

**TITLE:** EFFECT OF PROBIOTIC *L. rhamnosus* BY-PRODUCTS ON GINGIVAL EPITHELIAL CELLS CHALLENGED WITH *P. gingivalis*

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**ABSTRACT:**

Little is known about potential benefits of products derived from probiotic bacteria regarding periodontal disease. Therefore, the aim of this study was to evaluate whether these products (spent media and lysate) would attenuate the inflammatory process caused by *P. gingivalis* in gingival epithelial cells (GECs). For this, OBA-9 GECs ( $\sim 2,5 \times 10^5$  cells/well) were challenged with *P. gingivalis* ATCC33277, and co-infected with *L. rhamnosus* Lr-32 for 4h. The Lr-32 spent media and lysate were diluted in the media (1:4) and added to GECs for 4h either alone or with *P. gingivalis*. Additionally, another set of cells were first exposed to *P. gingivalis* ATCC 33277 for 2h and then the probiotic or its products were added and spent 2 more hours in contact with the cells. OBA-9 viability was measured by trypan blue exclusion assay. Levels of gene expression encoding cytokines (IL-1 $\beta$ , TNF, IL-6 and IL-8) and receptors (TLR2 and TLR4) were evaluated by RT-qPCR. The experiments were performed in triplicate and ANOVA was used as statistical test. Lr-32 spent media decreased cell viability, while other groups did not differ from control. Lr-32 lysate was able to increase the expression of cytokines encoding genes (IL-1 $\beta$ , TNF- $\alpha$ , IL-6 and IL-8) whereas spent media decreased their expression especially when used after prior infection with *P. gingivalis*, and overall the reduction was more pronounced than when the live probiotic was used. Transcription of *TRL2* was upregulated in all experimental groups compared to control. Both spent media and lysates reduced the growth of *P. gingivalis* in a competition assay. Thus, it can be concluded that spent media of *L. rhamnosus* Lr-32 rather than live probiotic can modulate the inflammatory process in gingival epithelial cells caused by *P. gingivalis* infection.

**Keywords:** Probiotics; *Porphyromonas gingivalis*; Gene expression; Spent media; Lysate.

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