinal related diseases. This is the first report on LA as an essential substance for the enhancement on the intestinal epithelial barrier from the dysfunction caused by H_2O_2, although many other effects of LA have been well-known so far. The enhancement on OCLN was much weaker than CLDN-4 especially by the supplementation of the CSs of two strains. This difference between CLDN-4 and OCLN was shown on both protein expressions and mRNA expression. By further experiments related to the ex-pression of AQP3, the difference of the enhancements between TJs-related proteins contributing to the protection of intestinal epithelial barrier function was investigated. As an endogenous reactive oxygen species, H_2O_2 is mostly produced in the lamina propria by antibacterial defense. Therefore, the monolayers in vitro model constructed by Caco-2 cells which imitated the intestinal epithelial barrier were treated with H_2O_2 from the basolateral side in this study. Also, it was known that AQP3 was expressed only on the basolateral side of the cell membrane. Combining the positions of CLDN-4 and OCLN in TJs structure, it was indicated that H_2O_2 added to the basolateral side of the monolayer in the model had two different invasion routes including intercellular invasion by simple passive diffusion and intracellular route by AQP3-facilitating diffusion. It was suggested that H_2O_2 intracellular invasion via APQ3 caused the suppression of the expression of TJs-related proteins especially CLDN-4. It was also suggested that the suppression by the CSs of the strains on AQP3 expression at the transcription level made the H_2O_2 preferentially target on OCLN via intercellular route than CLDN-4 via AQP3-facilitating intracellular invasion. The suppression was not shown by LA is mostly because as a small molecular substance, it prefers the paracellular route by TJs rather than transcellular route. Therefore, the relationship between the dysfunction of intestinal epithelium and H_2O_2 intracellular invasion has been clarified for the first time. In summary, CS of Lactiplantibacillus plantarum strain BF1-13 isolated from DSW protects the intestinal epithelial barrier from the dysfunction caused by H_2O_2 treatment. It was also elucidated that the mechanism of this supplementation effect was achieved by both the enhancement CLDN-4 expression and the suppression on AQP3-facililating H_2O_2 invasion. Still, the suppression of the AQP3 expression by the CSs remains to be researched.

Keywords: Aquaporin3, Deep seawater, Hydrogen peroxide, Lactic acid, Lactiplantibacillus plantarum, Tight junctions