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THE INFLUENCE OF ENVIRONMENTAL FACTORS ON THE PLANKTONIC GROWTH AND BIOFILM FORMATION OF *Escherichia coli*

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ABSTRACT. In this study, the effects of environmental factors (different media, temperature, pH, salt and sugar concentrations) on the planktonic growth, biofilm formation and formed biofilm of *Escherichia coli* KGPMF 16 and *Escherichia coli* KGPMF 17 were investigated. Tested bacteria were isolated from traditionally made cheese produced in Southeastern Serbia (Sokobanja region). The influence on planktonic growth, biofilm formation and formed biofilm was determined using spectrophotometric method. The limiting factors for the planktonic growth and biofilm formation were temperature of 4 °C and all tested concentrations of salt. The growth of tested bacteria was higher in media enriched with lactose than in media containing glucose. TSB was more congenial media to the planktonic growth of bacteria than MHB broth. None of the tested bacteria demonstrated the ability to form biofilm at 4 °C and 44 °C. Only *E. coli* KGPMF 17 showed ability to form biofilm in TSB at 37 °C. Different concentrations of salt, glucose and lactose exhibited inhibitory effect on biofilm formation, but all tested concentrations of lactose showed stimulating effect on formed biofilm of *E. coli* KGPMF 17. These results contribute to better understanding of the effects of environmental factors on the development of *E. coli* in cheese.

Keywords: biofilm formation, *E. coli,* planktonic growth, traditionally made cheese.

INTRODUCTION

Escherichia coli is one of the members of the microbiota of the intestinal flora. With other bacteria, they contribute 0.1% to the total flora (ECKBURG *et al.*, 2005). *E. coli* possesses the ability to survive outside the host for a certain time which makes it an important indicator of environmental condition (FENG *et al.,* 2002). ISHII and SADOWSKY (2008) confirmed that resistant *E. coli* may survive very long outside the host.

Environmental factors, such as concentration of nutrients, osmotic pressure, temperature, etc., affect bacteria in their environment (BRENHORVD *et al.,* 1992; BOUCHER *et*

al., 1994). In the environment, bacteria are more frequently found in biofilms than in planktonic (free-living) forms (HALL-STOODLEY *et al.,* 2004; HARRISON *et al.,* 2007). These biofilms are generally described as microbial cells attached to a surface and encased in an extracellular polysaccharide matrix (COSTERTON *et al.,* 1995). Several factors such as pH, temperature, concentration of O_2 and glucose can affect biofilm formation (COSTA *et al*., 2014). Biofilms demonstrate greater resistance to antimicrobial agents (XU *et al.,* 2000) which is significant due to the fact that biofilms cause food spoilage during production in food industry (TRÉMOULET *et al.,* 2002). Many investigations indicated that the temperature, type of medium and other growth conditions determined which genes were induced or repressed during biofilm development (SCHEMBRI *et al.,* 2003; BELOIN *et al.,* 2004; REN *et al.,* 2004; DOMKA *et al.,* 2007). According NESSE et al. (2014) potentially human-pathogenic *E. coli* from the ovine reservoir can form biofilm on various surfaces and at several temperatures relevant for food.

The aim of this study was to investigate the planktonic growth and ability of the bacteria from the genera *Escherichia* (*E. coli* KGPMF 16 and *E. coli* KGPMF 17) from autochthonous cheese, to form biofilm in two different broths, under the influence of different temperatures, pH, concentrations of NaCl, glucose and lactose, as well as the impact of the mentioned environmental factors on the formed biofilm.

MATERIALS AND METHODS

Strains and growth conditions

Bacteria used in this study were *E. coli* KGPMF 16 and *E. coli* KGPMF 17. *E. coli* ATCC 25922 was used as a positive control. The bacteria were previously isolated from Serbian cheese (Sokobanja region) and determined at the Laboratory for Microbiology at the Faculty of Science, University of Kragujevac (KGPMF) (MLADENOVIĆ *et al.,* 2018). The collection of identified bacterial species was kept in a 20% glycerol/medium mixture at -80 °C.

The effect of different temperatures on the planktonic growth of tested bacteria

The examination of the effect of temperature on the growth of tested bacteria was conducted in Tryptic soy broth (TSB) (Merck, KGaA, Darmstadt, Germany) and Muller-Hinton broth (MHB) (Torlak, Serbia) of standard or modified compositions. These two broths were used due to their different composition and different influence on the bacterial growth. 10 μl of initial bacterial suspension (10^8-10^9 CFU/ml) was added to 3 ml of each type of media. All samples were prepared in triplicate, each for one tested temperature (4 °C, 37 °C, 44 °C). The samples were incubated for 24 h. Pure TSB and MHB served as sterility controls. The results were obtained using spectrophotometer (SPEKOL 21, MA 9521, Iskra, Kranj, Slovenia) at 600 nm. Each experiment was performed in triplicate.

The effect of different pH and different concentrations of NaCl

For examining the effect of pH, the media whose pH values were 5.5, 6.5, 7, 7.5 and 8.5, were prepared. After adding HCl, acidic and neutral media were obtained (pH 5.5, 6.5 and 7), while after adding NaOH, we obtained basic media (pH 7.5 and 8.5). For TSB, the growth control was pH 7.5, while for MHB, growth control was pH 7. The effects of different salt concentrations were investigated in modified media with the addition of NaCl (4%, 6.5% and 8%). Growth in TSB containing 4% NaCl and in pure MHB served as growth controls. In 3 ml of each type of modified media, 10 μl of initial bacterial suspension (10^8-10^9 CFU/ml)

was added. The samples were incubated at 4 °C, 37 °C and 44 °C for 24 h. The results were obtained using spectrophotometer at 600 nm. Each experiment was performed in triplicate.

The effect of different concentrations of glucose and lactose

To modified TSB and MHB, different concentrations of glucose and lactose (0.5%, 1.5%, 2.5% and 3.5%) were added, respectively. 10 µl of initial bacterial suspension (10^8-10^9 CFU/ml) was added to 3 ml of each type of media. All samples were prepared in triplicate, each for one of tested temperatures (4 °C, 37 °C and 44 °C). The samples were incubated for 24 h. Growth and sterility controls were prepared, as well. The results were obtained using spectrophotometer at 600 nm. Each experiment was performed in triplicate. Growth in TSB containing 0.25% of glucose (pH 7.5) and in pure MHB served as growth controls for growth of bacteria in glucose and lactose.

Determination of antibiofilm activity

Pellicle test

The ability to form a biofilm phenotype or pellicle formation on the air-liquid interphase was demonstrated using pellicle assay in accordance with the method already described (VESTBY *et al.*, 2009), with modifications. 1.8 ml of TSB and MHB were inoculated with 0.2 ml of each isolate suspension, and then incubated for 96 h at 37 °C. The categorization of isolates and their ability to produce the biofilm were based on the production of pellicle on the surface of the liquid phase according to the following scheme: the solid fat formed pellicle (+++) - a good biofilm producer, a thin pellicle formed (++) - a moderate biofilm producer, very thin pellicle (+) - a weak biofilm producer, a complete absence of pellicle (-) - the absence of ability to produce the biofilm. Pellicle test was repeated three times for each tested isolate.

Biofilm formation assay and quantification

The ability of *E. coli* KGPMF 16, *E. coli* KGPMF 17 and *E. coli* ATCC 25922 to form biofilms at 4 °C, 37 °C and 44 °C, was assayed by O'TOOLE AND KOLTER (1998), with some modifications.

Two different broths (TSB or MHB) were used for the experiment. In sterile 96-well tissue culture plates (Sarstedt, Nümbrecht, Germany) containing 100 µl of modified broth per well (with different pH, different concentration of salt, glucose and lactose), 10 µl of fresh bacterial suspension (1.0 McFarland) was added. After incubation at 37 °C for 48 h, the content of each well was gently removed by tapping the plates. The wells were washed with 200 µl of sterile saline to remove free-floating bacteria. Biofilms formed by adherent cells in plate were fixed with 100 µl of methanol, and then stained with 100 µl (0.1%) of crystal violet and incubated at the room temperature for 20 min. Excess stain was rinsed off by thorough washing three times with 200 µl deionized water and then fixed with 100 µl of 96% ethanol. Optical densities (ODs) of stained adherent bacteria were determined using an enzyme-linked immunosorbent assay (ELISA) plate reader (RT-2100C, Rayto, Shenzhen, China) at 630 nm.

A sterile broth with different concentration of pH, NaCl and sugar served as control for checking sterility and nonspecific binding of media. In order to neutralize background absorbance, OD readings from sterile medium, modified broth, fixative, and dye were calculated and subtracted from all test values. All tests were performed in triplicate and their mean value was calculated.

The effect on formed biofilm

The tissue culture 96-well microtiter plates (Sarstedt, Nümbrecht, Germany) were prepared by dispensing 100 μl of TSB or MHB. 10 μl of fresh bacterial suspension (1.0 McFarland) was added into each well. The inoculated microtiter plates were incubated at 37 °C for 24 h. After the incubation, the content of each well was gently pulled out. Then, 100 μl of modified broth, which contained different pH, different concentration of salt, glucose and lactose was added and inoculated microtiter plates were incubated at 37 °C for 24 h. After the incubation, the content of each well was gently removed by tapping the microtiter plates. The rest of the experiment was performed as described above.

Data analysis

All data were presented as means ± standard deviations using Microsoft Excel (Redmond, Washington, DC, USA).

RESULTS

The influence of different temperature, pH, NaCl, glucose and lactose concentrations on the planktonic growth of tested bacteria

Tested bacteria were incubated in different media at three different temperatures (4 °C, 37 °C and 44 °C). After the incubation, it was noticed that there was no growth at 4 °C.

In TSB at 37 °C, the growth of *E. coli* KGPMF 16 was reduced in all pH, except in pH 8.5, where growth was higher compared to the growth control. The growth of *E. coli* KGPMF 17 and *E. coli* ATCC 25922 was reduced in pH 5.5 and 6.5, while in other tested pH growth was similar as growth control. In MHB at 37 °C, the growth of *E. coli* KGPMF 16 was reduced in all tested pH, except in pH 7.5, where growth was equal to the growth control. The growth of *E. coli* KGPMF 17 was reduced in pH 5.5 and 8.5, while growth of *E. coli* ATCC 25922 was reduced in all tested pH, except in pH 6.5.

In TSB at 44 °C, growth of *E. coli* KGPMF 16, *E. coli* KGPMF 17 and *E. coli* ATCC 25922 was reduced in all tested pH, except 8.5. In MHB, at 44 °C, growth of *E. coli* KGPMF 16 was reduced in pH 5.5 and 8.5, but in pH 6.5, the growth was stimulated. The growth of *E. coli* KGPMF 17 and *E. coli* ATCC 25922 was reduced in all pH, except in pH 6.5 (Tab. 1).

All tested salt concentrations in TSB and MHB produced reducing effect on the planktonic growth of *E. coli* KGPMF 16, *E. coli* KGPMF 17 and *E. coli* ATCC 25922, at both tested temperatures (37 °C and 44 °C) (Tab. 2).

All tested concentrations of glucose in TSB at 37 °C demonstrated reducing effect on the planktonic growth of *E. coli* KGPMF 16, *E. coli* KGPMF 17 and *E. coli* ATCC 25922. In MHB with different concentrations of glucose, at 37 °C, the growth of *E. coli* KGPMF 16 was reduced. The growth of *E. coli* KGPMF 17 was stimulated in all concentrations, except in 1.5%, while the growth of *E. coli* ATCC 25922 was reduced in all concentrations, except in 2.5%.

In TSB containing different concentrations of glucose, at 44 °C, growth of *E. coli* KGPMF 16, *E. coli* KGPMF 17 and *E. coli* ATCC 25922 was reduced. Exception is 0.5% of glucose, where growth of *E. coli* ATCC 25922 was stimulated. MHB containing 2.5% and 3.5% of glucose was stimulating for E. coli KGPMF 16 at 44 °C, while the growth of *E. coli* KGPMF 17 and *E. coli* ATCC 25922 was stimulated in all other pH (Tab. 3).

Table 1. The effect of different pH on the planktonic growth.

	TSB at 37 °C					MHB at 37 °C				
Species	5.5	6.5	7	7.5*	8.5	5.5	6.5	7*	7.5	8.5
***E. coli* KGPMF 16**	0.01±0.00	0.67±0.01	0.80±0.01	1.04±0.01	1.41±0.02	0.05±0.00	0.70±0.10	0.80±0.01	0.80±0.00	0.62±0.01
***E. coli* KGPMF 17**	0.02±0.00	1.46±0.04	1.63±0.01	1.64±0.00	1.60±0.00	0.06±0.02	0.70±0.01	0.70±0.03	0.70±0.01	0.40±0.06
***E. coli* ATCC 25922**	0.03±0.00	1.19±0.04	1.76±0.01	1.72±0.02	1.68±0.01	0.05±0.00	0.82±0.04	0.75±0.01	0.66±0.00	0.45±0.00
	TSB at 44 °C					**MHB at 44 °C**				
Species	5.5	6.5	7	7.5*	8.5	5.5	6.5	7*	7.5	8.5
***E. coli* KGPMF 16**	0.02±0.00	0.65±0.03	0.78±0.02	1.07±0.02	1.38±0.07	0.01±0.00	0.94±0.36	0.71±0.00	0.72±0.00	0.17±0.01
***E. coli* KGPMF 17**	0.03±0.00	0.62±0.26	0.82±0.25	1.07±0.02	1.13±0.04	0.02±0.00	0.29±0.05	0.24±0.04	0.21±0.04	0.12±0.02
***E. coli* ATCC 25922**	0.02±0.00	0.82±0.02	0.87±0.01	0.94±0.02	1.38±0.02	0.04±0.00	0.47±0.01	0.37±0.00	0.32±0.002	0.26±0.12

Values are presented as mean ± standard deviation measured at 600 nm; *growth control.

Table 2. The effect of different concentrations of NaCl on the planktonic growth.

	TSB at 37 °C				MHB at 37 °C		
Species	4%*	6.5%	8%	PMHB*	4%	6.5%	8%
***E. coli* KGPMF 16**	0.72±0.01	0.31±0.02	0.07±0.00	0.80±0.01	0.19±0.00	0.07±0.00	0.02±0.00
***E. coli* KGPMF 17**	1.15±0.02	0.44±0.06	0.12±0.01	0.70±0.03	0.24±0.03	0.09±0.02	0.02±0.00
***E. coli* ATCC 25922**	1.58±0.26	0.86±0.03	0.42±0.02	0.75±0.01	0.16±0.00	0.02±0.00	0.02±0.00
	TSB at 44 °C				**MHB at 44 °C**		
Species	4%*	6.5%	8%	PMHB*	4%	6.5%	8%
***E. coli* KGPMF 16**	0.60±0.02	0.06±0.02	0.03±0.00	0.71±0.00	0.06±0.02	0.03±0.00	n.g.
***E. coli* KGPMF 17**	0.54±0.00	0.07±0.01	0.05±0.01	0.24±0.04	0.06±0.00	0.03±0.00	n.g.
***E. coli* ATCC 25922**	0.62±0.00	0.54±0.03	0.12±0.02	0.37±0.00	0.12±0.00	0.04±0.00	0.01±0.00

Values are presented as mean ± standard deviation measured at 600 nm; n.g.- no growth; *growth control; PMHB-pure MHB- growth control.

Table 3. The effect of different concentrations of glucose on the planktonic growth.

	TSB at 37 °C						MHB at 37 °C			
Species	0.25% *	0.5%	1.5%	2.5%	3.5%	PMHB*	0.5%	1.5%	2.5%	3.5%
***E. coli* KGPMF 16**	1.04±0.01	0.84±0.01	0.81±0.02	0.73±0.00	0.68±0.01	0.80±0.01	0.63±0.03	0.63±0.03	0.72±0.04	0.65±0.12
***E. coli* KGPMF 17**	1.64±0.00	1.29±0.00	1.21±0.01	1.08±0.00	1.15±0.01	0.70±0.03	0.91±0.02	0.69±0.03	0.80±0.02	0.74±0.03
***E. coli* ATCC 25922**	1.72±0.02	1.19±0.04	1.15±0.01	1.06±0.02	1.03±0.02	0.75±0.01	0.51±0.00	0.55±0.01	0.75±0.00	0.52±0.03
	TSB at 44 °C						**MHB at 44 °C**			
Species	0.25% *	0.5%	1.5%	2.5%	3.5%	PMHB*	0.5%	1.5%	2.5%	3.5%
***E. coli* KGPMF 16**	1.07±0.02	0.95±0.03	0.95±0.02	0.86±0.01	0.81±0.02	0.71±0.00	0.63±0.03	0.67±0.02	0.76±0.05	0.72±0.04
***E. coli* KGPMF 17**	1.07±0.02	0.94±0.02	0.93±0.01	0.88±0.00	0.83±0.01	0.24±0.04	0.55±0.11	0.60±0.08	0.73±0.05	0.60±0.10
***E. coli* ATCC 25922**	0.94±0.02	0.98±0.04	0.93±0.02	0.84±0.06	0.65±0.44	0.37±0.00	0.63±0.02	0.65±0.05	0.44±0.04	0.51±0.10

Values are presented as mean ± standard deviation measured at 600 nm; * growth control; PMHB-pure MHB- growth control.

Table 4. The effect of different concentrations of lactose on the planktonic growth.

	TSB at 37 °C						MHB at 37 °C			
Species	0.25% [1]	0.5%*	1.5%*	2.5%*	3.5%*	PMH	0.5%	1.5%	2.5%	3.5%
***E. coli* KGPMF 16**	1.04±0.01	0.99±0.00	0.90±0.00	0.84±0.00	0.76±0.01	0.80±0.01	0.80±0.07	0.70±0.00	0.70±0.01	0.70±0.01
***E. coli* KGPMF 17**	1.64±0.00	1.67±0.00	1.62±0.00	1.53±0.00	1.44±0.00	0.70±0.03	1.10±0.03	0.92±0.02	0.84±0.01	0.85±0.00
***E. coli* ATCC 25922**	1.72±0.02	1.26±0.01	1.14±0.01	1.09±0.02	1.07±0.03	0.75±0.01	0.66±0.03	0.66±0.02	0.63±0.02	0.57±0.01
	TSB at 44 °C						**MHB at 44 °C**			
Species	0.25%[1]	0.5%*	1.5%*	2.5%*	3.5%*	PMH	0.5%	1.5%	2.5%	3.5%
***E. coli* KGPMF 16**	1.07±0.02	0.88±0.01	0.79±0-02	0.78±0.03	0.74±0.01	0.71±0.00	0.49±0.08	0.52±0.04	0.53±0.08	0.55±0.04
***E. coli* KGPMF 17**	1.07±0.02	0.89±0.01	0.80±0.00	0.78±.001	0.73±0.00	0.24±0.04	0.56±0.10	0.57±0.07	0.53±0.06	0.53±0.12
***E. coli* ATCC 25922**	0.94±0.02	1.10±0.01	1.01±0.05	0.99±0.03	0.87±0.02	0.37±0.00	0.51±0.03	0.52±0.05	0.51±0.04	0.54±0.04

Values are presented as mean ± standard deviation measured at 600 nm; [1] growth control with glucose;
* in TS with 0.25% of glucose, it was added 0.5%, 1.5%, 2.5% and 3.5% of lacose; PMHB- MHB without glucose or lactose (growth control).

In TSB containing different concentrations of lactose, at 37 °C the growth of *E. coli* KGPMF 16 and *E. coli* ATCC 25922 was reduced, while growth of *E. coli* KGPMF 17 was stimulated only in 0.5% of lactose. In MHB with different concentrations of lactose, at 37 °C the growth of *E. coli* KGPMF 17 was stimulated, while the growth of *E. coli* KGPMF 16 and *E. coli* ATCC 25922 were reduced. Only *E. coli* KGPMF 16 on 0.5% of lactose give the same value as in growth control.

In TSB containing lactose, at 44 °C the growth of E. coli KGPMF 16 and *E. coli* KGPMF 17 was reduced, while the growth of *E. coli* ATCC 25922 was stimulated in all tested concentrations, except in 3.5%. In MHB containing lactose, at 44 °C the growth of *E. coli* KGPMF 16 was reduced, while the growth of *E. coli* KGPMF 17 and *E. coli* ATCC 25922 was stimulated (Tab. 4).

Determination of antibiofilm activity

Pellicle test

E. coli KGPMF 16, and *E. coli* KGPMF 17 were tested on the ability to form pellicle in TSB and MHB at 37 °C. According to the results, *E. coli* KGPMF 16 and *E. coli* KGPMF 17 exhibited no ability to form pellicle in TSB or in MHB.

Biofilm formation

Bacteria isolated from traditionally made cheese were tested on the ability to form biofilm in two different media, at three temperatures (4 °C, 37 °C, 44 °C). After the incubation (48 h), it was noticed that none of the tested bacteria showed ability to form biofilm at 4 °C, 37 °C, 44 °C in MHB. It was also noticed that only *E. coli* KGPMF 17 demonstrated the ability to form biofilm in TSB at 37 °C.

The influence of different pH, NaCl, glucose and lactose concentrations on the biofilm formation and formed biofilm in TSB

On the biofilm formation of *E. coli* KGPMF 17, pH 5.5 showed stimulating effect, while in pH 6.5 and 8.5 biofilm was reduced. In pH 7, biofilm formation was equal to the biofilm formation of positive control. The growth of formed biofilm was stimulated in all pH values (Fig. 1).

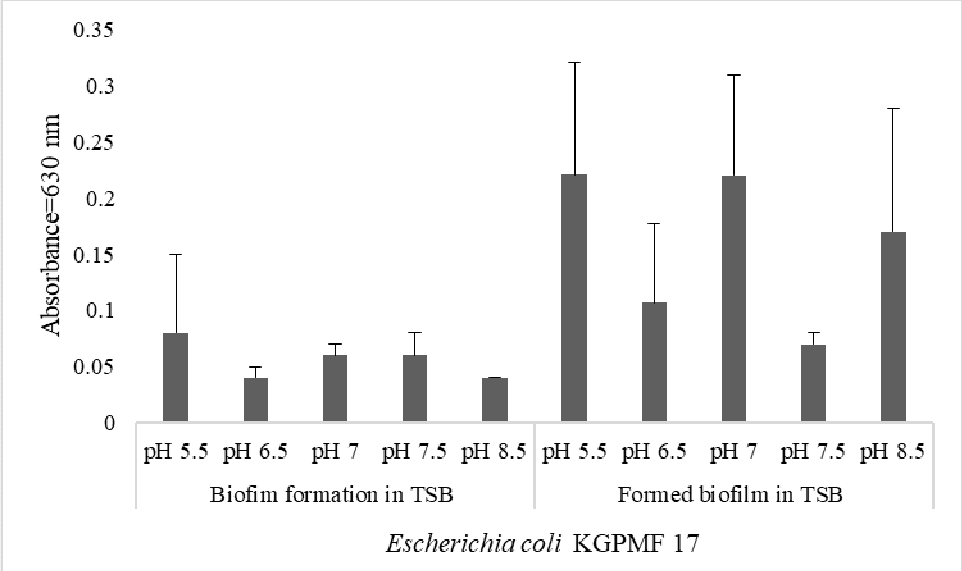

Figure 1. The influence of pH on the biofilm formation and formed biofilm (*growth control).

All concentrations of salt produced inhibitory effect on the biofilm formation of *E. coli* KGPMF 17, compared to the control (growth at 4%). All concentrations of salt demonstrated stimulating effect on the formed biofilm (Fig. 2).

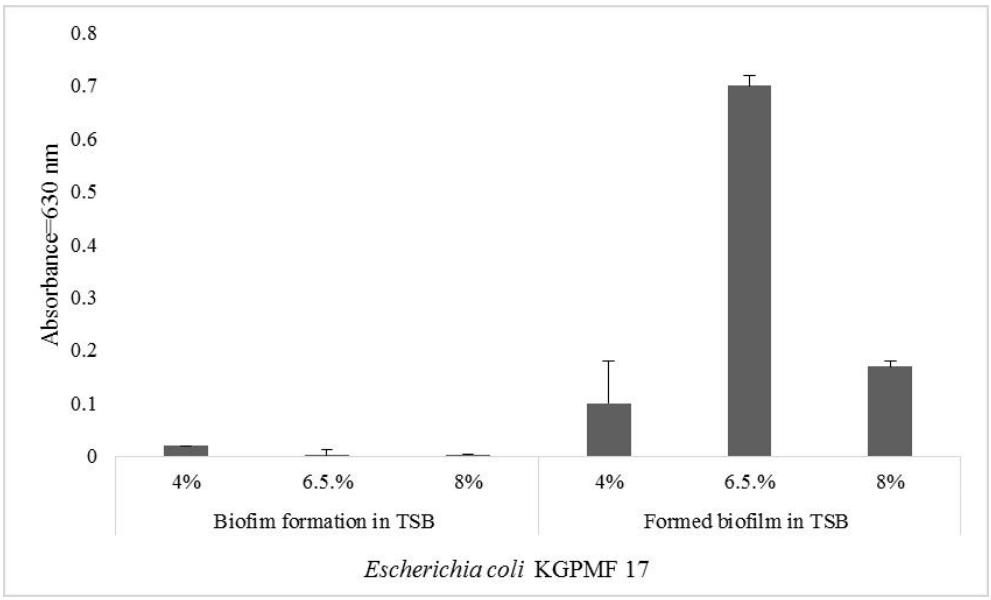

Figure 2. The influence of NaCl on the biofilm formation and formed biofilm (*growth control at 4%).

All tested concentrations of glucose showed inhibitory effect on the biofilm formation, except 0.5% glucose, where biofilm formation was equal to the growth control. 0.5% glucose demonstrated stimulating effect on the formed biofilm, 1.5% showed no influence on the formed biofilm, while higher concentrations of glucose demonstrated the ability to reduce the formed biofilm (Fig. 3).

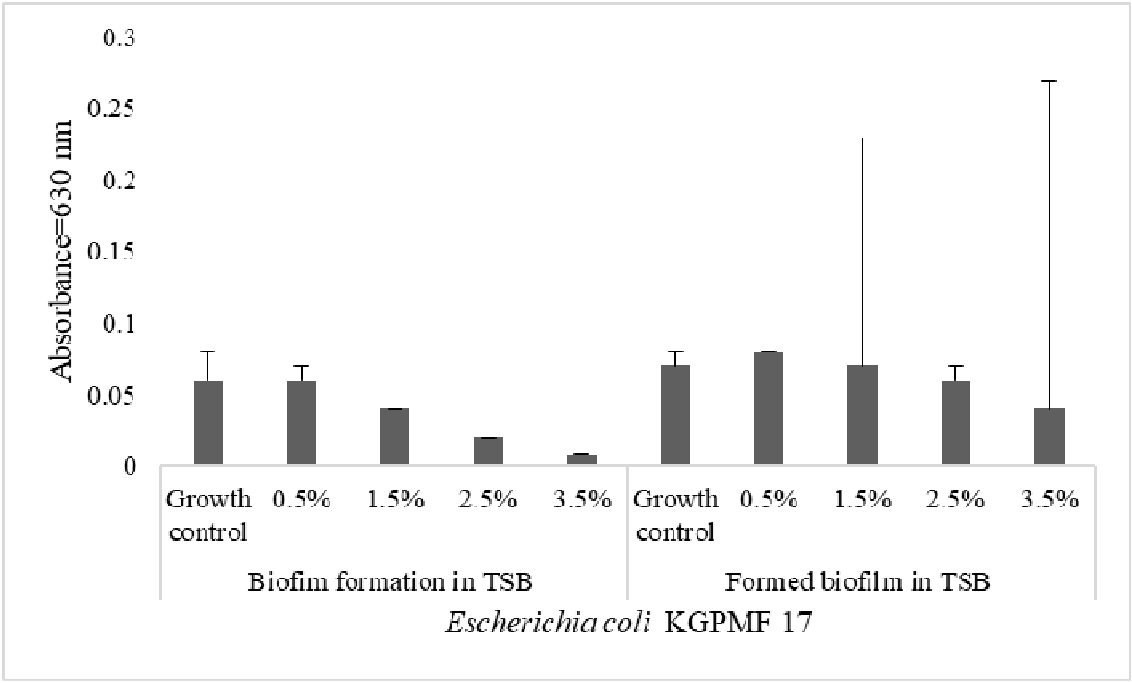

Figure 3. The influence of different concentrations of glucose on the biofilm formation and formed biofilm.

On the biofilm formation of *E. coli* KGPMF 17, 0.5% lactose, showed no influence. On the biofilm formation, 2.5% lactose demonstrated stimulating effect, while 1.5% and 3.5% lactose showed inhibitory effect on the biofilm formation. All concentrations of lactose showed stimulating effect on the formed biofilm (Fig. 4).

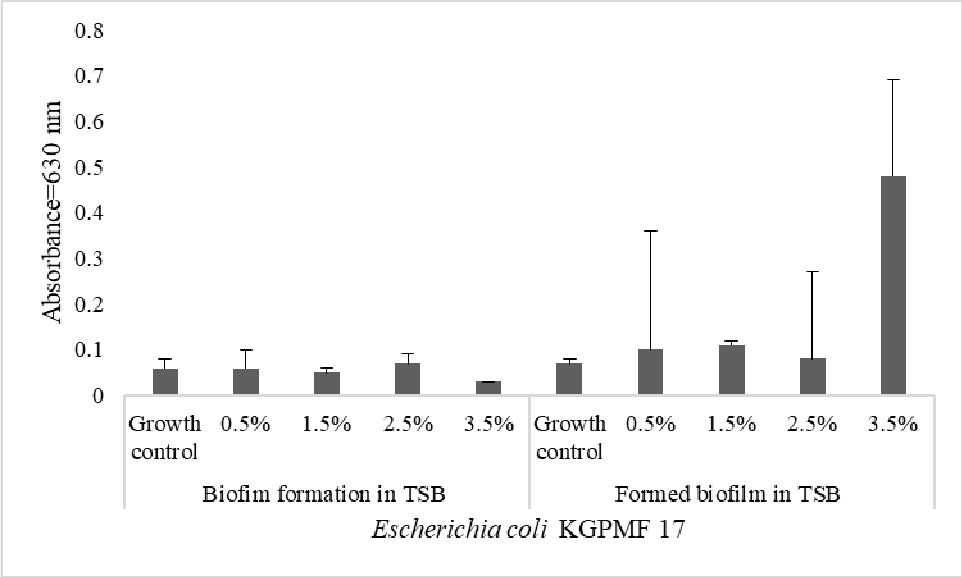

Figure 4. The influence of different concentrations of lactose on the biofilm formation and formed biofilm**.**

DISCUSSION

In this study, for the first time, the planktonic growth of *E. coli* KGPMF 16 and *E. coli* KGPMF 17 (isolated from Serbian cheese (Sokobanja region) made in a traditional way) was investigated.

It was noted that the growth was higher in TSB at 37 °C. The acidic medium was a limiting factor for the growth of *E. coli* KGPMF 16 and *E. coli* KGPMF 17, while the basic medium was more suitable for the growth. Salt was limiting factor for the planktonic growth of *E. coli* KGPMF 16 and *E. coli* KGPMF 17. Higher salt concentrations increased osmolarity of the medium, which led to hyper osmotic shock of *E. coli* cells causing the growth suppression. The significance of increasing the salt concentration of the medium for the growth of *E. coli* became more apparent when the temperature was raised from 37 °C to 44 °C. It was concluded that increasing the salt concentration in the medium partially surmounted the inhibition of growth of *E. coli* at high temperature. One of studies that examined the combined effect of salt and heat treatment on the bacterial growth were conducted (ABDULKARIM *et al.*, 2009). It was already established that high salt concentration, which increases osmolarity of the medium, also increasing the temperature, so both of this conditions limit the growth of bacteria (SCOTT, 1989; TROLLER, 1986). Based on the results, it could be concluded that the planktonic growth of tested bacteria was more stimulated in the presence of lactose than glucose, while different salt concentrations demonstrated reducing effect on the planktonic growth.

In our investigation, *E. coli* KGPMF 16 and *E. coli* KGPMF 17 demonstrated limited growth in TSB with different concentrations of glucose and lactose, at 37 °C and at 44 °C. In MHB with some concentrations of glucose and lactose, at 37 °C and at 44 °C, the growth was stimulated. The growth control was higher in the presence of lactose than in the presence of glucose, which was expected, due to the origin of these bacteria.

In vitro biofilm formation of *E. coli* is variable and depends on the growth conditions (REISNER *et al.,* 2006). Adding glucose to the medium at 37 °C is more significant for the growth than the addition of salts. Glucose provides a carbon source for the bacterial growth and metabolism. According KHANGHOLI and JAMALLI (2016) the influence of sugars and other factors on biofilm formation can depending on the type of bacteria. Sugars has no effect on the bacterial growth. The expression ycfR (multiple stress resistance protein BhsA precursor), which encodes the synthesis of the outer membrane protein, negatively influences biofilm formation of *E. coli*. Removing ycfR reduced the formation of the biofilm five times in the presence of glucose. The protein protects the cell from the various environmental conditions (ZHANG *et al*., 2007).

However, the addition of the certain concentration of glucose at 44 °C produced very slight effect on the growth (ABDULKARIM *et al.,* 2009). The differences in the production of biofilms under various environmental and nutritional conditions can be explained by the ability of some microorganisms to respond to external conditions, such as population density, limited nutrients, osmolarity, pH or composition of the medium. Bacteria in nature live far away from the optimal growth conditions. Therefore, the cell must have the ability to sense, integrate, and respond to a variety of stresses for survival (DRAGOSITS *et al*., 2013). Bacteria can activate genes responsible for the expression of surface proteins that enable the adhesion and production of EPS (extracellular polymeric substances), which are directly involved in the production of biofilms (FRANK *et al.,* 2007). It is very important to correctly understand metabolic regulation in response to stresses from environmental factors including protein expression, gene expression, and etc (SHIMIZU, 2014). According to TRÉMOULET *et al.* (2002), *E. coli* O157:H7 modified the expression of several proteins involved in biofilm growth mode.

CONCLUSION

Based on the results, it could be concluded that TSB was more suitable for the planktonic growth and biofilm formation of selected bacteria. All pH values produced stimulating effect on formed biofilm. All concentrations of salt exerted inhibitory effect on the biofilm formation of *E. coli* KGPMF 17, but demonstrated stimulating effect on the formed biofilm. All concentration of lactose demonstrated stimulating effect on the formed biofilm. Further studies need to include the investigation of additional environmental factors on the growth and biofilm formation of *E. coli* isolated from Sokobanja cheese.

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