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EFFECT OF STARVATION STRESS ON MORPHOLOGICAL CHANGES AND PRODUCTION OF ADHESIVE EXOPOLYSACCHARIDE (EPS) BY *PROTEUS VULGARIS*

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Background. *Proteus vulgaris* attach to available surfaces in industrial environments, can develop into extensive biofilm. Such bacterial layer is a potential source of contamination of foods that may lead to spoilage or transmission foodborne pathogens. The purpose of these investigations was to evaluate the influence of limited nutrients availability in the medium on the morphological changes and biosynthesis of bacterial surface-associated EPS by *P. vulgaris*. The relationship between the dimension of cells, EPS production and *P. vulgaris* biofilm development process on stainless steel surfaces (type 316L) was also examined.
Material and methods. *P. vulgaris* ATCC 6380 was used in this study. The cultures were incubated at 37°C on the *Enterobacteriaceae* enrichment broth according to Mossel [1962]. During the investigations the medium with optimal and 10 times diluted optimal of nutrient availability were used. For cells dimension analysis a Carl-Zeiss Axiovert 200 inverted microscope and a scanning electron microscope (LEO 435VP) was applied. Isolation of exopolysaccharides was based on the procedure employed by Forde and Fitzgerald [1999]. To determine the level of *P. vulgaris* adhesion to the surface of stainless steel, the method described by Le Thi et al. [2001] was used.
Results. In all experimental variants the area of *P. vulgaris* cells was changed upon long-term starvation. Altering of physical dimension of bacteria was effected by the decreasing value of the cell length. The change of *P. vulgaris* morphology promoted the beginning stages of biofilm formation process on the surface of stainless steel. Under starvation conditions *P. vulgaris* produced more EPS. It was observed with an increase of incubation period. These extracellular molecules initiated more advanced stages of *P. vulgaris* biofilm formation on examined surfaces.
Conclusion. The data support the notion that cellular factors influencing *P. vulgaris* adhesion process to abiotic materials should be examined under conditions in which marine bacteria are widely distributed. Analysis of both physical dimension of cells and EPS se-

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cretion by marine bacteria under starvation conditions will help to eradicate the attached bacteria.

Key words: *Proteus vulgaris*, starvation, biofilm, image analysis, exopolysaccharides

INTRODUCTION

Biofilm formation process on food contact surfaces can have detrimental effect on the microbial status of the food. The presence of biofilm on abiotic materials can contaminate the product through direct contact. As a consequence, there is an increased chance of food spoilage that may lead to reduced shelf life and an increase in the risk of food poisoning from pathogens [Gram et al. 2002, Fuster-Valls et. al. 2008]. Bacteria colonizing the processing equipments are extremely difficult to overcome. Biofilms can tolerate antimicrobial agents at concentrations of 10-1000 times that needed to inactivate genetically equivalent planktonic bacteria [Jefferson 2004]. A better understanding of bacterial adhesion process is needed for production of microbiologically safe and good quality products in the food industry.

In food processing plants abiotic materials are often colonized by Gram-negative bacteria that originally exist in aquatic environment (with limited nutrients availability) [Gram et al. 2002]. Among marine bacteria species, *Proteus vulgaris* is one of the most important food spoilage and human opportunistic microorganism [Różalski et al. 1997]. *P. vulgaris* may cause textural changes resulting in sensory rejection of fresh meat, poultry and seafood [Różalski et al. 1997, Kumar and Anand 1998]. These bacteria have also been described as etiological agents in urinary tract infections, as well as in gastroenteritis resulting from the consumption of contaminated food [Różalski et al. 1997].

The bacteria biofilm expansion process on food contact surface is due to morphological changes of the cells and to extracellular polysaccharide (EPS) production [Wai et al. 1999, Dunne 2002]. Changes in the physical dimensions of cells improved initial adhesion process to solid surfaces [Hood and Zottola 1997]. Pores and crevices at the abiotic materials increased the surface area available for cell contact. Moreover, bacteria located inside pores are sheltered from shear forces [Kumar and Anand 1998]. The production of exopolysaccharide is responsible for both adhesion and cohesion interactions and play a crucial role in maintaining structural integrity of mature biofilms [Sutherland 2001, Chen and Stewart 2002]. In some cases EPS can promote a preconditioning of surface, making the adhesion process more favourable [Dunne 2002].

The presence of *P. vulgaris* biofilm on food contact surfaces has not been eliminated yet. Most investigations have focused so far on the mechanisms determining the bacteria attachment process under optimal nutrients availability in the medium. These cultivation conditions do not correspond to natural environment, where *Proteus* spp. is widely distributed [Różalski et al. 1997]. Analysis of both physical dimension of cells and EPS secretion by marine bacteria under starvation conditions will help in the prevention of biofilm development process on solid materials [Bower et al. 1996].

The aim of this study was to define the influence of limited nutrients availability in the medium on the morphological changes and bacterial surface-associated EPS production by *P. vulgaris* cells. The relationship between the dimension of cells, EPS biosynthesis and *P. vulgaris* biofilm development process on stainless steel surfaces (type 316L) was also examined.

MATERIAL AND METHODS

Bacterial strains and growth conditions

P. vulgaris ATCC 6380 (American Type Culture Collection, Rockville, MD, USA) was used in this study. *P. vulgaris* is a straight gram negative rod, 0.5 μm in width and 1.5-5.0 μm in length. During the investigations the microorganisms were passaged three times after every 48 h on *Enterobacteriaceae* enrichment medium according to Mossel [1962]. The medium used in the work is included in official (ISO) standard for detecting *Enterobacteriaceae* in food products. In the study from each passage 10ml of inoculum of *P. vulgaris* was added to the fresh medium. The cultures were incubated at 35°C under shaking conditions (100 rpm/min) on the media with optimal and 10 times diluted of nutrients availability. The pH value of the culture medium at the beginning of incubation was 7. The incubation lasted in total 144 h.

Microscopic preparation for cells dimensions analysis

Microscopic preparations were carried out after 24, 72, 120 and 144 h of each experiment. The simple stain method with crystal violet was used. To avoid distortion of *P. vulgaris* cells, heat fixing process was not conducted. Images were captured using a Carl-Zeiss Axiovert 200 inverted microscope with a digital camera Carl-Zeiss AxioCam attached to a computer.

Cell dimensions analysis

Photographs were prepared from 30 randomly selected microscopic fields from each sample and examined using KS-300, Carl-Zeiss Soft. The image analysis steps included: image acquisition, image segmentation and measurement of the detected objects. Image acquisition enabled to create a 100 × 100 pixel numerical image, each pixel being coded into 256 gray levels. Image segmentation improved selection the objects of interest from the background. Measurements of the detected cells included cells area, cells length, cells width and width to length ratio.

Scanning electron microscopy

P. vulgaris morphology was also examined by a scanning electron microscopy. The bacteria were harvested by centrifugation at 3000 g for 20 min. The pelleted cells were mounted on the steel plates and fixed for 2 h in 2% glutaraldehyde (v/v) and 2% paraformaldehyde (v/v). After rinsing, the samples were dehydrated in 99.8% (v/v) ethanol. The plates were mounted on aluminum stubs and coated with gold-palladium. The samples were then examined in the scanning electron microscope (LEO 435VP) at an accelerating voltage of 5 kV [Arnold and Bailey 2000].

Isolation and quantification of bacterial surface-associated EPS

Isolation of exopolysaccharides was based on the procedure employed by Forde and Fitzgerald [1999]. The bacteria were harvested by centrifugation at 3000 g for 20 min

at the room temperature after 24, 48, 72, 96, 120 and 144 h of each experiment. The cells were resuspended in 1.5 ml of 30% (w/v) NaOH. Samples were boiled for 15 min, centrifuged at 15 000 g for 15 min and the supernatants fluid were added dropwise to 60% (v/v) ethanol. The total EPS (expressed as μg/CFU) was determined using acid hydrolysis method of Parkar et al. [2001]. The precipitated EPS was collected by centrifugation (15 000 g, 20 min) and resuspended in 1 ml of sterile water. The samples were mixed with 7 ml of 77% (v/v) H_2SO_4 and cooled for 10 min in an ice-bath. 1 ml of 1% (w/v) of cold tryptophan was added and the samples were heated in a boiling bath for 20 min to effect hydrolysis. The acid hydrolysis of EPS produced a furan which condenses with the tryptophan and forms a coloured product. This was evaluated after cooling the samples by measuring $O.D._{\cdot 500}$. Calibration curves were prepared against standard dextran (Mp. 40 000) solutions (Sigma, USA).

Stainless steel surface preparation

Stainless steel plates (type 316L) sized 1 cm × 6.5 cm × 1 mm was treated with 50% solution of HNO_3 for 10 min at 70°C. After soaking under distilled water the plates were put into glass containers and sterilized at 121°C for 15 min [Parkar et al. 2001].

Bacterial adhesion analysis

P. vulgaris adhesion analysis was started after 144 h in each experiments. The stainless steel plates were put into *P. vulgaris* cultures. At 145 h the plates were removed from the glass containers and washed with PBS solutions (pH 7.2) in order to remove unattached cells from the surfaces. The plates were stained with 0.01% solution of acridine orange (2 min at room temperature). For observation of bacteria adhering to the stainless steel surface a fluorescence microscope was used (Carl-Zeiss, Axiovert 200). To determine the level of *P. vulgaris* adhesion to the surface of stainless steel the method proposed by Le Thi et al. [2001] was used. This technique is based on the estimation of randomly selected 50 visual fields according to a 9-degrees scale:

1st degree: from 0 to 5 bacteria in visual field.
2nd degree: from 5 to 50 bacteria in visual field.
3rd degree: only single bacteria (above 50 bacteria cells in visual field); no microcolonies.
4th degree: single bacteria cells + small microcolonies.
5th degree: large but not confluent microcolonies + single bacteria cells.
6th degree: confluent microcolonies + single bacteria cells.
7th degree: 1/4 visual field covered by the biofilm.
8th degree: 1/2 visual field covered by the biofilm.
9th degree: visual field totally covered by the biofilm.

The classification procedure excluded bacteria situated on the edges of each visual field.

Statistical analysis

Presented results are the average of three independent experiments. Effect of different nutrients availability in the medium on *P. vulgaris* morphology was analysed using one-way ANOVA with *post-hoc* comparison (Tukey's test; program Statistica).

RESULTS

P. vulgaris morphology

To characterise the ability of *P. vulgaris* to accumulate on microroughness of the abiotic surface under starvation conditions, the cells dimension analysis were carried out. *P. vulgaris* dimension at different nutrients availability in the medium is shown in Table 1. Scanning electron micrographs of *P. vulgaris* cells on stainless steel surface upon nutrionally favourable and starvation conditions are presented in Figure 1. *P. vulgaris* cells alter their morphology in response to nutrients deprivation. In the first 72 h of the process, the area of examined cells equaled between 0.43 μm^2 and 0.50 μm^2. From 120 h of cultivation the cell area was significantly decrease to the level of 0.31 μm^2. The effect of change in *P. vulgaris* area upon starvation depends on shortening of the cell length from 0.94 μm to 0.79 μm. The width to length ratio indicates that *P. vulgaris* starved of nutrients form swollen cells. In contrast, favourable conditions induced a non-detectable or a small change in the examined cells morphology. During the cultivation process, mean values of *P. vulgaris* area, cells width and cells length equalled: 0.57 μm^2, 0.65 μm and 1.20 μm respectively.

Table 1. *P. vulgaris* dimension under different nutrients availability in the medium

Time of incubation h	Nutrients availability in the culture medium							
	optimal				reduced			
	observed and calculated mean cell dimensions							
	area μm^2	width μm	length μm	width to length ratio	area μm^2	width μm	length μm	width to length ratio
24	0.61^a ±0.01	0.66^a ±0.02	1.27^a ±0.02	0.52^a ±0.07	0.43^a ±0.01	0.64^a ±0.09	0.94^a ±0.01	0.70^a ±0.07
72	0.55^b ±0.01	0.66^a ±0.07	1.17^b ±0.02	0.56^a ±0.09	0.50^b ±0.01	0.59^a ±0.06	1.18^b ±0.02	0.50^b ±0.08
120	0.61^a ±0.02	0.65^a ±0.08	1.25^a ±0.02	0.54^a ±0.08	0.31^c ±0.09	0.57^a ±0.08	0.79^c ±0.01	0.75^a ±0.09
144	0.52^c ±0.01	0.63^a ±0.06	1.14^b ±0.02	0.56^a ±0.09	0.32^c ±0.09	0.58^a ±0.07	0.79^c ±0.01	0.75^a ±0.09

Average values ±standard deviations.
Values followed by different letters in columns differ significantly at the $P < 0.05$ level.

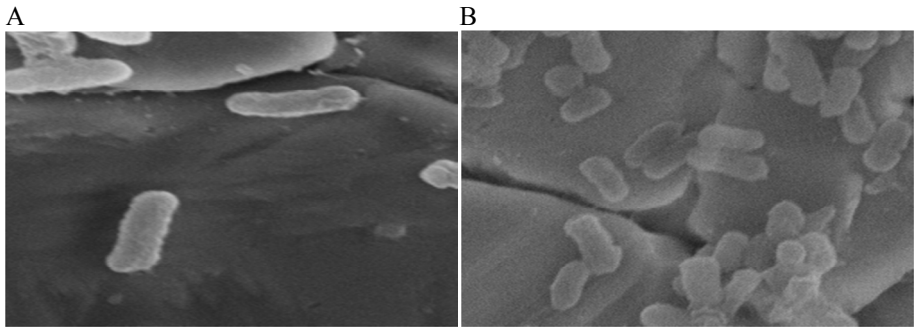

Fig. 1. Scanning electron micrographs of *P. vulgaris* cells on stainless steel surface (316L) upon different nutrients availability in the medium, ×10 000: A – optimal nutrients availability, B – reduced nutrients availability

Quantitative determination of bacterial surface-associated EPS

To define the potential of *P. vulgaris* cells to form mature biofilm structure on abiotic surfaces, the quantitative determination of bacterial surface-associated EPS was conducted. Figure 2 presents the EPS production capacity of *P. vulgaris* at different nutrients availability in the medium. *P. vulgaris* synthesized more EPS with an increased incubation period upon starvation. In the first 24 h of the experiment, the EPS production by examined bacteria was not higher than 10 μg/10^9 CFU. At 48 h of incubation the EPS synthesis was significantly increased to the value of 140 μg/10^9 CFU. From 120 h of the cultivation the EPS secretion by *P. vulgaris* remain relatively constant at the level of 125 μg/10^9 CFU. Upon nutrient-rich conditions, the maximum EPS production by *P. vulgaris* (105 μg/10^9 CFU) was observed in 48 h of the process. From 72 h the EPS synthesis decreased to the level of 18 μg/10^9 CFU.

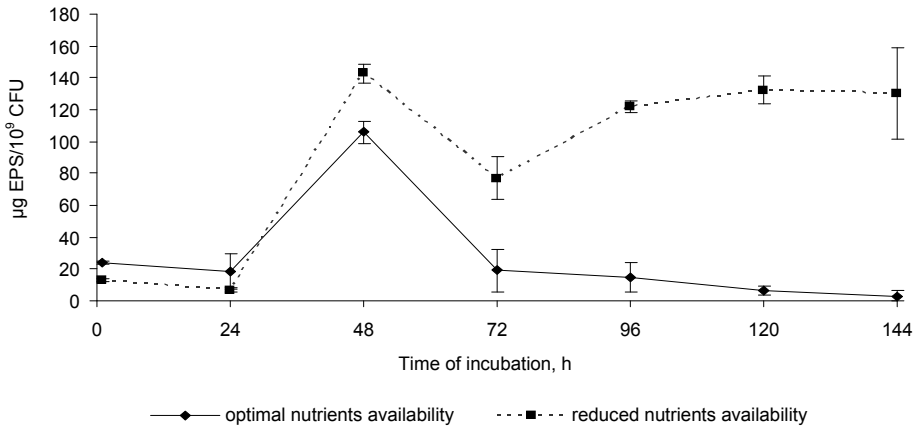

Fig. 2. EPS synthesis by *P. vulgaris* under different nutrients availability in the medium (error bars represent standard deviation)

Adhesion

In the study to define the rate of *P. vulgaris* biofilm development process, the 9--degree scale according to Le Thi et al. [2001] was used. The results of the influence of nutrients availability on the attachment of *P. vulgaris* cells to stainless steel (type 316L) are presented in Table 2. In the work, the adhesion analysis started when *P. vulgaris* alter their morphology and produced the high amount of EPS upon starvation conditions. Approximately 10^5-10^6 CFU/ml *P. vulgaris* cells were present in the culture medium during the experiments.

Table 2. The influence of nutrients availability on *P. vulgaris* adhesion to stainless steel surfaces (type 316L)

Time of incubation h	Nutrients availability in the culture medium					
	optimal			reduced		
	cell number in the medium CFU/ml	dominant adhesion degrees	appearance of higher adhesion degrees (6, 7, 8, 9)	cell number in the medium CFU/ml	dominant adhesion degrees	appearance of higher adhesion degrees (6, 7, 8, 9)
145	1.5×10^6	1, 2	–	8.2×10^5	3, 4	6

In this study when a particular degree of adhesion occurred with a minimum amount of 20% that degree became the dominant one. Stainless steel was efficiently colonized by *P. vulgaris* upon nutrients limited conditions. Starvation conditions induced more advanced stages of examined bacteria biofilm formation process on the surface of stainless steel (6^{th} adhesion degree). *P. vulgaris* grown under nutrient-rich conditions colonized the stainless steel surface at the 1^{st} and 2^{nd} adhesions degree and no developed stages of adhesion (6^{th}-9^{th} degrees) was noticed in this work.

DISCUSSION

The microbiological attachment to solid surfaces is a multi-step process. To predict the rate of biofilm formation process on abiotic materials under starvation conditions, both morphology and bacterial surface-associated EPS production by marine bacteria must be accounted for. In this work we aimed to evaluate the influence of limited nutrients availability in the medium on the morphological changes of *P. vulgaris* cells. According to Wai et al. [1999] and Shau-Yan et al. [2009], altering of physical dimension of cells is favoured by nutrients deprivation. However, in the literature very little information is available on this starvation-induced mechanism. Nutrient limited condition represents the natural aquatic habitat of *P. vulgaris* cells [Różalski et al. 1997].

Under starvation conditions rod-shaped bacteria, may change their size and become coccoid [Shau-Yan et al. 2009]. In our study, long-term starvation decreased the *P. vulgaris* area. Altering of physical dimension of *P. vulgaris* depended on shortening of the cell length. Haznedaroglu et al. [2008] noticed similar effects when monitoring *Escherichia coli* morphology upon starvation. According to this study, the change of *Escherichia coli* area was effected by the decreasing value of the cell length [Haznedaroglu

et al. 2008]. Siegele and Kolter [1992] reported that changes in cell size and shape are accompanied by changes in the subcellular compartments; cytoplasm is condensed and the volume of the periplasm increases. The change of marine bacteria morphology is believed to be a means of minimizing the requirements for cells maintenance and protects non-spore-forming bacteria against environmental stresses [Chaiyanan et al. 2007].

In this work, the EPS synthesis by *P. vulgaris* cells during growth on low nutrients availability in the medium was also investigated. In the food industry and clinical conditions, EPS-rich strains are difficult to overcome [Bower et al. 1996]. Fuster-Valls et al. [2008] reported that EPS surrounding the bacteria protect the cells from the effects of antimicrobial agents. This feature of microorganisms may seriously affect the quality and safety of the processed food and suppose a potential risk to patients [Dunne 2002]. In our work, EPS synthesis was affected by the increasing incubation period. The highest yield of EPS production by examined bacteria was observed after 120 h of cultivation process. Also Kiliç and Dönmez [2008] noticed that long-term starvation influenced higher productivity of EPS by marine bacteria. The highest production of extracellular matrix by the examined cells was observed after incubation of 96 h [Kiliç and Dönmez 2008]. Siegele and Kolter [1992] and Dunne [2002] concluded that extensive production of exopolysaccharides by marine bacteria is a starvation-induced mechanism. It helps in trapping and retaining the nutrients by the cells from surrounding environments [Siegele and Kolter 1992, Dunne 2002].

Researchers have concluded that low-nutrient environments may enhance adherence [Hood and Zottola 1997]. The main biofilm expansion is due to bacterial morphology and to extracellular polysaccharide production [Dunne 2002]. These features help the microorganisms become more closely associated with a surface where nutrient accumulation can take place [Bower et al. 1996]. In the work the adhesion analysis started when *P. vulgaris* alter their morphology and produced the high amount of EPS upon starvation conditions. This knowledge may improve the elimination the particular pathogenic and spoilage promoting mechanisms [Gram et al. 2002]. In this study, starvation conditions increased the adhesion of *P. vulgaris* to abiotic surfaces. Our study performed that changes in marine bacteria morphology are required for the first step in biofilm formation process (3^{rd} adhesion degree). According to Haznedaroglu et al. [2008] the morphological changes of cells affect microbial penetration in porous surface. Altering of physical dimension of cells also helps optimize interactions between cells and the surfaces to which they attach [Young 2006]. In our study we observed that high quantities of EPS are needed to develop a true biofilm matrix (6^{th} adhesion degree). Similar effects were noticed for a wild type of *Pseudomonas fluorescens* and a nonpolysaccharide-producing mutant [Allison and Sutherland 1987]. Both bacteria adhered to a glass surface, but over time, the wild strains formed three-dimensional structure while the mutant remained as single adherent cells on the surface.

CONCLUSION

Our data support the notion that cellular factors influencing *P. vulgaris* adhesion process to abiotic materials should be examined under conditions in which marine bacteria are widely distributed. In response to nutrient limitation in the medium the size of *P. vulgaris* cells was changed. Altering of physical dimension of bacteria was effected by the decreasing value of the cell length. This feature is required for only the beginning stages of biofilm formation process under starvation conditions. Extensive production of

EPS molecules by *P. vulgaris* cells under long-term starvation has greater importance in advanced stages of cells attachment process on the examined materials.

REFERENCES

Allison D.A., Sutherland I.W., 1987. The role of exopolysaccharide in adhesion of freshwater bacteria. J. Gen. Microbiol. 133, 1319-1327.

Arnold J.W., Bailey G., 2000. Surface finishes on stainless steel reduce bacterial attachment and early biofilm formation: scanning electron and atomic force microscopy study. Poultry Sci. 79, 1839-1845.

Bower C.K., McGuire J., Deaschel M.A., 1996. The adhesion and detachment of bacteria and spores on food-contact surfaces. Trends Food Sci. Technol. 7, 152-157.

Chaiyanan S., Grim C., Maugel T., Huq A., Colwell R.R., 2007. Ultrastructure of coccoid viable but non-culturable *Vibrio cholerae*. Environ. Microbiol. 9, 393-402.

Chen X., Stewart P.S., 2002. Role of electrostatic interactions in cohesion of bacterial biofilms. Appl. Microbiol. Biotechnol. 59, 718-722.

Dunne W.M., 2002. Bacterial adhesion: seen any good biofilms lately? Clin. Microbiol. Rev. 15, 155-166.

Forde A., Fitzgerald G.F., 1999. Annalysis of exopolysaccharide (EPS) production mediated by the bacteriphage adsorption blocking plasmid, pCI658, isolated from *Lactococcus lactis* ssp. *Cremoris* HO2. Int. Dairy J. 9, 465-472.

Fuster-Valls N., Hernández-Herrero M., Marin-De-Mateo M., Rodríguez-Jerez J.J., 2008. Effect of different environmental conditions on the bacterial survival on stainless steel surfaces. Food Control. 19, 308-314.

Gram L., Ravn L., Rasch M., Bruhn J.B., Christensen A.B., Givskov M., 2002. Food spoilage – interactions between food spoilage bacteria. Int. J. Food Microbiol. 78, 79-97.

Haznedaroglu B.Z., Bolster C.H., Walker S.L., 2008. The role of starvation on *Escherichia coli* adhesion and transport in saturated porous. Water Res. 42, 1547-1554.

Hood S.K., Zottola E.A., 1997. Adherence to stainless steel by foodborne microorganisms during growth in model food systems. Int. J. Food Microbiol. 37, 145-153.

Jefferson K.K., 2004. What drives bacteria to produce a biofilm? FEMS Microbiol. Lett. 236, 163-173.

Kiliç N.K., Dönmez G., 2008. Environmental conditions affecting exopolysaccharide production by *Pseudomonas aeruginosa*, *Micrococcus* sp., and *Ochrobacterium* sp. J. Hazard Mater. 154, 1019-1024.

Kumar G.C., Anand S.K., 1998. Significance of microbial biofilms in food industry: a review. Int. J. Food Microbiol. 42, 9-27.

Le Thi T.T., Prigent-Combaret C., Dorel C., Lejeune P., 2001. First stages of biofilm formation: characterization and quantification of bacterial functions involved in colonization process. Met. Enzymol. 336, 152-159.

Mossel D.A.A., 1962. Use of a modified MacConkey agar medium for the selective growth and enumeration of all *Enterobacteriaceae*. J. Bacteriol. 84, 381-386.

Parkar S.G., Flint S.H., Palmer J.S., Brooks J.D., 2001. Factors influencing attachment of thermophilic bacilli to stainless steel. J. Appl. Microbiol. 11, 675-685.

Różalski A., Sidorczyk Z., Kotełko K., 1997. Potential virulence factors of *Proteus* bacilli. *Microbiol.* Mol. Biol. Rev. 61, 65-89.

Shau-Yan C., Wann-Neng J., Yi-Shin C., Hin-Chung W., 2009. Morphological changes of *Vibrio parahaemolyticus* under cold and starvation stresses. Int. J. Food Microbiol. 129, 157-165.

Siegele D.A., Kolter R., 1992. Life after log. J. Bacteriol. 174, 345-348.

Sutherland I.W., 2001. The biofilm matrix – an immobilized but dynamic microbial environment. Trends Microbiol. 9, 222-227.

Young K.D., 2006. The selective value of bacterial shape. Microbiol. Mol. Biol. Rev. 70, 660-703.

Wai S.N., Mizunoe Y., Yoshida S., 1999. How *Vibrio cholerae* survive during starvation. FEMS Microbiol. Lett. 180, 123-131.

WPŁYW STRESU GŁODOWEGO NA ZMIANY MORFOLOGICZNE I BIOSYNTEZĘ ADHEZYJNYCH EGZOPOLISACHARYDÓW (EPS) PRZEZ *PROTEUS VULGARIS*

Wstęp. W warunkach przemysłowych adhezja *Proteus vulgaris* do powierzchni stałych rozpoczyna proces tworzenia się biofilmów. Powstawanie błon bakteryjnych stwarza ryzyko zanieczyszczenia żywności drobnoustrojami, powodującymi zepsucie organoleptyczne produktu, oraz mikroorganizmami chorobotwórczymi. Celem badań była ocena wpływu ograniczonej dostępności składników odżywczych w środowisku wzrostu na zmiany morfologiczne i biosyntezę EPS, związanych z powierzchnią komórki *P. vulgaris*. Oceniono również zależność pomiędzy morfologią komórek, biosyntezą EPS i tworzeniem się biofilmów *P. vulgaris* na powierzchni stali nierdzewnej (typ 316L).
Materiał i metody. W pracy wykorzystano gatunek *P. vulgaris* ATCC 6380. Hodowlę drobnoustrojów prowadzono w temperaturze 37°C na podłożu namnażająco-wybiórczym, zaproponowanym przez Mossel [1962]. W doświadczeniach zastosowano podłoża o optymalnej i 10-krotnie zredukowanej dostępności składników odżywczych. W badaniach nad właściwościami morfologicznymi komórek wykorzystano mikroskop odwrócony (Carl-Zeiss, Axiovert 200) oraz skaningowy mikroskop elektronowy (LEO 435VP). Do izolacji egzopolisacharydów bakteryjnych zastosowano metodę, którą opracowali Forde i Fitzgerald [1999]. Dynamikę procesu adhezji *P. vulgaris* do powierzchni stali nierdzewnej oceniano na podstawie metody, którą zaproponowali Le Thi i in. [2001].
Wyniki. We wszystkich wariantach doświadczenia zaobserwowano zmianę powierzchni komórek *P. vulgaris* pod wpływem stresu głodowego. Zmiana wymiarów komórek wynikała ze zmniejszania się długości drobnoustrojów. Zmiany morfologiczne *P. vulgaris* promowały początkowe etapy tworzenia biofilmów na powierzchni stali nierdzewnej. W warunkach głodowych zaobserwowano intensyfikację biosyntezy EPS przez *P. vulgaris*. Było to szczególnie widoczne w końcowych etapach procesu hodowlanego. Biosynteza zewnątrzkomórkowych polisacharydów inicjowała tworzenie się dojrzałych matryc biofilmu na testowanych powierzchniach stałych.
Podsumowanie. Wyniki przeprowadzonych doświadczeń wskazują, że czynniki wpływające ze strony komórek *P. vulgaris* na proces adhezji drobnoustrojów do powierzchni abiotycznych powinny być badane w warunkach, w których bakterie wodne występują naturalnie. Badania nad zmianami morfologicznymi oraz biosyntezą EPS przez bakterie wodne w warunkach głodowych mogą usprawnić procedury eliminacji błon biologicznych z powierzchni stałych.

Słowa kluczowe: *Proteus vulgaris*, warunki głodowe, biofilm, cyfrowa analiza obrazu, egzopolisacharydy

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