World Academy of Science, Engineering and Technology
International Journal of Biomedical and Biological Engineering
Vol:10, No:01, 2016

L. rhamnosus GG Lysate Can Inhibit Cytotoxic Effects of S. aureus on Keratinocytes in vitro

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Abstract : In the gut, probiotics have been shown to protect epithelial cells from pathogenic bacteria through a number of mechanisms: 1-Increasing epithelial barrier function, 2-Modulation of the immune response especially innate immune response, 3-Inhibition of pathogen adherence and down regulation of virulence factors. Since probiotics have positive impacts on the gut, their potential effects on other body tissues, such as skin have begun to be investigated. The purpose of this project is to characterize the potential of probiotic bacteria lysate as therapeutic agent for preventing or reducing the S. aureus infection. Normal human primary keratinocytes (KCs) were exposed to S. aureus (106/ml) in the presence or absence of L. rhamnosus GG lysate (extracted from 108cfu/ml). The viability of the KCs was measured after 24 hours using a trypan blue exclusion assay. When KCs were treated with S aureus alone, only 25% of the KCs remained viable at 24 hours post infection. However, in the presence of L. rhamnosus GG lysate the viability of pathogen infected KCs increased to 58% ($p=0.008$, $n=3$). Furthermore, when KCs co-exposed, pre- exposed or post-exposed to L. rhamnosus GG lysate, the viability of the KCs increased to ≈60%, the L. rhamnosus GG lysate was afforded equal protection in different conditions. These data suggests that two possible separate mechanisms are involved in the protective effects of L. rhamnosus GG such as reducing S. aureus growth, or inhibiting of pathogenic adhesion. Interestingly, a lysate of L rhamnosus GG provided significant reduction in S. aureus growth and adhesion of S. aureus that being viable following 24 hours incubation with S aureus. Therefore, a series of Liquid Chromatography (RP-LC) methods were adopted to partially purify the lysate in combination with functional assays to elucidate in which fractions the efficacious molecules were contained. In addition, the Mass Spectrometry-based protein sequencing was used to identify putative proteins in the fractions. The data presented from purification process demonstrated that L. rhamnosus GG lysate has the potential to protect keratinocytes from the toxic effects of the skin pathogen, S. aureus. Three potential mechanisms were identified: inhibition of pathogen growth; competitive exclusion; and displacement of the pathogen from keratinocyte binding sites. In this study, 'moonlight' proteins were identified in the current study's MS/MS data for L. rhamnosus GG lysate, which could elucidate the ability of lysate in the competitive exclusion and displacement of S. aureus from keratinocyte binding sites. Taken together, it can be speculated that L. rhamnosus GG lysate utilizes different mechanisms to protect keratinocytes from S. aureus toxicity. The present study indicates that the proteinaceous substances are involved in anti-adhesion activity. This is achieved by displacing the pathogen and preventing the severity of pathogen infection and the moonlight proteins might be involved in inhibiting the adhesion of pathogens.

Keywords : lysate, fractions, adhesion, L. rhamnosus GG, S. aureus toxicity
Conference Title : ICLMCC 2016 : International Conference on Laboratory Medicine and Clinical Chemistry
Conference Location : London, United Kingdom
Conference Dates : January 18-19, 2016