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INVESTIGATION OF BIOFILM FORMATION ABILITY OF COAGULASE-NEGATIVE STAPHYLOCOCCI ISOLATED FROM READY-TO-EAT MEAT

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ABSTRACT

The aim of the study was to analyze the biofilm production capacity of coagulase-negative staphylococci (CNS) isolated from ready-to-eat meat products including pork ham, chicken cold cuts, pork sausage, salami and pork luncheon meat, sliced in the store to the consumer's specifications, along with determination of occurrence of the adhesion genes determining the polysaccharide production (icaA and ica) and collagen binding protein (cna). The isolates investigated included *Staphylococcus equorum* (28%), *S. vitulinus* (16%), *S. carnosus* (14%), *S. succinus* (11%), *S. xylosus* (11%), *S. saprophyticus* (9%), *S. warneri* (9%), *S. haemolyticus* (1%) and *S. pasteuri* (1%). The assessment of biofilm production capacity by staphylococci was made using crystal violet, whereas evaluation of the frequency of occurrence of genes was performed using the PCR. Among 81 CNS included in the current study, 84% showed ability to form biofilm in the experimental *in vitro* conditions. High biofilm capacity was demonstrated in 54% of strains, the average in 14%, and low in 16%, while the lack of biofilm production capacity was found in 16% of the tested strains. The *ica*A and *ica*D genes responsible for the production of extracellular polysaccharide adhesins were detected in 5% and 16% of strains respectively. The gene determining the formation of collagen binding protein (cna) was detected in 14% of strains. From the results obtained, it can be concluded that food is a source of coagulase-negative staphylococci capable of forming biofilm, which is referred to as clinically important virulent factor of these bacteria.

Key words: meat products, biofilm, coagulase-negative staphylococci

INTRODUCTION

In the course of evolution, bacteria living in diverse environments have developed mechanisms that allow them to adhere to abiotic and biotic surfaces and to produce biofilms. Biofilm formation allows bacterial cells to have easier access to food substrates and protection against the harmful effects of external factors. Biofilm is a structure that stands out in the natural environment because of its heterogeneous structure, genetic diversity and the complexity of interactions occurring inside it [Kołwzan 2011]. A particularly important feature of the biofilm is the resistance of this structure to routinely used antimicrobials e.g. disinfectants and antibiotics. For this reason, bacteria in biofilm are difficult to eradicate from the surface [Simões et al. 2010]. The production of bacterial biofilm is a complex and multistep process starting with attachment to a surface then formation of microcolony that leads to the formation of three dimensional structure and finally ending with detachment and defragmentation. Numerous proteins, polysaccharides and enzymes synthesized by biofilm-producing microorganisms are involved in this process [de Oliveira 2016]. Coagulase-negative staphylococci (CNS), which are part of the microflora of skin and mucous membranes of animals and humans, are also commonly isolated from food and environmental samples. In food processing, CNS are used as starter cultures for the production of ripening food, e.g.

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cheese and fermented meat products. Their enzymatic activity influences the sensory characteristics of the product desired by the consumer [Irlinger 2008]. Among CNS, species most commonly found in fermented foods include: *S. xylosus*, *S. carnosus*, *S. equorum*, *S. vitulinus*, *S. succinus* and *S. warneri* [Irlinger 2008; Landeta et al. 2013]. For many years, CNS have been considered as saprophytes that have no impact on human health. However, this conviction has changed after the 1970, when number of studies have been carried out to show that this group of staphylococci can cause dangerous infections, especially in immunocompromised patients [Piette and Verschraegen 2009, Becker et al. 2014].

In recent years, species such as *S. equorum*, *S. succinus*, *S. pettenkoferi*, *S. warneri*, S. saphrophyticus, *S. haemolyticus*, *S. xylosus*, *S, pasteuri* and especially *S. epidermidis* were isolated from clinical specimens collected from humans [Nováková et al. 2006, Irlinger 2008 Piette and Verschraegen 2009, Becker et al. 2014]. There are also clinical data on the development of mastitis in dairy cattle and sheep by CNS strains [Taponen and Pyörälä 2009, Srednik et al. 2015]. Furthermore, various species of CNS have been isolated from clinical materials, which until now have been isolated only from foods or which have traditionally been considered as starter cultures of fermented products [Nováková et al. 2006, Irlinger 2008, Piette and Verschraegen 2009, Becker et al. 2014]. Food due to its ntritional value is an excellent substrate for the growth of microorganisms. It is recognized that food of animal origin is one of the major way of bacterial transmission between animals and humans. Food is an excellent way of transmitting pathogenic microorganisms to humans, especially if its preparation for consumption requires contact with hands or if it constitutes food ready for consumption without any thermal treatment beforehand [Podkowik 2013]. Potentially pathogenic CNS, which are part of the microflora of the skin and mucous membranes of humans and animals, can be found in food due to failure to maintain proper hygiene of raw material during its processing, transport or portioning of the finished product at the customer's request (through contact with the working surfaces, hands of workers or by air). Such food contaminated with potentially pathogenic CNS may pose a threat to human health [Bhatia and Zahoor 2007, Kadariya et al. 2014, Korpysa-Dzirba et al. 2012, Podkowik 2013, Podkowik et al. 2015]. Virulence factors produced by CNS include various bacterial cell components and number of substances produced extracellularly. Biofilm increase the invasiveness prosperities of CNS and their ability to cause infection [Becker et al. 2014, van Meervenne et al. 2014]. The components of the biofilm activate macrophages and stimulate the secretion of IL-1, IL-6 and TNF-α. In the course of the infection in which the biofilm is involved, the processes of phagocytosis, opsonization, chemotaxis and the blastogenesis of B and T cells are inhibited [Nowicka et al. 2012]. Polysaccharide intracellular adhesin (PIA), which is a part of the biofilm produced, participates in cell-cell communication and protection of bacterial cells against defense mechanisms of the host immune system [Moryl 2015]. Except to biofilm, CNS virulence factors include also various enterotoxins and enzymes such as hemolysins, fibrinolysins, gelatinases, DNAases, proteases, esterases and lipases [Nowicka et al. 2012].

The aim of the current study was to analyze the biofilm production capacity of CNS isolated from ready-to-eat meat products including pork ham, chicken cold cuts, pork sausage, salami and pork luncheon meat, sliced in the store to the consumer's specifications, along with determination of occurrence of the adhesion genes determining the polysaccharide production (*ica*A and *ica*D) and collagen binding protein (*cna*).

MATERIAL AND METHODS

Microorganisms

The research was carried out using 81 CNS strains isolated from ready-to-eat meat products including pork ham, chicken cold cuts, pork sausage, salami and pork luncheon meat, sliced in the store to the consumer's specifications. Isolates belonged to 12 species (number of isolates): *S. equorum* (21), *S. carnosus* (10), *S. vitulinus* (10), *S. succinus* (9), *S. saprophyticus* (8), *S. xylosus* (8), *S. warneri* (7), *S. lentus* (4), *S. epidermidis* (1), *S. haemoly-*

Table 1. Sequences of primers used for PCR reaction

Tabela 1. Sekwencje starterów użyte w badaniach

Gene Gen	Sequence Sekwencja	Product size, bp Wielkość produktu, pz	Annealing temperature Temperatura przyłączania	Reference Źródło
*ica*A	5′ACACTTGCTGGCGCAGTCAA3′ 5′TCTGGAACCAACATCCAACA3′	188	56°C	Szweda et al. 2012
*ica*D	5′ATGGTCAAGCCCAGACAGAG3′ 5′AGTATTTTCAATGTTTAAAGCAA3′	198	56°C	Szweda et al. 2012
cna	5'AATACAAAATGGTGACACGA3' 5'CTTGTGGAATTGTTACATCA3'	605	56°C	Ote et al. 2011

ticus (1), *S. pasteuri* (1), *S. pettenkoferi* (1) [Fijałkowski et al. 2016].

Detection of *ica*A, *ica*D and *cna* genes

The frequency of *ica*A and *ica*D genes determining the synthesis of intercellular polysaccharide adhesin and *cna* gene, which is responsible for the production of collagen binding protein, was determined using PCR. The primer sequences are presented in Table 1.

The composition of the reaction mixture used to perform the PCR reaction for all the analyzed genes was as follows: 6.5 µl of PCR Master Mix Plus High GC (A&A Biotechnology, Poland) 0.2 mM of each primer (10 mM), 1 µl of DNA, sterile deionized water to make up the volume of the reaction mixture equals 12,5 µl. The following PCR conditions were used: for *ica*A and *ica*D genes – 94°C for 4 min, 35 cycles: 94°C for 30 s, 56°C for 30 s and 72°C for 1 min, final elongation at 72°C for 10 min, and for *cna* gene – 94°C for 5 min, 35 cycles: 94°C for 30 s, 56°C for 30 s and 72°C for 40 s, final elongation at 72°C for 7 min (Ote et al. 2011; Szweda et al. 2012). PCR products were characterized by 1.5% agarose gel (peqGOLD, Peqlab, Germany) electrophoresis in 1× Tris-borate-EDTA (TBE) buffer (Bio-Rad, USA), followed by staining with ethidium bromide (Merck, Germany), visualization under UV light and analysis using GeneTools software (Syngene, UK).

As a positive control for the *ica*A and *ica*D genes *S. aureus* ATCC 6538 was used, while *Staphylococcus aureus* ATCC 25923 for the *cna* gene.

Phenotypic assessment of biofilm production ability

200 µl of a 24-hour bacterial culture in TSB medium (Oxoid) with 2% glucose was introduced into the wells of the 96-well plate. The plate was incubated for 48 hours at 37°C. After incubation, culture medium was removed, the wells of the plate were washed with sterile 0.85% sodium chloride solution, and biofilm was stained with 1% crystal violet solution for 10 min. The wells were again rinsed twice with sterile 0.85% sodium chloride solution and crystalline violet absorbed by biofilm was dissolved in 95% ethanol. The absorbance was read at 570 nm using microplate reader (Infinite® 200 PRO, Tecan, Switzerland).

Depending on the absorbance, the bacteria were classified as: having a high biofilm production capacity (OD $\geq$ 0.300), average biofilm production (OD of 0.200–0.299), low biofilm production capacity (OD of 0.199–0.100), lack of ability to produce biofilms (OD <0.100) [Solati et al. 2015]. As a positive control *Staphylococcus aureus* subsp. *aureus* ATCC 6538 was used.

Resistance to methicillin

Antibiotic susceptibility to methicilin was examined according to the guidelines of the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2016) with cefoxitin (30 g) disk (Biomaxima, Poland). *S. aureus* ATCC 25923 (MSSA, Oxoid) and *S. aureus* ATCC 43300 (MRSA, Oxoid) were used as a control.

RESULTS

Ability to produce biofilms

It was found that among all tested strains, 84% of them showed the ability to form biofilms on polystyrene titration plates. High ability to form biofilm was demonstrated in 54% of strains, the average in 14%, and low in 16%, while the lack of ability to produce biofilm was found in 16% of all strains (Table 2).

All strains belonging to the species *S. carnosus*, *S. epidermidis*, *S. haemolyticus*, *S. pasteuri*, *S. pettenkoferi*, *S. succinus* and *S. xylosus* showed the ability to form biofilms (Fig. 1). Among the remaining species, the percentage of strains with ability to form biofilm was lower and was 86% for *S. warneri*, 76% for *S. equorum*, 75% for *S. saprophyticus*, 75% for *S. lentus* and 60% for *S. vitulinus*

All analyzed food products contained strains with the ability to form biofilms. All strains isolated from pork ham, salami and pork sausage demonstrated the ability to form biofilms. The percentage of strains with ability to form biofilm from other food products was lower and equaled 83% for chicken cold cuts and 71% for pork luncheon meat.

Determination of the incidence of *ica*A, *ica*D and *cna* genes

A large variation in the occurrence of the *ica*A, *ica*D and *cna* genes has been demonstrated (Fig. 2, Table 3). The presence of the *ica*A gene was found in four (5%) of the isolates belonging to the species *S. carnosus*, *S. equorum*, *S. pettenkoferi* and *S. succinus*. All isolates that showed the presence of the *ica*A gene were also positive for the presence of the *ica*D and *cna*. The *ica*D gene was detected in 13 (16%) out of 81 analyzed isolates belonging to the 8 species: *S. carnosus*, *S. equorum*, *S. lentus*, *S. pettenkoferi*, *S. saprophyticus*, *S. succinus*, *S. vitulinus* and *S. warneri*. In turn, the *cna* gene was detected in 11 (14%) of 81 staphylococci represented 6 species: *S. carnosus*, *S. equorum*, *S. pettenkoferi*, *S. saprophyticus*, *S. succinus* and *S. vitulinus* (Table 3).

Table 3 presents a detailed distribution of the analyzed genes in all isolates, including their biofilm capacity

Table 2. Ability to produce biofilm on polystyrene titration plates

Tabela 2. Zdolność wytwarzania biofilmu

Species Gatunek	Number of isolates Liczba izolatów	The ability to produce biofilm Zdolność wytwarzania biofilmu			
		High – Wysoka	Average – Średnia	Low – Niska	None – Brak
S. equorum	21	10	0	6	5
S. carnosus	10	9	1	0	0
S. vitulinus	10	4	1	1	4
S. succinus	9	5	3	1	0
S. saprophyticus	8	3	2	1	2
S. xylosus	8	6	1	1	0
S. warneri	7	3	1	2	1
S. lentus	4	1	1	1	1
S. epidermidis	1	1	0	0	0
S. haemolyticus	1	1	0	0	0
S. pasteuri	1	0	1	0	0
S. pettenkoferi	1	1	0	0	0
Total – Razem	81	44 (54%)	11 (14%)	13 (16%)	13 (16%)

and source of isolation. Of the 15 isolates that showed the presence of genes responsible for adhesion to collagen or the accumulation of cells in the biofilm, 14 (93%) demonstrated the ability to produce biofilm on polystyrene titration plates. Out of all 68 isolates capable to form biofilm, only 14 (21%) possessed genes involved in biofilm formation process.

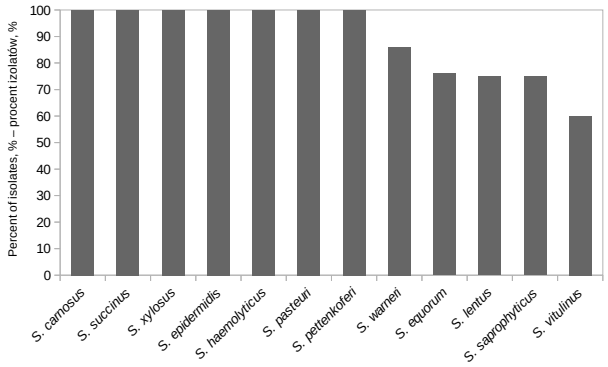

Fig. 1. Percentage of strains with biofilm-forming ability

Rys. 1. Odsetek biofilmujących szczepów z uwzględnieniem przynależności gatunkowej

In the case of *S. epidermidis*, *S. haemolyticus*, *S. pasteuri* and *S. xylosus* species, any of the analyzed genes were not detected. In contrast, strains of *S. carnosus*, *S. equorum*, *S. pettenkoferi* and *S. succinus* possessed all investigated genes (Fig. 3).

Among analyzed isolates, 12% (10 isolates) were methicillin resistant and nine of them demonstrated biofilm-forming ability (Table 4). The 6 out of 10 of methicillin-resistant strains were isolated from salami. All of these strains showed biofilm-forming ability and all belonged to the species normally included in starter cultures of fermented sausages (*S. carnosus*, *S. xylosus*).

DISCUSSION

The studies showed that 84% of investigated CNS isolated from meat products showed ability to form biofilm, and high ability to form biofilm was detected in 54% of the isolates. Only 20% of all strains with ability to form biofilm showed the presence of genes responsible for adhesion to the substrate and accumulation of cells in the biofilm. The results obtained are consistent with the results of the studies published by Møretrø et al. [2003] and with literature data indicating alternative biofilm formation pathways in coagulase-negative staphylococci, independent of the presence of operon genes *icaADBC* [Becker et al. 2014].

The highest number of strains with biofilm-forming ability was isolated from salami. All these strains belonged to *S. carnosus* and *S. xylosus* species, which are CNS typically isolated from fermented sausages. Of all meat products from which staphylococci were isolated, only salami production is based on the use of fermenting strains. The ability to create a biofilm can enable them to survive in the product and environment of food processing, by protecting against high temperatures, changes in the pH of the environment and substances used for cleaning and disinfecting the surface [Irlinger 2008; Resch et al. 2008]. Six strains isolated from salami showed the ability to form biofilms and methicillin resistance. These strains belonged to species normally included in starter cultures of fermented sausages.

In the present study, the *ica*A gene was found in 5% and the *ica*D gene in 16% of all investigated strains. Such a disproportion may be related to the possibility of occuring genes from the *icaADBC* operon alone (not in the form of the entire operon) [Podbielska-Kubera, 2015]. According to several authors, the presence of genes from the *icaADBC* operon can be used to differentiate virulent and commensal strains. As an example, research carried out by Frebourg et al. [2000], using 138 *S. epidermidis* strains collected from patients and from healthy volunteers showed a significant relationship between the presence of genes from the *icaADBC* operon and the origin

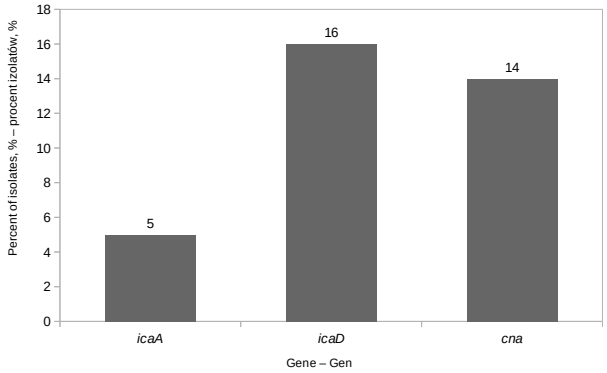

Fig. 2. The percentage of isolates in which the *ica*A, *ica*D and *cna* genes have been detected

Rys. 2. Odsetek izolatów, u których wykazano obecność poszukiwanych genów

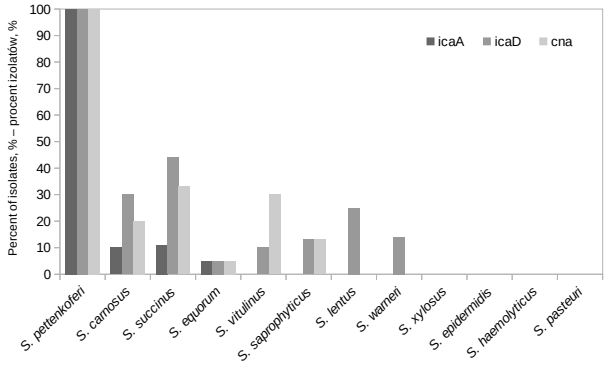

Fig. 3. Frequency of *ica*A, *ica*D and cna genes among CNS species

Rys. 3. Częstość występowania genów *ica*A, *ica*D i *cna* wśród poszczególnych gatunków CNS

of the strain – these genes are present more frequently in strains isolated from the clinical material than in those taken from healthy volunteers. Similar results were obtained in study carried out by Arciola et al. [2001], in which 68 *S. epidermidis* strains isolated from infections related to intravenous cannulas and 10 strains isolated from healthy volunteers were analyzed. In these studies the genes from the ica operon were also identified as an important virulence marker. On the other hand, studies carried out by Rhode et al. [2004] showed that genes from the *icaADBC* occurred more often in commensal strains of *S. epidermidis* as compared to clinical ones.

The *cna* gene, which determines the synthesis of the collagen binding protein that is the *Staphylococcus aureus* virulence factor, has been never previously detected in any CNS. In the current study, this gene was detected in *S. vitulinus*, *S. succinus*, *S. equorum*, *S. pettenkoferi*, *S. saprophyticus* and *S. carnosus*. Although, we did not perform sequencing of the PCR product suggesting *cna* gene presence, the size of the PCR product recognized as cns were the same as the size of the *cna* gene found in *Staphylococcus aureus* [Ote et al. 2011].

Among 12 species of CNS investigated in the present study, seven species (*S. epidermidis*, *S. xylosus*, *S. warneri*, *S. saphrophyticus*, *S. haemolyticus*, *S. pasteuri* and *S. pettenkoferi*) constitute a part of the microflora of human skin and mucous membranes. Strains of *S. xylosus*, *S. warneri* and *S. epidermidis* are isolated from the entire surface of the skin. *S. haemolyticus* is isolated particularly from regions rich in apocrine glands. *S. pettenkoferi* is usually isolated from the surface of the skin. *S. saprophyticus* colonizes the anus area of human and pigs, hence it is a frequent contamination of pork food products. *S. pasteuri* is isolated from a wide range of environments, including human skin and mucous membranes [Becker et al. 2014]. Species such as *S. carnosus*, *S. equorum*, *S. succinus* and *S. xylosus* are usually associated with fermented food and starter cultures [Irlinger 2008].

In recent years, some of the aforementioned CNS species, e.g. *S. equorum*, *S. epidermidis*, *S. saprophyticus*, *S. pettenkoferi*, *S. xylosus*, *S. warneri*, *S. haemolyticus* and *S. pasteuri* have been isolated from different human clinical samples, e.g. blood, cerebrospinal fluid, pus, swab from the wound, swab from the eye and exudate [Nováková et al. 2006, Irlinger 2008, Savini et al. 2009, Becker et al. 2014]. Strains belonging to these species were also isolated in the current study, and most of these strains were able to produce biofilm.

Interestingly, a strain belonging to the species *Staphylococcus pettenkoferi* analyzed in the current study was for the first time isolated by Trülzsch et al. [2002]. The authors isolated two strains from this species, one from the blood (patient with extrapulmonary tuberculosis) and other from a wound formed in the course of bursitis of the elbow joint (patient with lymphocytic leukemia). Strains belonging to this species were also isolated by other authors, however always from clinical materials obtained from immunocompromised patients [Loïez et al.

Table 3. Isolates in which *ica*A, *ica*D or *cna* genes were detected

Tabela 3. Izolaty, u których wykazano obecność genów *ica*A, *ica*D lub *cna*

No. Nr	Species – Gatunek	Isolation source Źródło izolacji	Gene – Gen			Ability to produce biofilms Zdolność wytwarzania biofilmu
			*ica*A	*ica*D	*cna*	
1.	*S. vitulinus*	pork ham – szynka wieprzowa			+	High – Wysoka
2.	*S. succinus*	pork ham – szynka wieprzowa		+	+	High – Wysoka
3.	*S. succinus*	pork ham – szynka wieprzowa		+		Average – Średnia
4.	*S vitulinus*	pork ham – szynka wieprzowa		+	+	High – Wysoka
5.	*S. succinus*	pork ham – szynka wieprzowa	+	+	+	High – Wysoka
6.	*S. succinus*	chicken cold cuts – szynka drobiowa		+	+	Low – Niska
7.	*S. warneri*	chicken cold cuts – szynka drobiowa		+		None – Brak
8.	*S. lentus*	chicken cold cuts – szynka drobiowa		+		High – Wysoka
9.	*S. vitulinus*	chicken cold cuts – szynka drobiowa			+	High – Wysoka
10.	*S. equorum*	chicken cold cuts – szynka drobiowa	+	+	+	High – Wysoka
11.	*S. saprophyticus*	pork sausage – kiełbasa wieprzowa		+	+	Low – Niska
12.	*S. pettenkoferi*	pork sausage – kiełbasa wieprzowa	+	+	+	High – Wysoka
13.	*S. carnosus*	salami	+	+	+	Average – Średnia
14.	*S. carnosus*	salami		+	+	High – Wysoka
15.	*S. carnosus*	salami		+		High – Wysoka

Table 4. Methicillin-resistant strains and their ability to form biofilm

Tabela 4. Szczepy oporne na metycylinę z uwzględnieniem zdolności tworzenia biofilmu i źródłem izolacji

No. Nr	Species Gatunek	Isolation source Źródło izolacji	Ability to produce biofilms Zdolność wytwarzania biofilmu
1	*S. carnosus*	salami	High – Wysoka
2	*S. carnosus*	salami	High – Wysoka
3	*S. carnosus*	salami	High – Wysoka
4	*S. carnosus*	salami	Average – Średnia
5	*S. epidermidis*	pork luncheon meat – mielonka wieprzowa	High – Wysoka
6	*S. equorum*	pork ham – szynka wieprzowa	Low – Niska
7	*S. lentus*	pork luncheon meat – mielonka wieprzowa	None – Brak
8	*S. saprophyticus*	pork ham – szynka wieprzowa	High – Wysoka
9	*S. xylosus*	salami	High – Wysoka
10	*S. xylosus*	salami	High

2007, Trülzsch et al. 2007, Song et al. 2009, Mammina et al. 2011). Until now, there is just one study with the confirmed isolation of the above species from the food (Fijałkowski et al. 2016). It should be also noticed, that the strain analyzed in the current study, showed a high biofilm formation capacity and possessed all investigated genes, which may indicate the pathogenic potential of this strain.

In conclusion, ready-to-eat meat products sliced at the customer's request can be a source of biofilm-forming CNS. The presence of genes *ica*A, *ica*D and *cna* responsible for adhesion of the bacteria to the substrate and their accumulation in the biofilm correlated with the phenotypic capability of the bacteria to produce biofilms. The production of biofilm by CNS may occur regardless of the presence of genes from the operon *icaADBC*.

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BADANIE ZDOLNOŚCI TWORZENIA SIĘ BIOFILMU KOAGULAZO-UJEMNYCH GRONKOWCÓW IZOLOWANYCH Z GOTOWYCH PRODUKTÓW MIĘSNYCH

STRESZCZENIE

Celem badań była analiza zdolności produkcyjnej biofilmów, koagulazo-ujemnych gronkowców (CNS) izolowanych z gotowych do spożycia produktów mięsnych, w tym szynki wieprzowej, wędlin drobiowych, kiełbasy wieprzowej, salami i mięsa wieprzowego, wraz z określeniem występowania genów adhezji określających produkcję polisacharydów (*ica*A i *ica*D) i białka wiążącego kolagen (*cna*). Badane izolaty obejmowały gatunki takie jak *Staphylococcus equorum* (28%), *S. vitulinus* (16%), *S. carnosus* (14%), *S. succinus* (11%), *S. xylosus* (11%), *S. saprophyticus* (9%) , *S. warneri* (9%), *S. haemolyticus* (1%) i *S. pasteuri* (1%). Ocenę zdolności do produkcji biofilmu przez gronkowce wykonano za pomocą fioletu krystalicznego, natomiast ocenę częstości występowania genów przeprowadzono za pomocą PCR. Spośród 81 szczepów CNS objętych badaniami 84% wykazało zdolność do tworzenia biofilmu w doświadczalnych warunkach *in vitro*. Wysoką zdolność do tworzenia biofilmu wykazano w przypadku 54% szczepów, średnią w 14%, a niską w 16%, podczas gdy brak zdolności produkcyjnej biofilmu stwierdzono u 16% badanych szczepów. Geny *ica*A i *ica*D odpowiedzialne za wytwarzanie pozakomórkowych adhezyn polisacharydowych wykryto odpowiednio u 5% i 16% szczepów. Gen determinujący tworzenie białka wiążącego kolagen (cna) wykryto u 14% szczepów. Z otrzymanych wyników można wnioskować, iż żywność jest źródłem koagulazo-ujemnych gronkowców zdolnych do tworzenia biofilmu, który jest określany jako klinicznie ważny czynnik wirulentny tych bakterii.

Słowa kluczowe: produkty mięsne, biofilm, koagulazo-ujemne gronkowce