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THE ANTIBACTERIAL PROPERTIES OF SILAGE FROM DIFFERENT VARIETIES OF SORGHUM

WŁAŚCIWOŚCI ANTYBAKTERYJNE KISZONEK Z RÓŻNYCH ODMIAN SORGO

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Streszczenie. Ze względu na zadowalającą produktywność, niską pojemność buforową i wysoką zawartość rozpuszczalnych węglowodanów, sorgo cukrowe wydaje się być obiecującym surowcem kiszonkarskim. Celem przeprowadzonego doświadczenia była analiza właściwości antagonistycznych bakterii obecnych w kiszonkach przygotowanych z różnych odmian sorgo, wobec patogennych pałeczek jelitowych. Potencjał antybakteryjny mikroorganizmów pochodzących z zakiszonej masy roślinnej badano za pomocą metody studzienkowej i pomiaru wielkości stref inhibicji wzrostu obserwowanych na murawkach wykonanych z bakterii należących do rodzaju *Salmonella* spp., *Shigella* spp. oraz gatunku *Escherichia coli*. Identyfikacji dokonano na podstawie analizy jakościowej DNA. Gatunkiem wyraźnie dominującym w kiszonkach był *Lactobacillus plantarum.*

Key words: *Enterobacteriaceae, Lactobacillus* spp., sorghum.
Słowa kluczowe: *Enterobacteriaceae, Lactobacillus* spp.*,* sorgo.

INTRODUCTION

The large production potential of sorghum (*Sorghum saccharatum*) causes that it is being introduced as a crop also in temperate climate zones. Being a plant of tropical origin, it is perfectly adapted to periodical droughts. Sweet sorghum silages play a significant role in feeding cattle. The Polish climate and soils guarantee high yield of the crop, so it can provide a sufficient volume of fodder. Sorghum green fodder contains much less starch and total protein, but it has much more water-soluble saccharides, raw fibre and NDF and ADF cell wall fractions than maize (Michalski 2008). Good quality sorghum is characterised by high nutritional value and the content of vitamins in seeds is comparable to the content in maize. However, there are slightly greater amounts of pantothenic acid, nicotinic acid and biotin in sorghum (Wall and Blessin 1970). The study by Śliwiński et al. (2013) pointed to lower concentration of energy in silages prepared from Sucrosorgo 506. The low content of dry

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weight is a disadvantage of sorghum green fodder as a silage material. Ensiling high humidity sorghum involves the risk of considerable loss of nutrients caused by leakage of silage juice. In sorghum with low content of dry weight and high content of water-soluble carbohydrates the fermentation process is very intensive and silages have high content of lactic acid (Zwi G. Weinberg et al. 2011, Chahrour et al. 2013, Santos et al. 2013). Active production of this metabolite results in reduced pH and it limits animals' intake of fodder. Farmers are apprehensive about the presence of prussic acid in sorghum. Experiments unquestionably prove that if silage is appropriately prepared, the compound decomposes spontaneously after about three weeks. The aim of the study was to analyse the antagonistic properties of bacteria found in silages made from different sorghum varieties against potentially pathogenic enterobacteria of the S*almonella* spp. and *Shigella* spp. genera and *Escherichia coli* species and to identify lactic bacteria found in silages.

MATERIAL AND METHODS

The plant material came from the Department of Agronomy, Poznań University of Life Sciences, Poland. It consisted of the following sorghum cultivars: AC10, Aron, Bovital, Exp 702, Frigo, Goliath, Hercules, Monori Edes, Nutri Honey, Rona 1, Sucrosorgo 506, Topsilo. The silages were prepared in 4 dm^3 PVC microsilos (10 replications for each cultivar) with closures enabling discharge of gas products. The ensiling lasted 150 days. During ensiling the average room temperature was 2ºC ±1ºC. *E. coli, Salmonella* spp. and *Shigella* spp. bacterial isolates came from the collection of the Department of Biochemistry and Biotechnology. The excitation of the microorganism culture was achieved through passage of the input culture into enrichment broth and breeding with shaking for 16 h at a temperature of 37ºC.

Microbiological analysis. 5 g samples of individual silages were weighed and suspended in 25 ml liquid LB medium. The samples were incubated in a shaker for 16 h at a temperature of 37ºC. Next, the bacterial suspension was separated from solid parts of the silage and adjusted to pH 7. At the next stage, bacterial lawns were prepared from potentially pathogenic bacteria by pouring the bacterial inoculum containing 25 ml of liquid LB medium with agar inoculated with 100 µl of respective bacteria (*E. coli,* S*almonella* spp.*, Shigella* spp.) on petri plates. The samples were incubated for 16 h at a temperature of 37ºC. The use of the well method enabled examination of the antibacterial properties of proliferated silage microorganisms and determination of their antagonistic potential against enterobacteria of the S*almonella* spp. and *Shigella* spp. genera and *E. coli* species. Wells (14 mm in diameter) were made in each bacterial lawn. 120 µl of liquid LB medium with agar was poured to the bottom of each well. Next, 100 µl of 16-hour bacterial culture obtained from silages was placed in the well. Once again, 120 µl of liquid LB medium with agar was added and incubated at a temperature of 37ºC. After 24 h and 48 h the zones where the growth of pathogenic organism was inhibited were observed. In order to identify the predominant lactic fermentation bacteria in sorghum silages the bacteria were cultured on selective MRS Agar medium (Oxoid) and incubated at a temperature of 37ºC for 48–72 h. The species identification of lactic bacteria found in the silages was conducted by means of PCR with primers identifying *Lactobacillus plantarum* bacteria (gen recA, product volume – 249 bp), *Lactobacillus buchneri* bacteria

(16S rRNA, product volume – 193 bp) and *Lactobacillus brevis* bacteria (gen gyrB, product volume – 273 bp). The PCR conditions for the *Lactobacillus* bacteria were as follows: volume of reaction mixture – 25 µl, number of cycles – 35: 94ºC 10 min, 94ºC 30 s, 52ºC 30 s (primer bonding for *L. plantarum* and *L. brevis*), 74ºC 60 s, 74ºC 5 min, primer bonding temperature for *L. buchneri* – 58ºC.

RESULTS AND DISCUSSION

The analysis of the antagonistic potential revealed the presence of growth inhibition zones on most of the bacterial lawns prepared from *E. coli*, *Salmonella* spp. and *Shigella* spp. The 'halo' effect was observed around 170 out of 270 wells, i.e. about 63% of the total number.

The greatest growth inhibition zones were observed on lawns with *Salmonella* spp. This dependence was illustrated in Fig. 1. There were analogical dependences observed for *Salmonella* bacteria as for the *E. coli* bacterial lawns. The main difference was much greater antagonistic potential of the bacteria from the silages made from the Nutri Honey and Hercules cultivars. The microorganisms present in the silage made from the Frigo sorghum cultivar were characterised by the poorest antibacterial properties.

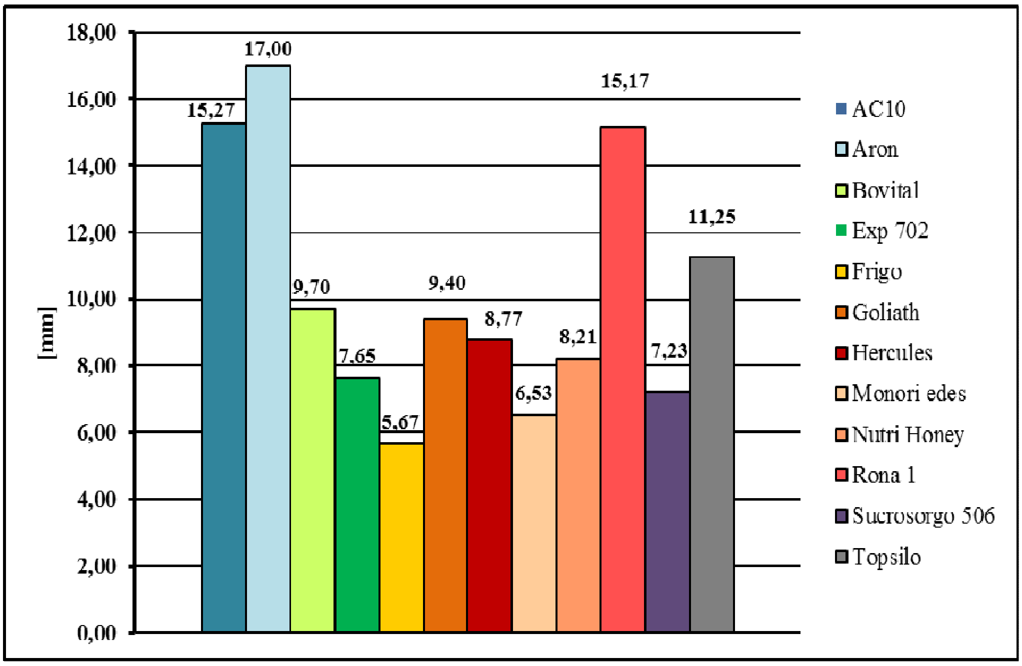

Fig. 1. The growth inhibition zones of *Salmonella* spp.
Rys. 1. Wielkości stref zahamowania wzrostu bakterii z rodzaju *Salmonella* spp.

The research findings point to the absence of statistically significant differences in the size of growth inhibition zones observed in the *E. coli* bacterial lawns prepared from the silages made from the Bovital, Exp 702, Sucrosorgo 506 and Topsilo sorghum cultivars. The microorganisms present in the silage sample made from the Aron sorghum cultivar exhibited the strongest antagonistic properties against potentially pathogenic microorganisms. On the other hand, the bacteria from the silages made from the Nutri Honey cultivar were characterised by the weakest antagonistic potential (Fig. 2).

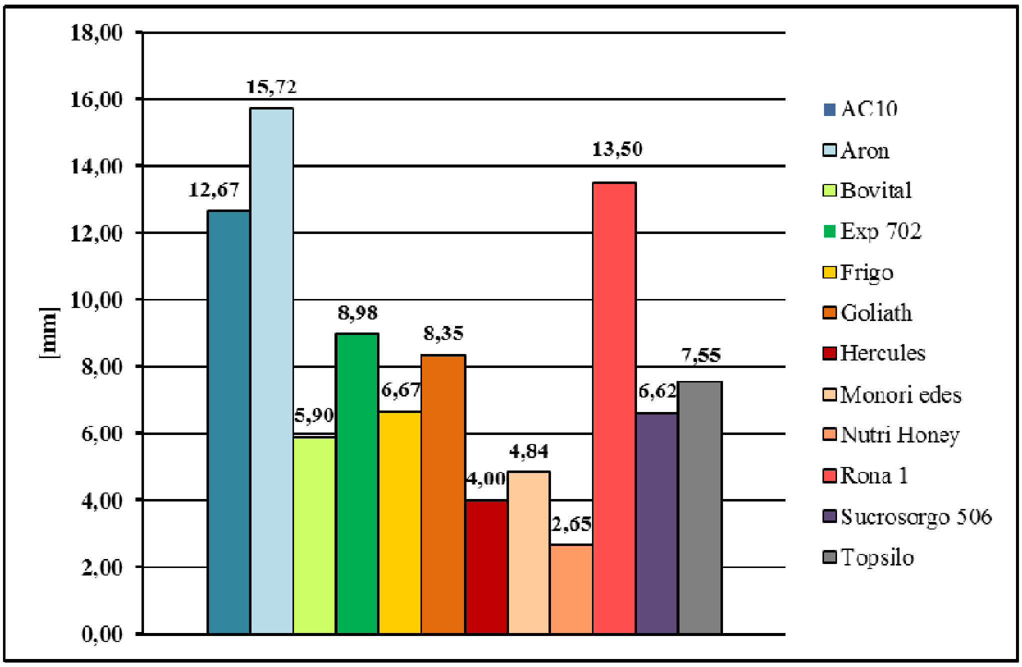

Fig. 2. The growth inhibition zones of *E. coli*
Rys. 2. Wielkości stref zahamowania wzrostu bakterii *E. coli*

As far as the bacterial lawns prepared from the bacteria of the *Shigella* spp. genus are concerned, there were high values of the radius of the pathogenic microorganism growth inhibition zone observed for the microorganisms present in the silage samples made from as many as four sorghum cultivars: Rona 1, AC10, Topsilo and Aron. As far as the 'halo' area is concerned, the sorghum samples made from the Bovital cultivar were noticeably the weakest (Fig. 3).

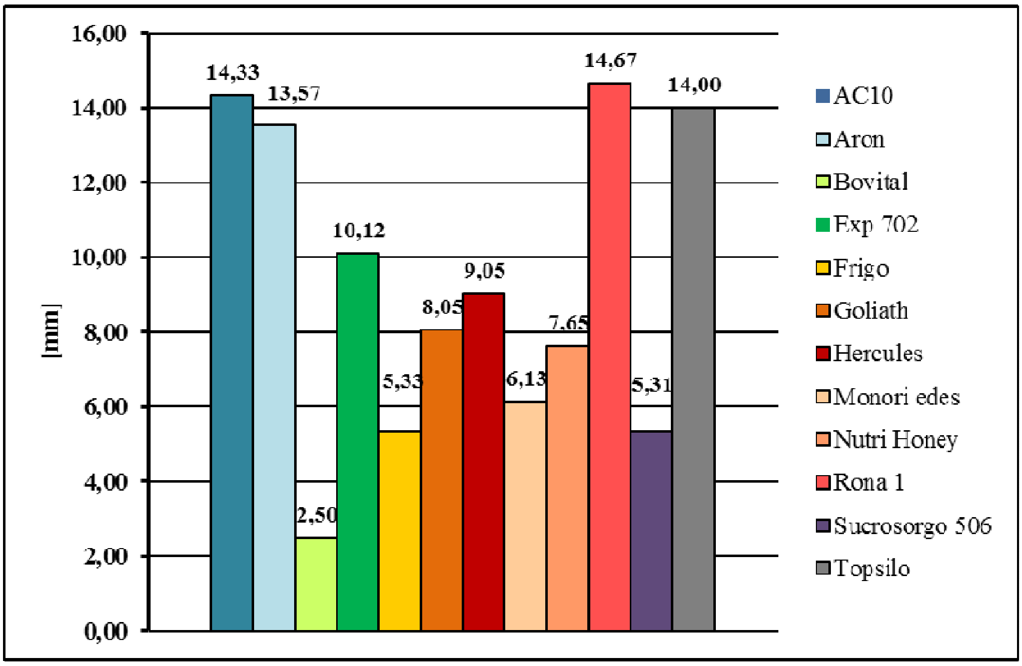

Fig. 3. The growth inhibition zones of *Shigella* spp.
Rys. 3. Wielkości stref zahamowania wzrostu bakterii z rodzaju *Shigella* spp.

The identification of bacterial species of the *Lactobacillus* genus was carried out by means of PCR in all the 12 silage samples. Figure 4 shows an example of electrophoretic image.

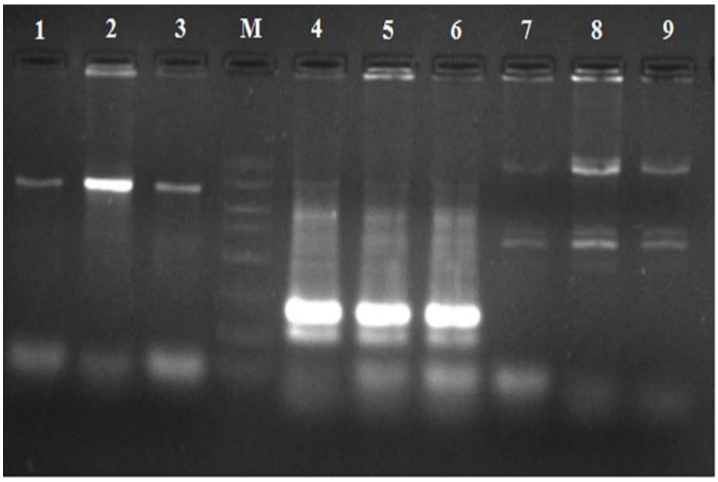

Fig. 4. PCR products amplified with different primers and separated on 1.5% agarose gel electrophoresis: 16S rRNA gene fragment *L. buchneri* (193 bp), recA gene fragment *L. plantarum* (249 bp), gyrB gene fragment *L. brevis* (237 bp). Line 1, 4, 7 – Goliath variety, Line 2, 5, 8 – Monori Edes variety, Line 3, 6, 9 – Sucrosorgo 506 variety, M – PCR marker (Sigma-Aldrich)
Rys. 4. Obraz elektroforetyczny rozdziału produktów PCR w 1,5% żelu agarozowym: fragmentu genu 16S rRNA *L. buchneri* (193 pz), fragmentu genu recA *L. plantarum* (249 pz), fragmentu genu gyrB *L. brevis* (237 pz). Tory 1, 4, 7 – odmiana Goliath, tory 2, 5, 8 – odmiana Monori Edes, tory 3, 6, 9 – odmiana Sucrosorgo 506, M – PCR marker wielkości (Sigma-Aldrich)

According to Śliwiński et al. (2013), the deficit of ground water in some regions of Poland and the ban on growing genetically modified maize, which is resistant to the pest European corn worm, may reduce the productivity of fodder plants. During a soil drought the maize yield reaches 50–60% of its potential, so the amount of silage is reduced. Studies have proved that sorghum may be a good substitute due to its limited transpiration and a different rooting manner. It produces higher dry weight yield in low humidity. Sorghum in the form of various hybrid cultivars is a valuable fodder plant due to its considerable yield potential, effective growth on most soil types and low susceptibility to diseases. As Grant and Stock (1996) reported, feeding cattle with silage made from sorghum harvested at an early stage of ripeness does not infringe the basic parameters of cows' metabolism and it should not have negative influence on their productivity. Animals are ready to eat sorghum silage due to its sweet taste and juiciness. On the other hand, Michalski (2008) noted the problem of high fibre content and lesser digestibility due to the high content of stalks in ensiled green fodder. According to the author, it is necessary to give animals nutritive mixtures, which will compensate for the lower energetic value of sorghum. In spite of these drawbacks sorghum is gaining popularity and farmers' interest.

The aim of the study was to determine the antagonistic properties of microorganisms in silages prepared from different sorghum cultivars without chemical additives and microbiological inoculants against potentially pathogenic bacteria from the S*almonella* spp. and *Shigella* spp. genera and *Escherichia coli* species. The enterobacteria of the *Enterobacteriaceae* family can be found in animals' alimentary tracts in normal physiological states, but they may cause serious diseases under favourable conditions for the development of pathogens. *Shigella* spp. bacteria are an exception, because they only infect the organisms of humans and higher primates, but they are not dangerous to farm animals. The presence of three species of lactic acid bacteria, i.e. *L. plantarum* (homofermentative), *Lactobacillus brevis* and *L. buchneri* (heterofermentative), was proved in the silages under analysis. The research findings are in agreement with the data documented by other researchers in the literature (Pahlow et al. 2003). It is noteworthy that during the experiment the agro-meteorological conditions in experimental plots favoured the normal vegetation of sorghum plants. The study of literature and analysis of the results obtained in the experiment let us formulate the following conclusions.

CONCLUSIONS

1. The bacteria found in silages exhibit antagonistic properties against potentially pathogenic enterobacteria of the *Salmonella* spp. and *Shigella* spp. genera and *Escherichia coli* species.
2. The largest growth inhibition zones were measured for *Salmonella* spp.
3. The research proved the dependence between the antibacterial potential of microorganisms isolated from silages and the sorghum cultivar from which they were prepared. As far as the size of the 'halo' zone observed on bacterial lawns made from potentially pathogenic microorganisms is concerned, the silages prepared from the Aron, Rona 1 and AC10 cultivars proved to be the best.
4. Lactic acid bacteria of the *Lactobacillus* genus were identified in the samples under analysis. *L. plantarum* was the predominant species in the sorghum silages.

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Abstract. Sorghum is a good silage material due to its good productivity, low buffer capacity and high content of soluble carbohydrates. The aim of the study was to examine the antagonistic properties of bacteria present in the silage prepared from different varieties of sorghum, against pathogenic enterobacteria. Potentially antibacterial microorganisms from the silages were investigated by means of the well method and by measuring the size of growth inhibition zones observed on bacterial lawns made from the *Salmonella* spp. and *Shigella* spp. genera and *Escherichia coli* species. The PCR method was used for qualitative detection of the DNA of antagonistic bacteria. *Lactobacillus plantarum* was the predominant species in the silage.