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RAPID COMMUNICATION

Monitoring the occurrence of bacteria in stored cabbage heads

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Abstract

Twenty-six cabbage heads stored under typical conditions in a storage hall in Moravia, Czech Republic, were tested for the presence of bacteria by the method of isolation from three different parts of the cabbage heads. Isolations were carried out from stalks, inner and superficial leaves. Two samplings were done; in November 2015 and February 2016. Bacterial cultures were sequenced in the part of 16S rRNA region; bacteria were identified according to the sequences obtained. The most prevalent bacteria were of the genus *Pseudomonas*. Genera: *Klebsiella*, *Erwinia, Pantoea, Bacillus* were also identified. The results provided an interesting insight into the bacterial spectrum in stored cabbage heads and their dynamics during storage. The nucleotide sequences which were found were saved in GenBank/NCBI under accession numbers KX160104-KX160145.

Key words: *Bacillus,* bacteria, cabbage, identification, isolation, MPA medium, *Pseudomonas*, sequencing, 16S rRNA

Introduction

Bacterial soft rot pathogens may cause serious losses in stored cabbage heads (*Brassica oleracea* var. *capitata* L.). Vegetables may already be infected at harvest even though they do not show visible symptoms (Bhat *et al*. 2012). Due to the ability to cause latent infection, the appearance of symptoms is strongly dependent on environmental conditions (Bretschneider *et al*. 1989; Czajkowski *et al*. 2011). However, postharvest bacterial soft rot losses caused by various species of *Bacillus*, *Pseudomonas* and *Erwinia* have been estimated to vary between 15–30% of the harvested crop (Agrios 2006). Bacteria which infect cruciferous plants are primarily gram-negative. The most frequently identified genera are *Pseudomonas* (Mauzey *et al*. 2015), *Erwinia* (Togashi *et al*. 2001), *Pectobacterium* (Nazerian *et al*. 2011), *Xanthomonas* (Roberts *et al*. 1999; Eichmeier *et al*. 2015) and *Bacillus* (Agrios 2006). The bacteria mainly attack the fleshy storage organs of their hosts (Bhat *et al*. 2010). Soft rot pathogens are transmitted by contact with infected plants or by their persistence on postharvest residues (Davidsson *et al*. 2013). Infection can also be spread by insects (Nadarasah *et al*. 2011).

Control of the disease is not always very effective, however, sanitary practices in production, storage and processing can slow the spread of the disease and protect yields (Czajkowski *et al*. 2011). Furthermore, dispersal of bacteria can also happen via usage of surface water for irrigation, aerosols generated by rain, movement of bacteria in soil water or mechanically via contaminated agricultural equipment (Perombelon and Kelman 1980; Czajkowski *et al*. 2011).

This work is focused on the determination of bacterial communities present in different parts of cabbage heads and their dynamics during cold storage.

Materials and Methods

Sources of materials and samplings

The bacterial isolates originated from naturally infected plants of cabbage *B. oleracea* var. *capitata* cv. Fundaxy, the cultivar of white cabbage suitable for storage. Cabbage heads were harvested in October 2015 in Otice (GPS coordinates: 49.91683940000001, 17.86982660000001) and they were stored at 1–2°C and high humidity (95%) in a storage hall located in Otice. Two samplings were done: in November 2015 and February 2016. At both samplings, 13 cabbage heads were analysed.

Isolation of bacteria from cabbage heads

From each head, three samples from different locations within the cabbage head were taken as follows: A – cabbage stalk, B – inner leaves and C – superficial leaves. The heads were cut with a knife and scalpel. Approximately 4 × 4 mm pieces were disinfected in 2% sodium hypochlorite solution and washed 2× in sterile distilled water. The pieces from parts A, B and C were placed on Petri dishes. Samples were cultivated on MPA (meat-peptone agar, Sigma-Aldrich, St. Louis, USA) on Petri dishes at 25°C in the dark. Most of the cabbage heads were without symptoms but some of them showed symptoms e.g. black spots on the superficial leaves – C. Parts A and B were entirely without symptoms (Tables 1 and 2).

PCR reaction and sequencing

The DNA from cultures colonising each Petri dish was isolated from approximately 5 mg of bacterial culture according to Roohie and Umesha (2012). Target amplicon, the part of the 16S rRNA, V3 and V4 region (Klindworth *et al*. 2013) was amplified with GoTaq® G2 Flexi kit (Promega, Madison, USA). The PCR products corresponding to the expected size were gel-purified using NucleoSpin Gel and PCR Clean-up (Macherey-Nagel, Düren, Germany) and subjected to nucleotide sequencing as described by Eichmeier *et al*. (2010). The obtained nucleotide sequences were analysed using CLC Genomics Workbench 6.0 (CLC Bio, Aarhus, Denmark). The genus of bacteria was determined with similarities greater than 90% in GenBank/NCBI as recommended by Klindworth *et al*. (2013). The species level was based on 100% nucleotide identity.

Results

In the first sampling, bacterial colonies grew on 56% of the Petri dishes (Table 1). Two bacterial colonies (12C and 13A) were not identified because of the very high similarity with undetermined bacteria in GenBank/NCBI. Most of the identified colonies belonged to the genus *Pseudomonas*. Bacterial spectrum isolated from the first sampling is shown in Fig. 1 (Bacterial spectrum from the 1st sampling).

Bacterial cultures in the second sampling were obtained on 51% of Petri dishes (Table 2). The rates of bacteria isolated from the second sampling are shown in Fig. 2 (Bacterial spectrum from the second sampling).

Six genera of bacteria in 26 stored cabbage heads were detected based on DNA sequencing of cultivated colonies. In the first sampling five genera were present: *Erwinia*, unclassified *Gammaproteobacterium*, *Klebsiella*, *Pantoea* and *Pseudomonas*. In the second sampling only *Pseudomonas* and *Bacillus* colonies occurred.

Discussion

Plants and their products gradually lose their defence mechanisms against invading microorganisms after harvest. Therefore they are generally more susceptible to infection by natural pathogens or even to attack by bacteria that normally do not cause diseases of plants growing in the field (Liao and Wells 1987). King and Bolin (1989) indicated the increase of microbial populations of lettuce under storage conditions which makes our results surprising since we found that the spectrum of identified bacterial pathogens was lower in the second sampling than in the first one. This is one of the first studies which focused on identification of bacteria in different parts (A, B, C) of cabbage heads. A comparison of symptomatic and asymptomatic heads was carried out. We detected only *Pseudomonas* spp. in the first sampling in the asymptomatic heads. In the second sampling we also detected *Bacillus* spp. (5A, 7B) (Tables 1 and 2). In the symptomatic heads, we detected *Klebsiella*, *Erwinia*, *Pantotea* and *Pseudomonas* species in the first sampling and in the second sampling only *Bacillus* and *Pseudomonas* species. The occurrence of *Pseudomonas* spp. in both sampling periods and in both symptomatic and asymptomatic heads was expected because bacteria belonging to this genus are able to grow at low temperatures. Brocklehurst and Lund (1981) isolated five strains of *Pseudomonas* sp. from cabbage stored at 1°C.

No *Bacillus* spp. were observed in the first sampling. The presence of *Bacillus* spp. in the second sampling

Table 1. List of detected bacteria in cabbage heads in storage hall: A – stalk, B – inner leaves, C – superficial leaves, 1st sampling

Isolate	S/A*	Detected bacteria	blastN [%]	Acces. Number [GenBank]
1A	A	*Pseudomonas* sp.	95	KX160117
4A	A	*Pseudomonas* sp.	98	KX160118
4C	A	*Pseudomonas* sp.	98	KX160119
5C	A	*Pseudomonas* sp.	97	KX160120
6A	A	*Pseudomonas fluorescens*	100	KX160121
6C	A	*Pseudomonas* sp.	99	KX160122
8A	S	*Pseudomonas* sp.	96	KX160123
8B	S	*Pseudomonas* sp.	97	KX160124
8C	S	*Klebsiella* sp.	92	KX160125
9A	S	*Gammaproteobacterium* sp.	92	KX160126
9B	S	*Pseudomonas* sp.	99	KX160127
9C	S	*Pseudomonas* sp.	99	KX160128
10A	S	*Pseudomonas* sp.	93	KX160129
10B	S	*Pseudomonas* sp.	96	KX160130
10C	S	*Pseudomonas* sp.	98	KX160131
11A	S	*Erwinia* sp.	99	KX160104
11C	S	*Pantoea* sp.	99	KX160105
12A	S	*Pseudomonas* sp.	99	KX160106
12C	S	not identified	93	KX160107
13A	S	not identified	94	KX160108
13B	S	*Gammaproteobacterium* sp.	91	KX160109
13C	S	*Pseudomonas* sp.	92	KX160110

*S/A – symptomatic/asymptomatic heads

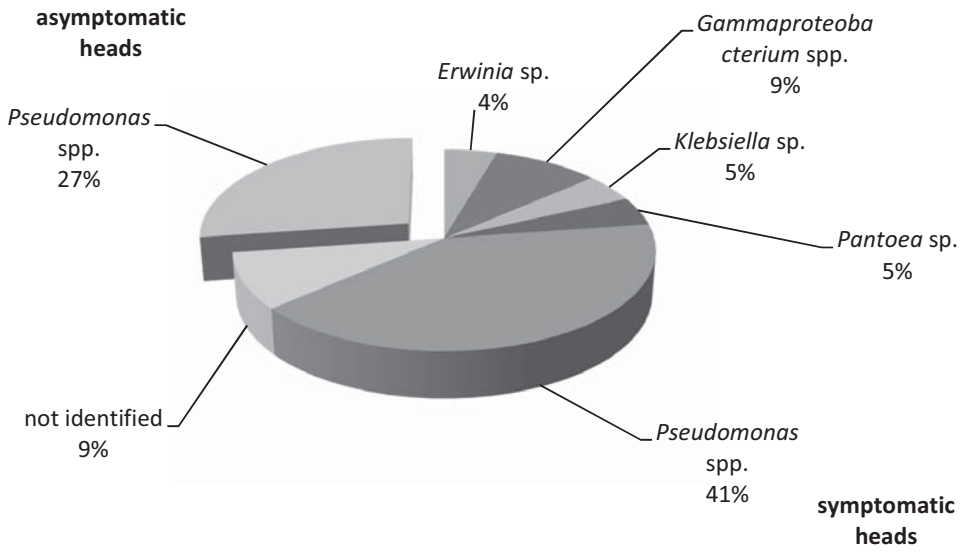

Fig. 1. Bacterial spectrum from the 1st sampling. The part of the circle which emerges from the unit belongs to asymptomatic cabbage heads (*Pseudomonas* spp.). The rest of the circle belongs to symptomatic heads.

was not surprising because spores of genus *Bacillus* have the ability to germinate and grow at refrigeration temperatures in different vegetable substrates (Nicholson *et al.* 2000; Valero *et al.* 2003).

Warth (1978) reported that the *Bacillus* spp. grow at 5°C as a minimal temperature. Valero *et al.* (2003) published results about *Bacillus* spp. growth at low temperatures and indicated that the pH strongly affects the

Table 2. List of detected bacteria in cabbage heads in storage hall: A – stalk, B – inner leaves, C – superficial leaves, 2nd sampling

Isolate	S/A*	Detected bacteria	blastN [%]	Acces. Number [GenBank]
2C	A	*Pseudomonas* sp.	98	KX160132
3C	A	*Pseudomonas* sp.	99	KX160133
4B	A	*Pseudomonas* sp.	100	KX160134
4C	A	*Pseudomonas* sp.	99	KX160135
5A	A	*Bacillus* sp.	87	KX160136
6A	A	*Pseudomonas* sp.	98	KX160137
6B	A	*Pseudomonas* sp.	94	KX160138
6C	A	*Pseudomonas* sp.	99	KX160139
7B	A	*Bacillus* sp.	96	KX160140
7C	A	*Pseudomonas* sp.	99	KX160141
8B	S	not identified	99	KX160142
8C	S	*Pseudomonas fluorescens*	100	KX160143
9C	S	not identified	99	KX160144
10C	S	*Pseudomonas* sp.	99	KX160145
11B	S	*Pseudomonas* sp.	99	KX160111
11C	S	*Pseudomonas* sp.	100	KX160112
12A	S	*Bacillus* sp.	99	KX160113
12B	S	*Bacillus* sp.	99	KX160114
12C	S	*Bacillus* sp.	99	KX160115
13A	S	*Pseudomonas* sp.	100	KX160116

*S/A – symptomatic/asymptomatic heads

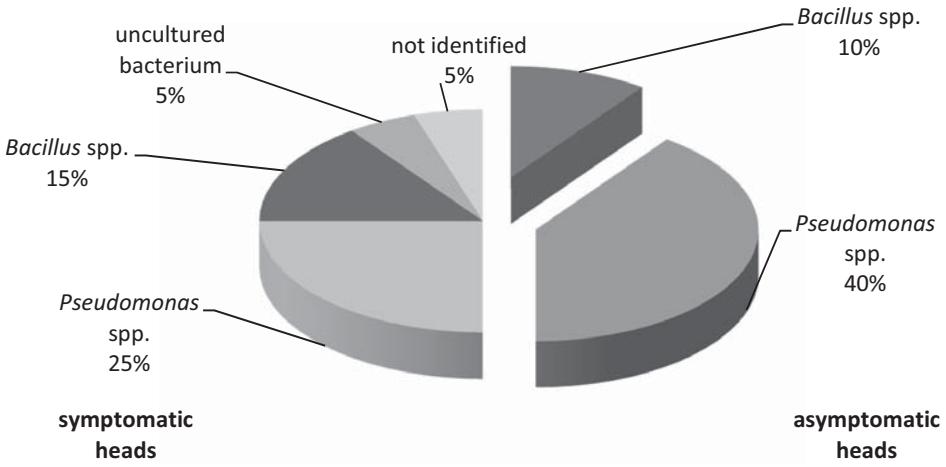

Fig. 2. Bacterial spectrum from the 2nd sampling. The part of the circle which emerges from the unit belongs to asymptomatic cabbage heads (*Pseudomonas* spp. and *Bacillus* spp.). The rest of the circle belongs to symptomatic heads.

growth of colonies. The vegetable substrates provide suitable pH conditions. Our study confirms the presence of *Bacillus* spp. in cabbage stalks (Table 2) also at low temperatures. *Pseudomonas* spp. were present in the first sampling in 36% and in the second one in 33% of isolations, throughout the whole cabbage head. *Pseudomonas* spp. is able to grow at 4°C as the lowest temperature (De Jonghe *et al.* 2011). Strong antagonistic behaviour of *Pseudomonas* spp. (Afsharmanesh *et al.* 2010) and *Bacillus* spp. against other bacteria (Zhao *et al.* 2013, 2014) could explain why the colonies of other genera declined with time inside the cabbage heads. *Bacillus* species are considered to be a potential biological control against postharvest diseases of vegetables and fruits, due to their antagonistic behaviour (Zhao *et al.* 2013; Arzanlou *et al.* 2016).

Conclusions

In the current study, the bacterial spectrum of genera *Pseudomonas*, *Klebsiella*, *Erwinia, Pantoea* and *Bacillus* was detected. Bacteria were detected in all of the examined parts of cabbage heads: stalk, inner leaves and superficial leaves.

Differences in bacterial spectrum present in the stored cabbage heads, depending on the time of sampling term were observed.

Development of *Pseudomonas* and *Bacillus* bacterial species at the later period of storage and dieback of other genera colonies may be due to the antagonistic behaviour of *Pseudomonas* and *Bacillus* spp.

The study proves the change of the bacterial spectrum through the whole cabbage heads under storage conditions even when the heads did not show significant and visible symptoms. Our results also show that the diversity of bacterial communities decreased during cold storage. Based on the obtained results future studies will focus on confirming the antagonistic effects of *Pseudomonas* and *Bacillus* spp. against the other genera.

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