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Mutational Improvement of *Lactobacillus acidophilus* GH 201 Intended for Ground Beef Preservation in Refrigerator Storage

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Abstract: Lactic acid bacteria widely used in food preservation at refrigerator temperatures due to their ability produce high amount of hydrogen peroxide and/or other antibacterial substances at refrigerator temperatures to inhibit food-borne pathogens and psychrophilic spoilage microorganisms. In order to improve of bio-preservation efficacy of *Lactobacillus acidophilus* GH 201 mutations causing resistance to rifampicin (*rif*) were used. Among UV-mutagenized population of *L. acidophilus* five *Rif* mutants producing high amounts of H_2O_2 were selected. *Rif* mutants produced significant amounts of hydrogen peroxide 50-55 µg/ml in sodium phosphate buffer (0.2 M, pH 6.5) and in beef broth (BB) at 5°C for 5 days submerged cultivation without of growth. The mutants possess higher impact against food-borne pathogen *Escherichia coli* O157: H7 at refrigeration temperatures and for 3 days reduces the pathogen total amount practically undetectable level. *Rif* mutants *L. acidophilus* reduced initial amount $2x10^5$ of *E. coli* O157: H7 in ground beef up to 3 log for 3 days of solid-state cocultivation when the wild strain reduced only 2 log. The application of *L. acidophilus* GH 201 *mutants* did not cause any changes in sensory characteristics of ground beef, moreover promotes expanding of shelf-life due to inhibition of psychrophilic spoilage microorganisms.

Keywords: Biopreservation, *Lactobacillus acidophilus*, *rif* Mutation, Refrigerator Temperatures, Hydrogen Peroxide, *E. coli* O157: H7

1. Introduction

For ground meat shelf life prolongation, synthetic chemicals have been traditionally used to inhibit resident pathogenic and spoilage microorganisms in refrigerated products. The increasing consumer concerns of potential health risks associated with some of synthetic preservatives has led researchers to evaluate the opportunity of using natural bio-preservatives such as Lactic Acid Bacteria (LAB) selected for their inhibitory activity towards undesirable microorganism. [1, 2]. Lactic acid bacteria produce a wide range of inhibitory compounds such as organic acids, hydrogen peroxide, diacetyl and bacteriocins and thus, expanding shelf life and increasing food safety and have GRAS (Generally Recognized As Safe) status. [3-7]. The inhibitory actions of LAB toward food-borne pathogens and spoilage organisms in non-processed foods occur during entire storage period by continuous production of inhibitory compounds instead of a one-time reduction, as occurs with antimicrobial substances interventions. It has been shown that for bio-preservation the most effective are LABs able to produce hydrogen peroxide at refrigerated temperatures in absence of growth [8-10].

Most raw foods are contaminated with pathogenic and spoilage microorganisms. Ground beef products are common sources of *E. coli* O157:H7 and its reduction is an important concern in the beef industry. It was shown LAB impact on *E. coli* O157:H7 viability in ground beef and the sensory properties of these products [6, 11, 12]. The species *L. delbrueckii* subsp. lactis more of all used in food

preservation, but hydrogen peroxide production is variable amongst strains [13, 14]. But the use of limited number of LAB strains may cause decreasing of treatment efficacy due to accumulation of deleterious mutations and/or adapting of pathogens to antibacterial substances which are produced [3]. In order to enhance biopreservation efficacy LAB mutant producing high amount of antimicrobial substances should be selected [15-17]. Because hydrogen peroxide production plays the major role in elimination of the pathogens at refrigerator storage, it levels should be assessed for newly selected strains. There aren't methods for direct selection of antimicrobials producing mutants, so we need in methods to enhance the likelihood of this mutants occurrence by help of mutations affecting global regulatory network of cell. Enhancement of secondary metabolites yield remains great challenge in biotechnology. Earlier we successfully used ribosome and RNA polymerase mutations for improvement of technological characteristics and enhancement secondary metabolites (polysaccharides, aroma substances, etc.) yields of dairy starters and other industrial microorganisms [18, 19].

There are fundamental connections between rifampicin resistance, RNA polymerase structure and function and global gene expression. Resistance to rifampicin is associated with mutations in the gene coding for the beta subunit of RNA polymerase (*rpoB*) [20, 21]. *Rif* mutations of RNA polymerase have been found involved in a variety of physiological processes and possessing pleiotropic effects, including: cell growth [22-24]; the ability of mutants to support the growth of various bacteriophages; the ability to maintain the F' episome; interaction with other genes mutant alleles; uracil sensitivity [22]; exopolysaccharides oversynthesis and thermosensitivity of LABs [18, 19]. A spontaneous *rif* mutation of *Saccharopolyspora erythraea* caused slow-growth and stimulated erythromycin production [25].

The objective of this study selection of *rif* mutants *L. acidophilus* GH 201 possessing high hydrogen peroxide production ability and evaluation their impact on *E. coli* O157:H7 in ground beef during refrigerated storage.

2. Materials and Methods

2.1. The Bacterial Cultures

L. acidophilus GH 201 and *Escherichia coli* O157: H7 MDC 5003 obtained from Microbial Depository Center of Armenian National Academy of Sciences (http://www.armbiotech.am).

2.2. Media

MRS agar and broth obtained from Hi Media (India). Nutrient broth (NB) [Serva, Germany], Tryptose agar (T-agar) [Merck, Germany]. Beef broth (BB) prepared by dissolving of 20 g Sigma beef extract powder in 1 L distilled water. Fresh ground meat was purchased from Yerevan butcher store. Sodium phosphate buffer (pH 6.5). Physiological saline - 0.9% NaCl. Merckoquant Peroxide Test strips [Merck, Germany].

2.3. Mutagenesis and Selection of Rifampicin Resistant Mutants

L. acidophilus GH 201 cells grown at 37°C in MRS (OD 0.6) were harvested by centrifugation at 12,000 x g for 15 min and resuspended in PBS. Aliquots of cell suspensions (2 ml) were poured into sterile petri dishes and irradiated with UV light (254 nm) for 5, 10, 20 and 40 sec. Irradiated cells diluted tenfold into fresh MRS broth and were grown at 37°C for 4 h to permit 3 - 4 division cycles. For obtaining *rif* mutants cells were plated on MRS agar containing 100 μg/ml of rifampicin and incubated at 37°C till resistant colonies appearance.

2.4. Bacteriological Analysis

Bacterial count in liquid media was made using standard methods [26]. For enumeration of *E. coli* and lactobacilli in ground beef 1 g infected meat sample was inoculated in 9 ml of sterile physiological, homogenized, made serial ten-fold dilutions and plated on Tryptose and MRS agars for determination of *E. coli* and LAB counts, respectively.

2.5. Hydrogen Peroxide Assay

Hydrogen peroxide concentration measured by Merckoquant Peroxide Test strips with measuring ranges 0.5 – 2 – 5 -10 - 25 and 1 – 3 – 10 – 30 – 100, according to the manufacturer instruction.

2.6. Agar Disk Diffusion Method

Agar disk diffusion method was used to evaluate the antimicrobial effect of LAB suspensions. *E. coli* O157: H7 culture grown in NB broth for 18 h at 37°C diluted to concentration of 10^7 cells/ml and 0.1 ml spread onto Tryptose agar. The paper discs (diameter, 5 mm) were soaked with LAB culture liquids and placed on the test culture lawn. After 2 h exposition in cold the plates were incubated at 37°C for 18 h and examined for size of clear inhibitory zones.

2.7. Quantification of Antimicrobial Activity of LAB Cultures in Cold Cultivation

Lactobacilli were grown in MRS broth for 18 h at 37°C divided in four 10 ml aliquots, centrifuged at 12,000 × g for 10 min and each pellet resuspended in 10 ml of cold medium; sodium phosphate buffer and beef broth and incubated at 5°C. Every two days for 7 days and then each week antimicrobial activity, hydrogen peroxide amount, OD600 and pH of the cell cultures were determined.

2.8. Submerged Cocultivation of LAB and E. coli in Nutrient Broth (NB)

For evaluating *rif* mutants antagonistic activity against of *E. coli* O157:H7, the pathogen overnight culture was diluted in 200 ml of fresh NB to obtain cell concentration of approximately 10^5 CFU/ml, divided in two equal portions and supplied LAB in ratios 1: 100 and 1: 10. Both samples stored at 5°C and subjected to microbial analysis on days 0, 1, 3, 5, and 7.

2.9. Agar Based Solid State Cocultivation of LAB with Pathogen

Cells from overnight cultures were harvested by centrifugation, washed twice in physiological saline, impregnated by paper disks and placed on *E. coli* O157:H7 lawn on T-agar kept for 24 hours at 5°C then transferred at 37°C and inhibitory zones around disks were examined on the next day.

2.10. LAB Antimicrobial Activity Determination in Ground Meat

150 g of fresh ground beef was inoculated with *E. coli* O157:H7 to obtain a pathogen concentration of approximately 10^5 CFU/g and divided into three equal portions. LAB cultures were prepared as described previously and at final concentrations of 10^7 CFU/ml added into two samples of infected ground beef mixed and packaged in vacuum polyethylene bags. The control portions of the ground meat with *E. coli* O157:H7 were processed in the same manner. All samples were kept at 5°C and subjected for microbiological analysis on days 0, 1, 3, 5 and 7.

2.11. Statistical Analysis

Statistical analysis was performed using SPSS program (Version 16). Standard deviation of mean was used to describe data. Fisher's range test was used to determine differences between tested groups. P value < 0.05 and 0.001 were considered as significant and highly significant, respectively.

3. Results and Discussion

3.1. UV Mutagenesis and rif Mutants Obtaining

Earlier we have shown that the maximum yield of UV induced resistant to antibiotics mutants in LABs occurs at survival about 0.1% [27]. For maximum yield of *rif* mutants *L. acidophilus* GH 201 cells suspension was irradiated by UV light for 20 sec giving survival ~ 0.1 % and plated on T-agar containing 100 µg/ml rifampicin. The UV induced yield of *rif* mutants was 3.2 ± 04 ppm which about 10 – 20 folds higher than of spontaneous yields.

3.2. Total Selection of H_2O_2 Active Producers Among of L. Acidophilus GH 201 rif Mutants

The dominant inhibitory factor produced by lactobacilli at refrigerating temperatures was identified as hydrogen peroxide [13, 28-30].

Among tested overnight cultures of 300 *rif* mutants grown in MRS broth by Merckoquant Peroxide Test strips, four mutants expecting for active H_2O_2 formation were isolated. The mutants were examined for production of antimicrobial substances at 5°C by Tryptose agar based solid-state cultivation on *E. coli* O157:H7 lawn. The growth inhibitory zones of pathogen around disks impregnated by washed cells of *rif* mutants presented in Figure 1.

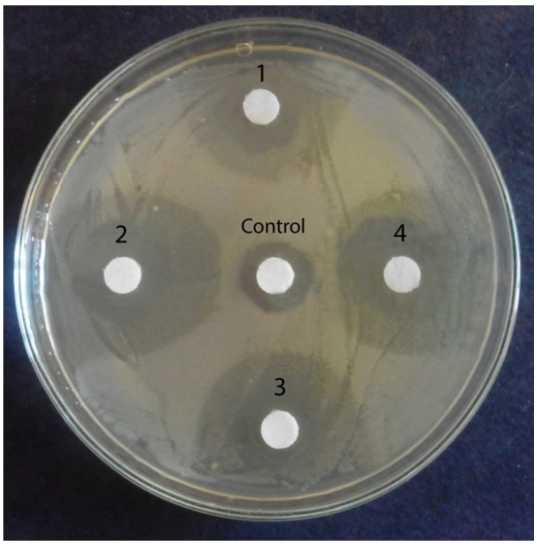

Figure 1. *Inhibitory zones on E. coli O157:H7 around disks impregnated by mutants L. acidophilus GH 201 Rif mutants cell suspensions after 24 h exposure at 5°C and further incubation at 37°C. 1- LR-128, 2-LR-195, 3-LR-247, 4- LR-286, 5- the wild strain in the center.*

The larger inhibitory zones formed around disks impregnated by cells of LR-105, LR-136 and LR-181 mutants. The storage in refrigerator more than one day was not significantly reflected on the size of inhibitory zones. For one-day solid-state cultivation LAB cells produced as much antimicrobial substances as in submerged cultivation for about three days, probably due to stimulation of hydrogen peroxide production by available oxygen.

3.3. Hydrogen Peroxide Production in BB by L. Acidophilus GH 201 rif Mutants During Storage at 5°C

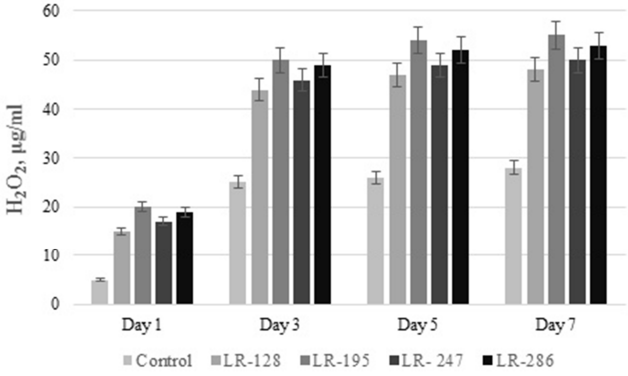

Figure 2. *Hydrogen peroxide production by L. acidophilus GH 201 and rif mutants at 5°C in beef broth.*

Three mutants forming larger inhibitory zones were investigated for hydrogen peroxide formation in beef broth. In laboratory experiments for evaluation of hydrogen peroxide production by LAB cells at 5°C usually was used sodium phosphate buffer [1, 31]. In order to estimate mutant strains potential ability in hydrogen peroxide formation in this study we used beef broth which composition is consimilar with ground beef. Beside this, for full expression of LABs hydrogen peroxide production activity, the MRS is

not satisfy because include yeast extract and high concentration of glucose which possesses respectively peroxidase and hydrogen peroxide formation inhibitory activities [32, 33]. The hydrogen peroxide accumulation in BB gradually increased and after five days of cold storage riches maximum in case of all tested strains (fig. 2). *Rif* mutants by hydrogen peroxide production 70-80 % exceeds parental strain L. acidophilus GH 201. The most active mutant LR-195 produces H_2O_2 mean 55 µg/ml.

3.4. Cells Viability and pH Changes in LAB Cultures During Cold Storage

During the entire period of storage at 5°C live cells count and beef broth culture pH were monitored (Table.1).

Table 1. *L. acidophilus GH 201and rif mutants viability and pH in BB during storage at 5°C.*

	Day zero		After 7 days	
Strain	**pH**	**Viable cells, CFU/ml**	**pH**	**Viable cells, CFU/ml**
L. acidophilus GH 201	6.5 ± 0.2	7.6×10^8	6.4 ± 0.2	7.2×10^8
LR-128	6.7 ± 0.2	7.8×10^8	6.5 ± 0.2	7.7×10^8
LR-195	6.8 ± 0.2	8.5×10^8	6.5 ± 0.2	8.6×10^8
LR-247	6.8 ± 0.2	8.3×10^8	6.6 ± 0.2	8.5×10^8
LR-286	6.8 ± 0.2	8.4×10^8	6.6 ± 0.2	8.6×10^8

No significant differences were found in the population levels of LAB cultures during over 7 days storage at 5°C indicating that cells reproduction was not necessary for hydrogen peroxide formation. These findings come in accordance with the observations of Amezquita and Brashears 2002, Ruby and Ingham 2009 who suggested that the production of inhibitory metabolites can occur by LAB during storage in the absence of growth [1, 31].

3.5. Antagonistic Action of LABs on E. coli O157:H7 in Submerged Cocultivation at 5°C

L. acidophilus GH 201 and most active *rif* mutant LR-195 about 10^7 CFU/ml were separately added into NB broth along with *E. coli* O157:H7 10^5 CFU/ml in order to determine their comparative antagonistic action against the pathogen at 5°C. The total number of *E. coli* O157:H7 cells in both treatments were determined on days 0, 1, 3 and 5 by plating appropriate dilutions on Tryptose agar and incubation at 37°C for 24 hours.

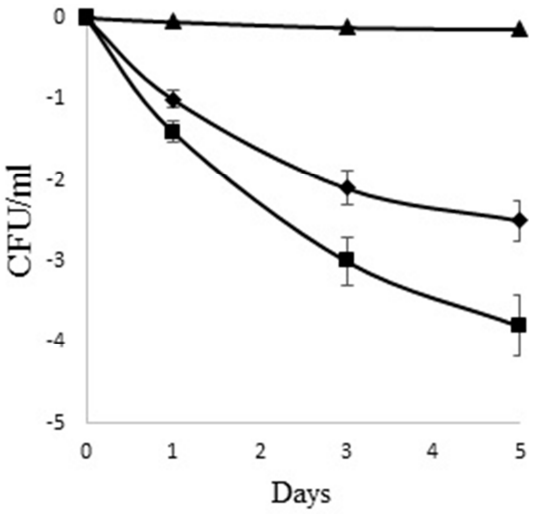

Figure 3. *E coli O157:H7 inactivation during submerged co-cultivation in NB at 5°C by LAB strains. (♦) - L. acidophilus GH 201, (■) - rif mutant LR-195, (▲) - E coli O157:H7control.*

The impact of mutant and parental strains of *L. acidophilus* GH 201 on *E. coli* O157:H7 in nutrition broth significantly differ (Figure. 3). For 3 days of storage, the mutant LR-195 reduces the initial populations of E. *coli* O157:H7 ~ 3.5 log whereas the wild strain only 2.3 log. In the control sample viable cell number of *E. coli* O157:H7 was not significantly changed for 7 days.

3.6. LABs Inhibitory Effect on E. coli O157:H7 in Ground Meet at 5°C

L. acidophilus GH 201 and *rif* mutant LR-195 were tested in packaged ground meat for their ability to reduce the viability of *Escherichia coli* O157:H7 during storage at 5°C in plastic vacuum bags. Each ground meat sample was infected with 10^5 CFU/g of *E. coli* O157:H7 and treated with 10^7 CFU/g of LABs. Samples were analyzed for *E. coli* O157:H7 survivors and lactic acid on days 1, 3, 5 and 7.

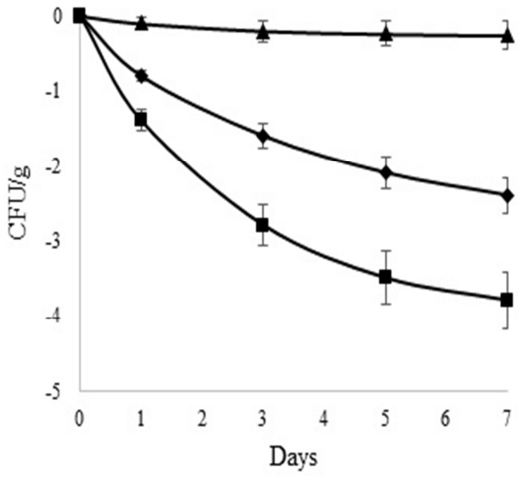

Figure 4. *Kinetic of E. coli O157:H7 inactivation by L. acidophilus GH 201 and rif mutant LR-181 in ground meat at 5°C. (■) – E. coli O157:H7 + LR-195 (♦) E coli O157:H7 + L. acidophilus GH 201, (▲) – E coli O157:H7 control.*

Towards the end of ground meat vacuum storage *E. coli* O157:H7 quantity was 3-4 log lower than those in the

control. The impact of rif mutant on the pathogen was significantly higher of *L. acidophilus* GH 201.

Growth of LABs in a fresh meat held at refrigeration temperature is not desirable because it would lead to premature spoilage of the product. The count LABs in treated samples for 7 days wasn't significantly changed. It was revealed that the application of rif mutant hasn't any influence on sensory characteristics of ground beef, moreover promote expanding of shelf-life due to inhibition of psychrophilic spoilage microorganisms which is in agreement with other authors [6, 34-36].

Experiments suggest that treated by rif mutant ground beef keeps good quality for entire period of storage; avoid appearance of undesirable odor, greening and smooth on meat surface by synthesizing antimicrobial compounds in amounts sufficient to inhibit the growth of pathogens and spoilages. Thus, *rif* mutations have high potential to be used for improvement antimicrobial compounds production LABs intendent for biopreservation meat products in cold storage.

It was shown that hydrogen peroxide producing ability of LAB at 5°C is strongly dependent on nutrition media composition used for their prior propagation as well as media for sub cultivation at refrigeration temperatures. For largest amount of hydrogen peroxide production LAB cells must priory propagated in rich medium and then transferred in sodium phosphate buffer at 5°C [1, 32]. Cell suspensions in phosphate buffer without glucose showed high accumulation of H_2O_2 in contrast to phosphate buffer containing glucose where produced undetectable amounts of H_2O_2. High concentrations of glucose appeared to inhibit the production of H_2O_2 by the cells [29, 32, 37]. In our experiments at the first time we used beef powder broth very close to the ground beef by composition as model for evaluation H_2O_2 production and found yield significantly higher than in phosphate buffer.

A characteristic feature of *rif* mutations is pleiotropy of their phenotypic realization. They are able to cause misreading of genetic information at the level of transcription over whole genome that brings to simultaneous alterations of large spectrum phenotype features of bacteria e. g. colony morphology, growth rate, sensitivity to temperature, requirements in growth factors, production of secondary metabolites etc., by global changing of transcriptional profile [38-43].

Thus, it was possible to show that mutations deter mining resistance to rifampicin may be an efficient instrument for selecting strains of lactobacilli with enhanced yield of antimicrobial substances including hydrogen peroxide.

4. Conclusion

Resistant to rifampicin mutants of mutants *L. acidophilus* GH 201 produced significant amounts of hydrogen peroxide at 5°C in beef broth without of growth. They exert higher inhibitory action against *E. coli* O157:H7 and practically fully eliminate the pathogen bacteria from ground beef at refrigeration temperatures. *Rif* mutations can be successfully used as a tools in biotechnology for improving preservative properties of LABs intended for commercial applications.

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References

[1] Amézquita A, Brashears MM. Competitive inhibition of *Listeria monocytogenes* in ready-to-eat meat products by lactic acid bacteria. J Food Prot. Vol. 65(2), pp. 316–325, 2002.

[2] Gyawali R, Ibrahim SA. Natural products as antimicrobial agents. Food Control. Vol. 46, pp. 412–429, 2014.

[3] Davidson PM, Harrison MA. Resistance and Adaptation to Food Antimicrobials, Sanitizers, and Other Process Controls. Scientific Status Summary, Food Technology. Vol. 56(11), pp. 69–78, 2002.

[4] Muhialdin BJ. Hassan Z. Screening of Lactic Acid Bacteria for Antifungal Activity against Aspergillus oryzae. American Journal of Applied Science. Vol. 8, pp. 447–451, 2011.

[5] Dalié DKD, Deschamps AM. Richard F. Lactic acid bacteria Potential for control of mould growth and mycotoxins. A review Food Control. vol. 21, pp. 370–380, 2010.

[6] Smith L, Mann JE, Harris K, Miller MF, Brashears MM. Reduction of *Escherichia coli* O157:H7 and Salmonella in ground beef using lactic acid bacteria and the impact on sensory properties. J Food Prot. Vol. 68(8), pp. 1587–92, 2005.

[7] Favaro L, Penna ALB, Todorov DD. Bacteriocinogenic LAB from cheeses -Application in Biopreservation. Trends in Food Science Technology. Vol. 41, pp. 37–48, 2015.

[8] Daly CW, Sandine E, Elliker PR. Interaction of food starter cultures and food-borne pathogens: Streptococcus diacetilactis versus food pathogens. J. Milk Food Technol. vol. 35, pp. 349–357, 1972.

[9] Daeschel MA. Antimicrobial substances from lactic acid bacteria for use as food preservatives. Food Technology. Vol. 1, pp. 164–167, 1989.

[10] Dahiya RS, Speck ML. Hydrogen peroxide formation by lactobacilli and its effect on *Staphylococcus aureus*. J. Dairy Sci. Vol. 51, pp. 1568–1572, 1968.

[11] Holzapfel WH, Geisen R, Schillinger U. Biological preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes. Int J Food Microbiol. Vol. 24(3), pp. 343–62, 1995.

[12] Salem A M. Bio-Preservation Challenge for Shelf-Life and Safety Improvement of Minced Beef. Global Journal of Biotechnology and Biochemistry. Vol. 7 (2), pp. 50–60, 2012.

[13] Yap PS, Gilliland SE. Comparison of Newly Isolated Strains of *Lactobacillus delbrueckii* subsp. *lactis* for Hydrogen Peroxide Production at 5°C. J Dairy Sci. Vol. 83, pp. 628–632, 2000.

[14] Gilliland SE. Use of lactobacilli to preserve fresh meat. Proc Recip Meat Conf. Vol. 33, pp. 54–58, 1980.

[15] Jones RJ, Hussein HM, Zagorec M, Brightwell G, Tagg JR. Isolation of lactic acid bacteria with inhibitory against pathogens and spoilage organisms associated with fresh meat. Food Microbiology. Vol. 25, pp. 228–234, 2008.

[16] Kostrzynska M, Bachand A. Use of microbial antagonism to reduce pathogen levels on produce and meat products: a review. Canadian Journal of Microbiology. Vol. 52, pp. 1017–1026, 2006.

[17] Maragkoudakis PE, Mountzouris KC, Psyrras D, Cremonese S, Fischer J, Cantor MD, Tsakalidou E. Functional properties of novel protective lactic acid bacteria and application in raw chicken meat against *Listeria monocytogenes* and *Salmonella enteritidis*. International Journal of Food Microbiology. Vol. 130, pp. 219–226, 2009.

[18] Hovhannisyan, H. G., Barseghyan, A. A., Grigoryan, N. G., Topchyan, A. V. Genetic improvement of technological characteristics of starters for fermented milk products. Applied Biochemistry and Microbiology. Vol. 46(4), pp. 395–399. 2010.

[19] Hovhannisyan H, Barseghyan A. The influence of rifampicin resistant mutations on the biosynthesis of exopolysaccharides by strain *Escherichia coli K-12 lon.* Applied Biochemistry and Microbiology. Vol. 51(5), pp. 546–550, 2015.

[20] Garibyan L, Huang T, Kim M, Wolff E, Nguyen A, Nguyen T, Diep A, Hu K, Iverson A, Yang H, Miller JH. Use of the rpoB gene to determine the specificity of base substitution mutations on the Escherichia coli chromosome. DNA Repair (Amst). Vol. 13; 2(5), pp. 593-608, 2003.

[21] Lai CY, Jaruga E, Borghouts C, Jazwinski SM. A mutation in the ATP2 gene abrogates the age asymmetry between mother and daughter cells of the yeast Saccharomyces cerevisiae. Genetics. Vol. 162(1), pp. 73-87, 2002.

[22] Jin DJ, Gross A. Characterization of the pleiotropic phenotypes of rifampin-resistant *rpoB* mutants of *Escherichia coli.* J. Bacteriol. Vol. 171(9), pp. 5229 5231, 1989.

[23] Kogoma T. *Escherichia coli* RNA polymerase mutants that enhance or diminish the SOS response constitutively in the absence of RNase HI activity. J. Bacteriol. Vol. 176(5), pp. 1521-1523, 1994.

[24] Maughan H, Galeano B, Nicholson WL. Novel *rpo*B mutations conferring rifampin resistance on *Bacillus subtilis*: global effects on growth, competence, sporulation, and germination. J. Bacteriol. Vol. 186, pp. 2481 2486, 2004.

[25] Elisabetta C, Clelia P, Salvatore MT, Francesco F, Adelfia T, Giorgio C, Silvio B, Gianluca, De B, Pietro A. Phenotypes and gene expression profiles of *Saccharopolyspora erythraea* rifampicin-resistant (rif) mutants affected in erythromycin production. Microbial Cell Factories. Vol. 8(18), pp. 1-15, 2009.

[26] Gerhardt P. Manual of methods for general bacteriology. Washington, D. C., American Society for Microbiology. 1981.

[27] Alireza G. 2016. "UV - Induced Mutagenesis in Lactic Acid Bacteria." International Journal of Genetics and Genomics. Vol. 4(1), pp. 1-4.

[28] Ammor S, Tauveron G, Dufour E. Chevallier I. Antibacterial activity of lactic acid bacteria against spoilage and pathogenic bacteria isolated from the same meat small-scale facility 1-Screening and characterization of the antimicrobial compounds. Food Control. Vol. 17, pp. 454–461, 2006.

[29] Villegas E, Gilliland SE. Hydrogen Peroxide Production by Lactobacillus delbrueckii subsp. lactis I at 5°C. Journal of food science. Vol. 63(6), pp. 1070–1074, 1998.

[30] Collins EB, Aramaki K. Production of hydrogen peroxide by *Lactobacillus acidophilus*. J. Dairy Sci. Vol. 63, pp. 353–357, 1980.

[31] Ruby JR, Ingham SC. Evaluation of potential for inhibition of growth of *Escherichia coli* O157:H7 and multidrug-resistant *Salmonella* serovars in raw beef by addition of a presumptive *Lactobacillus sakei* ground beef isolate. Journal of Food Protection. Vol. 72, pp. 251–259, 2009.

[32] Berthier, F. "On the screening of hydrogen peroxide-generating lactic acid bacteria." Letters in Applied Microbiology. Vol. 16(3), pp. 150–153, 1993.

[33] Rodríguez JM, Martínez M I, Suárez AM, Martínez JM, Hernández PE. Unsuitability of the MRS medium for the screening of hydrogen peroxide-producing lactic acid bacteria. Letters in Applied Microbiology. Vol. 25(1), pp. 73–74, 1997.

[34] Gilliland SE, Speck ML, Morgan C G. Detection of *L. acidophilus* in feces of humans, pigs, and chickens. Appl. Microbiol. Vol. 30, pp. 541–545, 1975.

[35] Senne MM, Gilliland SE. Antagonism action of cells of *Lactobacillus delbrueckii* subsp. *lactis* against pathogenic and spoilage microorganisms in fresh meat systems. Journal of Food Protection. Vol. 66, pp. 418–425, 2003.

[36] Sakaridis I, Soultos N, Batzios Ch, Ambrosiadis I. Koidis P. Lactic Acid Bacteria Isolated from Chicken Carcasses with Inhibitory Activity against *Salmonella* spp and *Listeria monocytogenes*. Czech J. Food Sci. Vol. 32(1), pp. 61–68, 2014.

[37] Jaroni D, Brashears MM. Production of Hydrogen Peroxide by *Lactobacillus delbrueckii* subsp. lactis as Influenced by Media Used for Propagation of Cells. Journal of Food Science. Vol. 65(6), pp. 1033–1036, 2000.

[38] Ingham CJ, Furneaux PA. Mutations in the β subunit of the *Bacillus subtilis* RNA polymerase that confer both rifampicin resistance and hypersensitivity to Nus G. Microbiology. Vol. 146(12), pp. 3041-3049, 2000.

[39] Jin DJ, Gross CA. Mapping and sequencing of mutations in the *Escherichia coli* rpoB gene that lead to rifampicin resistance. J Mol Biol. Vol. 202(1), pp. 45-58, 1988.

[40] Kogoma T. *Escherichia coli* RNA polymerase mutants that enhance or diminish the SOS response constitutively expressed in the absence of RNase H1 activity. J. Bacteriol. Vol. 176, pp. 1521-1523, 1994.

[41] Maughan H1, Galeano B, Nicholson WL. Novel rpoB mutations conferring rifampin resistance on Bacillus subtilis: global effects on growth, competence, sporulation, and germination. J Bacteriol. Vol. 186(8), pp. 2481-2486, 2004.

[42] Yanofsky C, Horn V. Rifampin resistance mutations that alter the efficiency of transcription termination at the tryptophan operon attenuator. J. Bacteriol. Vol. 145(3), pp. 1334-1341, 1981.

[43] Yura T, Ishihama A. Genetics of Bacterial RNA Polymerases. Annual Review of Genetics. Vol. 13, pp. 59-97, 1979.