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COEXISTENCE OF SALMONELLA
ENTERICA AND HUMAN EPITHELIAL
CELLS-IN VITRO SYSTEM

KOEGZISTENCIJA SALMONELLA
ENRERICA I HUMANIH EPITELIJALNIH
ĆELIJA- IN VITRO SISTEM

Correspondence to:

Ksenija Durgo,

Faculty of Food Technology and
Biotechnology,
University of Zagreb, Pierottijeva 6,
10000 Zagreb, Croatia,

E-mail: kdurgo@pbf.hr
ksenija974@gmail.com

Ksenija Durgo¹, Jelena Miđić¹, Ana Butorac¹,
Mario Cindrić² and Višnja Bačun-Družina¹

¹ Faculty of Food Technology and Biotechnology, Zagreb, Croatia

² Institute Rudjer Bošković, Bijenička 4, 10000 Zagreb, Croatia

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Ključne reči

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Abstract

Salmonella enterica is an ultimate human pathogen that causes various health problems. On the other hand, *Lactobacillus plantarum* has numerous positive effects on human health. The objective of this study was to investigate the influence of enterobacteria *S. enterica* LT21 on human laryngeal carcinoma cell line (HEp2). The ability of the adhesion of *S. enterica* LT21 as an independent culture and in combination with *L. plantarum* 16.4 to HEp2 cells was tested as well as metabolic activity of *S. enterica* LT21 after adhesion to HEp2 cells. The protein content in HEp2 cells after the incubation with *S. enterica* LT21 was investigated and compared with the protein content in the untreated HEp2 cells. Adhesion results show better binding of *S. enterica* LT21 than *L. plantarum* 16.4 when bacteria are mixed in a 1:1 and 10:1 ratios, respectively. Acetic and lactic acid in the sample of HEp2 cells incubated with *S. enterica* LT21 were detected, as well as lactic acid in a sample of HEp2 cells. Proteomic studies were also performed using tandem mass spectrometry analysis (MS/MS). Results imply that *S. enterica* LT21 affects the expression of proteins of HEp2 cells and moreover HEp2 cells after incubation with *S. enterica* LT21 additionally expressed protein troponin T type 1.

INTRODUCTION

Interactions between bacteria and human organism are intriguing issue over decades and there are still a lot of unanswered questions concerning mechanisms responsible for disease development, resistance against certain pathogens and also interactions among pathogens and normal human microflora. To get the answer on, at least, few of these questions it has to be used a suitable system for investigation. First organ which is in the contact with numerous numbers of bacteria (beside a skin) is larynx. It is important organ responsible for breathing, swallowing, voice formation and removal of foreign particles which have entered into body during breathing process. Its role is not dedicated to respiration only, but it also represents a crossroad between gastrointestinal and respiratory system. Epithelial tissue which covers larynx consists of two layers, epithelial cells and *lamina propria*, which are bounded to throat muscle and cartilage tissue. Epithelial cells are rich on different bacteria among

which some of them can cause severe diseases in the case of their overgrowing of other members of epithelial microflora. Since majority of these microorganisms can easily enter into gastrointestinal system, it is highly expected that in the case of severe disturbances pathogens present on larynx can easily enter into gastrointestinal system as well. Investigations showed that epithelial cells express different antigen proteins pointing out that epithelia is a lot more than just a barrier between environment and organism ⁽¹⁾.

Contrary to the pathogens, probiotic microorganisms can change the balance of colon microflora, improving colon integrity and mobility, preventing growth of pathogens and increasing resistance of the organism against disease development⁽²⁾. But real question is what is really happening on relation pathogen-human epithelia and pathogen-probiotic bacteria-human epithelia.

RESULTS AND DISCUSSIONS

In order to determine relationship and influence of *Salmonella enterica* on human epithelial cells, an *in vitro* system was prepared; the idea was to see how will the intensity of the *Salmonella* binding for epithelial cells surface change during 4 hours of incubation. Real problem in this experiment is the difference in division time between microorganisms and human cells; for one cell division bacterial cell needs only 20 minutes and human cells need 24 hours to do the same. So, if you want to examine relationship between bacteria and epithelia tissue, you have to be careful with the time of incubation-any kind of incubation longer than two hours would mean classic contamination of human cells and their senescence caused by inflammatory processes and lack of nutrients in growth media. After inoculation of human epithelial cells with *S. enterica* LT1 (optical density 1) it was noticed that after 30 minutes of incubation, the number of bacterial cells which are bounded for laryngeal cells increase and that increasing trend was noticed after 2 hours of incubation. There are several explanations for this phenomenon; one is that *Salmonella* cells bind for membrane proteins slowly and another is that during these 2 hours some increase in the number of epithelial cells is increasing, so consequently, the number of bounded *Salmonella* cells grows also. Expectedly, after 2 hours, the number of bacterial cells bounded for epithelial cells has started to decrease, and microscopic analysis of epithelial cells reveal their shrinkage, detachment and apparent decrease in cell viability.

What about metabolism of epithelial cells in this dual system? HPLC analysis revealed that *S. enterica* LT21 has significant influence on the production of acetic and lactic acid in epithelial cells. Normally, epithelial cells produce carbonate and lactic acid as a consequence of more less anaerobic condition⁽³⁾. In comparison to the epithelial cells, *S. enterica* by itself produces 4 times higher quantity of lactic acid. After attachment of *S. enterica* LT1 cells to epithelial cells, the quantity of produced lactic acid decreases significantly. The level of lactic acid produced by pathogen decreases also and explanation for this effect lies in the fact that all excessive quantity of lactic acid is used by epithelial cells as preferable substrate⁽³⁾. Acetic acid was determined in the growth media in which bacteria were grown and also in the media in which both, bacteria and epithelial cells were

co-incubated. Its concentration was 2-3 times lower than concentration of lactic acid, but when compared to the concentrations of acetic acid in growth media, it was seen that it was significantly higher in the media in which bacterial cells and epithelial cells were grown together than in the media in which only *Salmonella enterica* LT1 was grown. There are several studies which have shown that lactic acid bacteria could prevent pathogen adhesion on the surface of epithelial cells, preventing in such way level of colonization and consequently, infection⁽⁴⁾. Competitive behavior of pathogen (*S. enterica* LT21) and lactic acid bacteria (*L. plantarum* 16.4.) was determined by measurement of adhesion of bacterial cells to epithelial cells. After one hour of incubation it was concluded that same ratio of both, pathogen and lactobacilli, goes into the favor of pathogen binding - pathogens inhibited all active sites on epithelial cells, preventing binding of *Lactobacillus plantarum*. Unexpectedly, when the ratio was increased in the favor of pathogenic bacteria, binding of lactobacilli was increased. It is possible that *Lactobacillus plantarum* uses some metabolic products of *Salmonella enterica* as a growth factor or binding factor which increases their capacity for binding. Two dimensional gel electrophoresis and mass spectrometry revealed that *S. enterica* LT21 induced troponine T type 1 when epithelial cells were grown with pathogen. It is a small protein that was first detected in *Escherichia coli*, but later, it was determined in the majority of prokaryotic and eukaryotic cells. It can be found in cytosol (Trx-1) and in mitochondria (Trx-2). Both types prevent oxidation destruction of the cells and are important during embryonic development and often Trx-1 is expressed as a response to the oxidative stress⁽⁵⁾. In such way it is responsible for redox equilibrium in the cell and consequently, cell survival.

CONCLUSION

From this work it can be concluded that *Salmonella enterica* adhere to laryngeal epithelial cells. The strongest adhesion was seen after 1 hour of incubation at optical density 1. *Salmonella enterica* induced epithelial cells to produce higher concentrations of lactic and acetic acids. During incubation period *Salmonella* did not cause cytotoxic effect against epithelial cells. *Salmonella enterica* induced expression of troponine T type 1 protein.

Sažetak

Salmonella enterica predstavlja ultimativni humani patogen koji izaziva čitav spektar zdravstvenih problema. S druge strane, *Lactobacillus plantarum* pokazuje brojne pozitivne efekte po zdravlje ljudi. Stoga je cilj ovog pregleda da predstavi ispitivanje uticaja enterobakterije *S. enterica* LT21 na human ćelijsku liniju carcinoma grlića materice (HEp2). Opisana je sposobnost adhezije *S. enterica*LT21 kao nezavsne kulture i u kombinaciji sa *L. plantarum* 16,4 za HEp2 ćelije kao i metabolička aktivnost *S. enterica* LT21 nakon adhezije za HEp2. Sadržaj proteina u HEp2 ćelijama nakon inkubacije sa *S. enterica* LT21 je poređena sa sadržajem proteina u netretiranim HEp2 ćelijama. *S. enterica* LT21 se bolje vezuje od *L. plantarum*16.4 kada su bakterije pomešane u brojčanom odnosu 1:1 i 10:1. U inkubiranim uzorcima HEp2 ćelija sa *S. enterica* LT21 detektovane su sirćetna i mlečna kiseline. Studije o proteomicima su sprovedene korišćenjem tandem masene spektrometrijske analize (MS/MS) i pokazuju da *S. enterica* LT21 utiče na ekspresiju proteina HEp2 ćelija, štaviše HEp2 ćelije nakon inkubacije sa *S. enterica* LT21 ekspimiraju gene za sintezu troponina T tipa 1.

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