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CULTIVABLE MICROORGANISMS INHABITING THE AERIAL PARTS OF *Hypericum perforatum*

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Abstract. The present investigation was carried out to analyse the presence of endophytes in the above-ground parts of *Hipericum perforatum* and to analyse the biodiversity and enumeration of epiphytes. Plant material was collected in Poland three times during the growing season. Phenotypic and genotypic diversity of all the endophytes and the most abundant epiphytes were researched. We analysed fungistatic activity of this isolates. From the endosphere of tested plant *Alcaligenes faecalis* and *Bacillus licheniformis* were isolated. The most numerous epiphytes were the copiotrophs and a bit less numerous were oligotrophs, bacteria cultivated on Bunt and Rovir's medium and fungi. The least numerous bacteria were *Azotobacter* sp. Among all the molds dominant were: *Cladosporium herbarum*, C. *cladosporioides* and *Alternaria consortialis*, *A. alternata*, *Clonostachys rosea f. catenulata* (*Gliocladium catenulatum*), *Scopulariopsis brevicaulis* and *Penicillium terrestre*. Among phyllobacteria there were found mostly the following species: *Burkholderia cepacia*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas fluorescens*, *P. putida*, *Pantoea agglomerans*, *Paenibacillus polymyxa*, *Bacillus cereus*, *Rhodococcus* sp., *R. erythropolis* and *Cellulosimicrobium cellulans*. The broadest spectrum of antifungal activity was examined for the following species: *Paenibacillus polymyxa*, *Pseudomonas putida* and *Pantoea agglomerans*. *P. polymyxa* limited the growth of over 82% tested molds, so did the other two strains: *P. agglomerans* over 77% and *P. putida* over 73%.

Key words: microorganisms colonizing herbs, St. John's wort, endophytes, epiphytes, fungistatic activity

INTRODUCTION

Plants are ecological niche for many groups of microorganisms. We can consider plant as three environments: rhizosphere, phyllosphere and endosphere. Due to the

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physico-chemical conditions they are extremely different environments. Rhizosphere is rich in nutrients such as sugars, organic acids and growth factors, so the growth of bacteria and fungi is facilitated. Phyllosphere, in contrast, is poor in nutrients and this is an extreme environment because of continuous changes in temperature, plentiful sunshine, frequent droughts and winds [Hirano and Upper 2000, Lindow and Brandl 2003, Whipps et al. 2008]. Nevertheless phyllosphere is settled by many bacterial populations. Morris and Kinkel estimated that the total number of phyllospheric bacteria is 10^{26}, while global surface of phyllosphere is 4×10^8 km^2 [Whippes et al. 2008]. Among the phyllosphere inhabiting microorganisms there are casual colonizers and residents, that is, organisms adapted to life in these conditions. Researches also divide epiphytes in four groups: (1) plant pathogens, (2) biological disease control agents, (3) bacteria with ice-nucleation activity, (4) biofilm forming bacteria [Gnanamanickam and Ommanuel 2006]. Endosphere is the environment inside plants. It is formed by internal plant tissues, mostly by their intercellular spaces. This area is settled by pathogens or endophytes. Endophytes are define as bacteria or fungi inhabiting inner plan tissues without causing any harm to their host plant [Bacon and Hinton 2006].

All the microorganisms inhabiting plants without causing them any harm are called plant associated microorganisms. There are plant hormone producing bacteria, bacteria that produce growth factors and plant pathogen antagonists [Bcon and Hinton 2006, Gnanamanickam and Ommanuel 2006]. The major interes is in bacteria with antagonistic activity because they can find uses as biocontrol agents in crops cultivations including herbs. Herbs cultivation in Poland is a large part of the EU countries [Zimowska 2007]. Recently the search for plant associated microorganisms inhabiting medicinal plants has intensified. Herbs are a very rich source of bacteria and fungi with different biochemical properties. As for now, the community of bacterial endophytes associated with many important herbs is unknown. One of them is *Hypericum perforatum*.

Hypericum perforatum (St. John's wort) is a common herb found on fresh loamy-stony and clay soils. Its properties make it a very interesting plant. Depending on dosage and the way it is used, its action can be either toxic or therapeutic. This is caused by the presence in the plant of compounds with pharmacological properties [Dias et al. 1998, Turek 2005, Kurkin and Pravdivtseva 2007, Silva 2008]. The herb has been found to contain at least ten classes of biologically active compounds. These include the naphthodianthrones, phloroglucinols, flavonoids, procyanidins, tannins, essential oils, amino acids, phenylpropanes and xanthones. Some of these compounds are fungicidal and bactericidal [Schempp 1999, Greeson 2001, Mazandarani 2007, Milosevic 2007]. There currently is growing interest in St. John's wort in view of its antidepressant properties. This is related to the discovery that hypericin – the compound responsible for these properties – is produced by endophytic fungi of St. John's wort [Kusari 2008]. In the available literature there are no reports on the bacteria inhabiting *Hypericum perforatum*. In this study we have attempted to determine the occurrence of endophytic bacteria in the aerial parts of the plant and we have investigated the biodiversity of microorganisms inhabiting the phyllosphere of *Hypericum perforatum*.

MATERIALS AND METHODS

The plants used in the studies were collected in Poland three times during the vegetative season: on July 1 from meadowlands at the edge of the forest in Łomianki, on July 24 from a meadow at Skarpa Ursynowska and on August 18 from the Opaleń forest glade in Kampinos Forest.

Determination of the presence of endophytes in samples of stems and leaves from St. John's wort. At each collection time material from five healthy, freshly collected plants was used. The plants were washed in running water, and the stems were cut into sections several centimeters long, which were rinsed with distilled water and then sterilized as described by Hung and Annapurna [2004]. The sterilized plant sections were then washed four times in sterile distilled water and after drying on sterile filter paper, the wrapped ends of the stems were cut off and each stem with leaves was incised lengthwise and placed on media in Petri dishes (TSA, PDA, King B, agar with 1000-fold diluted nutrient broth). Sterilized, uncut surface disinfected plants were a control of sterility. The plates were incubated for five days at 28ºC (±2°C). It was assumed that growth of bacteria along the cut parts of the plant indicates the presence of endophytic bacteria.

Quantitative determinations for selected groups of microorganisms inhabiting the phyllosphere of St. John's wort. The aerial parts of the plants (the leaves and stems) were taken to prepare 10 gram aliquots, which were placed in 100 cm^3 sterile saline (Rf) and shaken for 20 min to wash the microorganisms off the surface of the plants. Dilutions of the thus prepared stock suspensions were plated out on the recommended media. The number of microorganisms in the studied plant material was determined by the plate method as follows: microscopic fungi on Martin's medium [Martin 1950]; heterotrophic bacteria on Bunt and Rovira medium [Bunt and Rovira 1955]; copiotrophs and oligotrophs on nutrient agar and agar with 1000-fold diluted nutrient broth, respectively and bacteria of the genus *Pseudomonas* on King's B medium [King et al. 1954]. The numbers of the studied groups of microorganisms per 1 gram fresh mass was determined according to z ISO standard 7218. Moreover, the Most Probable Number of bacteria belonging to the genus *Azotobacter* in semi-liquid nitrogen-free medium with glucose acc. to Fiodorov and in Nfb medium supplemented with malic acid [Rodina 1967, Hegazi et al. 1979] was estimated. The cultures were incubated at 28ºC (±2°C). The presence of Azotobacter was estimated based on microscopic observations of live bacteria after 2 and 7 days of incubation. The results of the numbers of all studied groups of microorganisms were calculated per 1 g dry mass of the plants. To this end, the aerial parts of the plants that were originally used to prepare the stock suspension were dried at 60°C (±2°C) and weighed.

Determination of the taxonomic position of endophytic bacteria and epiphytes dominating the phyllosphere of St. John's wort. Isolates of endophytic bacteria and bacterial epiphytes dominating in the phyllosphere of St. John's wort were subjected to diagnostic examinations based on Bergey's Manual of Determinative Bacteriology. To identify the species of bacteria belonging to the genus *Bacillus* keys for the identification of bacteria [Slepecky and Hemphill 2006] and biochemical tests produced by the firm BioMérieux: Api NE, Api 20E, Api 50CH, were used.

In the case of isolates, for which morphological and biochemical tests did not give explicit results (most of which were G+ rods) molecular determinations were made. Genomic DNA of the studies strains was isolated using a commercial A&A Biotechnology kit (Genomic Mini Ax Bacteria). In turn, for each of the isolates PCR to amplify gene 16S rRNA fragments with size 1300bp was carried out. The primers 63f (5'-CAGGCC TAA CAC ATG CAA GTC-3') and 1387r (5'-GGG CGG WGT GTA CAA GGC-3') [Marchesi et al. 1997] were used. The reaction mixtures contained 100 ng DNA, 10 pM of each starter, 1 × Taq pol buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP and 1 U Taq DNA polymerase. Total sample volume was 25 µl. The reactions were carried out in the following conditions: 5 minutes at 95°C, followed by 30 cycles of 1 minute at 95°C, 1 minute at 55°C, 1.5 minute at 72°C, and 5 minutes at 72°C. Amplification was in a thermal cycler (PTC-1148 MJ Mini Gradient Thermal Cycler, Bio-Rad). The amplified fragments were run in 1% agarose gel, purified with Clean up kit (A@A Biotechnology) and subjected to sequencing using both primers (63f and 1387r) in ABI 3730 Genetic Analyzer (Applied Biosystems) Sequenator. The obtained nucleotide sequences were compared and analyzed using the NCBI GenBank database.

Determination of the taxonomic position of epiphytic microscopic fungi. To identify the epiphytic moulds of St. John's wort diagnostic keys [Gilman 1957, Fassatiová 1983, Piontek 1999] were followed.

Determination of the antifungal activity of endophytic bacteria and epiphytes of St. John's wort was carried out using the diffusion method. Moulds isolated from the phyllosphere of different plants were used as test strains.

Preparation of the mould test strains. The mould test strains were grown on potato-glucose medium until strong spore formation was observed. The cultures were then used to prepare a suspension of spores in sterile saline.

Preparation of bacterial cultures. In order to examine the antifungal activity of epiphytes and endophytes from St. John's wort cultures were set up in nutrient broth and incubated for 48 hours.

Determinations. 0.1 ml of a suspension of mould spores was spread on a Petri dish containing Czapek medium, after which droplets (0.05 ml) of broth cultures of bacteria were deposited on the surface. The plates were incubated for 7 days at 30°C (±2°C). After this time the presence of zones of mould growth inhibition were assayed. Positive results were confirmed using the well method. A suspension of mould spores was plated out as above. Wells were then cut in the medium and the bottom part of each well was filled with a water solution of agar and allowed to solidify. Broth cultures of the different suspensions were added to the separate wells and the plates were then incubated and the results read as described above.

RESULTS

Biodiversity of *Hypericum perforatum* endophytes. Four strains were isolated from the internal stem and leaf tissues of St. John's wort. Two of them were classified as *Alcaligenes faecalis* (isolates E_1 and B_2), and the other two as *Bacillus licheniformis* (isolates B_4 and B_{10}).

Table 1. Endophytic bacteria isolated from the aerial parts of *Hypericum perforatum*

Collection dates	Taxons
July 1	*Alcaligenes faecalis*
July 24	*Bacillus licheniformis*
August 18	*B. licheniformis and A. faecalis*

Number and biodiversity of epiphytes in the phyllosphere of *Hypericum perforatum.* The highest number of epiphytic bacteria was found on nutrient agar. Slightly lower numbers were found on the three other media used, that is agar with 1000-fold diluted broth, Bunt and Rovira medium and King's B medium (tab. 2). The number of phyllobacteria growing on Bunt and Rovira medium and on King's B medium was similar at each collection date. The aerial parts of the plant contained over one hundred thousand cells per 1 gram of dry mass. The number of *Azotobacter* sp. was very low. In general 1 g of dry plant mass contained several score azotobacter cells. The exception were the results obtained for the first collection date when the MPN index for this group of bacteria was ten times higher. The phyllosphere of St. John's wort was not found to contain any *Azospirillum* sp.

Table 2. Enumeration of microorganisms from different physiological groups inhabiting the phyllosphere of *Hypericum perforatum*

Group of microorganisms		cfu × g^{-1} dry plant mass for the different collection dates		
		July 1	July 24	August 18
Bacteria	copiotrophs	3.78×10^6	1.38×10^7	7.54×10^6
	oligotrophs	3.84×10^5	1.81×10^6	5.54×10^5
	on Bunt and Rovira medium	4.42×10^5	7.33×10^5	6.31×10^5
	on King's B medium	4.53×10^5	8.43×10^5	5.35×10^5
Azotobacter sp.	on Fiodorov medium	2.21×10^2	5.11×10	1.26×10
	on NFb medium	4.98×10	2.05×10	2.35×10
Moulds		3.86×10^3	1.63×10^4	3.12×10^4
Yeasts		6.91×10^4	2.33×10^4	3.73×10^4

The number of yeasts in the phyllosphere of St. John's wort was relatively high. In July and August 1 g of dry mass of the aerial parts of the plants was found to contain over ten thousand cells. The number of moulds, however, was lower at the beginning of July than in the second part of the same month or in August.

The dominating moulds were *Cladosporium herbarum*, C. *cladosporioides* and *Alternaria consortiale* (tab. 3), which were present for all three collection date. For two out of the three studied collection dates in the phyllosphere of St. John's wort among the dominating moulds such species as *Alternaria alternata*, *Clonostachys rosea f. catenulata* (*Gliocladium catenulatum*), *Scopulariopsis brevicaulis* and *Penicillium terrestre* were found.

Table 3. Biodiversity of moulds inhabiting the phyllosphere of *Hypericum perforatum*

Mould taxons isolated during collection dates		
July 1	July 24	August 18
Alternaria alternata	*A. consortiale*	*A. alternata*
A.consortialis	*C. cladosporioides*	*A. consortialis*
Cladosporium herbarum	*C. herbarum*	*C. herbarum*
C. cladosporioides	*Fusarium poae*	*C. cladosporioides*
Penicillium terrestre	*Clonostachys rosea f. catenulate*	*Scopulariopsis brevicaulis*
Scopulariopsis brevicaulis	*Penicillium terrestre*	*Trichoderma viride*
	P. chermesinum	*Clonostachys rosea f. catenulata*

Based on phenotypic and molecular analysis of isolates dominating the phyllosphere of St. John's wort the following species were identified: *Burkholderia cepacia* (isolates 3 and 7), *Klebsiella pneumoniae* (isolate 8), *Acinetobacter baumannii* (isolate L7), *Pseudomonas fluorescens* (isolate K2), *Bacillus polymyxa* (isolate B7), *Bacillus cereus* (isolate B9), *Rhodococcus erythropolis* (isolates K14 and L18), *Cellulosimicrobium cellulans* (L11) *Pantoea agglomerans* (*Erwinia herbicola*, *Enterobacter agglomerans*) (isolate 2, L15 and L20), *Pseudomonas putida* (isolate L22), *Rhodococcus* spp. (isolate L13), *Pseudomonas* spp. (isolate 11) (tab. 4).

Table 4. Biodiversity of bacteria inhabiting the phyllosphere of *Hypericum perforatum*

Taxons		Symbol of isolate	Identification method*
Proteobacteria	**β-proteobacteria**	3	a)
	Burkholderia cepacia		
	Burkholderia cepacia	7	a)
	γ-proteobacteria		
	Klebsiella pneumoniae	8	b) 97.7%
	Acinetobacter baumannii	L7	b) 99.4%
	Pseudomonas trivialis or *P. poae* or *P. fluorescens*	11	c) 98%
	Pseudomonas fluorescens	K2	b) 92.3%
	Pseudomonas putida	L22	c) 99%
	Pantoae agglomerans (Erwinia herbicola)	2	b) 100%
	Pantoae agglomerans	L20	c) 100%
	Pantoae agglomerans	L15	c) 100%
Actinobacteria	*Rhodococcus erythropolis*	L13	c) 99%
	Rhodococcus erythropolis	L18	c) 99%
	Rhodococcus erythropolis or *R. globerulus*	K14	c) 99%
	Cellulosimicrobium cellulans	L11	c) 99%
Firmicutes	*Paenibacillus polymyxa*	B7	a)
	Bacillus cereus	B9	a)

* a) Analysis of physiological and morphological properties according to Bergey's Manual of Determinative Microbiologył
* b) API system used for identification (percent of probability in parentheses);
* c) Analysis of 16S rRNA sequences of tested strains-levels of similarity (in parentheses) based on 16S rDNA sequences for tested strains and sequences from GenBank database

Table 5. Antifungal activity of the epiphytic bacteria of *Hypericum perforatum*

Mould	Species of epiphytic bacteria (symbol of isolate)												
	B. polimyxa 41 (B7)	*Pseudomonas sp* (11)	*P. agglomerans* (2)	*R. erythropolis* (L13)	*P. putida* (L22)	*R. erythropolis* (L18)	*B. cepacia*	*A. baumannii* (L7)	*P. agglomerans* (L15)	*B. cereus* (B9)	*B. cepacia*	*P. agglomerans* (L20)	*K. pneumoniae* (8)
A. fumigatus	+	-	-	-	+	-	-	+	+	-	-	-	-
A. flavus	+	+	-	nt.	+	-	nt.	-	+	nt	nt	-	-
A. ochraceus	+	+	+	nt	+	+	nt.	+	+	nt	nt	-	-
A. nidulans	+	+	+	nt	-	+	-	-	+	-	-	+	-
A. niger	+	+	+	-	nt	-	-	+	+	+	-	+	+/-
A. ustus	+	+	+	-	+	-	-	+	+	S	-	S	-
S. chartarum	+	+/-	+	nt	+	-	nt.	+	+	nt	nt	+/-	+
T. viride	+	+	+	nt	+	+	nt	+	+	nt	nt	+	+
B. cinerea	+	nt	-	-	+	nt	-	+	+	nt	-	-	-
F. poae	+	-	-	+	+/-	-	-	-	+	+	-	-	-
F. solani	+	+/-	+	-	+	-	-	+	+	-	-	+	+
F. oxysporum	+	nt	nd	nt	+	nt	nt	-	+	nt	nt	-	-
A. consortiale	+	-	-	nt	nt	nt	nt	+	+	nt	nt	-	-
A. alternata	-	-	-	nt.	+	-	-	-	+/-	nt	-	-	+/-
P. terrestre	+	-	-	-	-	-	-	-	+	-	-	-	-
M. spinosus	-	+	-	-	-	-	-	+/-	-	-	-	-	-
Byssochlamys sp.	+	nt	+	+	+	+	-	-	+	-	-	+	+
Percentage of positive tests	82	50	50	22	73	28	0	50	78	25	0	28	22

* nt – not tested

Antifungal activity of St. John's wort phyllobacteria. The results of studies on the antagonistic properties of the epiphytic bacteria of St. John's wort are presented in Table 5, which show that the broadest spectrum of antifungal activity against the tested moulds was observed for the strains: *Paenibacillus polymyxa* (B7), *Pseudomonas putida* (L22) and *Pantoea agglomerans* (L15). *P. polymyxa* inhibited the growth of over 82% of the studied moulds, *P. agglomerans* (L15) over 77% and *P. putida* over 73%. Two isolates of *P. agglomerans* (2) and *Acinetobacter baumannii* inhibited the growth of 50% of the studied moulds.

DISCUSSION

The studies carried out revealed that the internal tissues of *Hypericum perforatum* leaves and stems can be inhabited by bacteria. However, only two bacterial species were identified, i.e. *Alcaligenes faecalis* and *Bacillus licheniformis*. *A. faecalis*, is known to

occur both in the soil and in clinical material. Consequently, it has to be considered whether the presence of these bacteria in herbal plants could pose a potential threat. On the other hand, bacteria of this species are known to show antagonistic activity towards plant pathogens [Liu et al. 2007, Honda et al. 1998]. Moreover, the antagonistic activity of *A. faecalis* towards larvae of the parasite *Hylesia metabus* [Osborn et al. 2002] has been described. The second endophytic species was *Bacillus licheniformis*. Like *A. faecalis*, it can be classified to the group of plant-associated bacteria. It has been found that the species is able to produce certain plant growth hormones as well as deaminase ACC [Sgroy et al. 2009].

The phyllosphere of *Hypericum perforatum* has been found to be inhabited by a numerous population of bacteria – from $6.02 \cdot 10^6$ to $8.35 \cdot 10^7$ cfu per 1 g of dry mass. Studies of the phyllosphere show that depending on the host organism and external conditions the number of cells in the phyllobacteria community can vary. Karamanoli et al. [2000] has demonstrated that the mean total number of phyllobacteria of the aromatic plants fluctuate between $4 \cdot 10^2$ to $5 \cdot 10^6$ cfu per 1 g of leaf of fresh or dry matter. The smallest quantification of bacteria was detected on Rosemary, Eucaliptus, Salvia and Origanum: 10^2–10^3 cfu per 1 g of leaf [Karamanoli et al. 2000, 2005]. In the phyllosphere of Celery and Lavender $1 \cdot 10^6$ and $5 \cdot 10^5$ cfu per 1 g of fresh weight was detected, respectively. Whereas the enumeration of phyllobacteria on Aloe vera and Oat varied between $1.1–26 \cdot 10^5$ cfu per 1 g of fresh matter [Amir et al. 2007] and $4.6 \cdot 10^5$ cfu per 1 g of dry matter, respectively [Rekosz-Burlaga and Garwolińska 2006]. It seems that the role of the plant host may be of considerable importance. This stems above all from the presence of specific compounds stimulating or inhibiting the growth of microorganisms. The bactericidal properties of St. John's wort have been documented in numerous studies [Mazandarani et al. 2007]. It should be stressed that copiotrophs were the most numerous group among the phyllobacteria of St. John's wort. Their number per 1 g dry mass of the aerial parts of St. John's wort was 10^6, and in July even 10^7. These are very high numbers considering how extreme the phyllosphere habitat is. It is well known that epiphytes are subject to UV radiation and continuous changes in humidity and temperature [Jacobs and Sundin 2001, Lindow and Brandl 2003]. The lack of continuous access to nutrients in the phyllosphere is also of importance. This may explain why oligotrophs accounted for a large part of the population of epiphytic bacteria in St. John's wort. For each of the three collection dates the number of oligotrophs was lower than that of copiotrophs by only one order of magnitude. There are no data in the available literature documenting the number of oligotrophs in a population of epiphytes. The concepts of oligotrophs and copiotrophs are used for populations of water and soil bacteria [Koch 2001]. Jacques and Morris [Jacques and Morris 1995] suggested that to characterize the phyllobacteria community the division adopted earlier for soil bacteria, based on the growth strategies of microorganisms, can be applied. The first of these, strategy-*k*, is characteristic for bacteria with slow growth rate, which are able to cope in environments poor in nutrients. This group corresponds to oligotrophs, which are defined as organisms capable of growth at extremely low concentrations of organic compounds (from 0.2 to 16.8 mg of dissolved $C \cdot dcm^{-3}$) [Schut et al. 1997]. For comparison, phyllobacteria can be distinguished that grow according to strategy-*r* – these are fast-growing bacteria. This group corresponds to copiotrophs/zymogens. Their ana-

logs are saprophytes, eutrophs and heterotrophs, which grow only in environments rich in organic compounds (ca. 1000 mg dissolved C·dcm^{-3}) [Langer et al. 2004]. It seems that the quantitative ratio of these two groups of bacteria may reflect the nutrient properties of the plant host and thus give an answer to the size of the population the phyllosphere can be host to.

Among the phyllobacteria the presence of diazotrophs belonging to the genus *Azotobacter* was also determined. Their number was very low relative to other heterotrophic bacteria, but they can be considered a very valuable source of nitrogen.

The number of yeasts and moulds was similar except for the first collection date when the number of yeasts was more than ten times higher than the number of moulds. Studies conducted by Zimowska and Machowicz-Stefaniak [2004] have demonstrated that the surface of the aerial parts of St. John's wort is colonized by pathogenic and potentially toxin-producing fungi which poses the danger of lowering the quality of the herbal material, and can even be a threat for the maintenance of a plantation. The roots of St. John's wort can be colonized by different species of fungi belonging to the genera: *Fusarium* (*F. avenaceum, F. culmorum*, *F. equniseti*, *F. oxysporum*, *F. solani*), *Rhizoctonia* and *Phoma* [Zimowska and Machowicz-Stefaniak 2004]. Diseases are also caused by *Cladosporium cladosporioides*, *Alternaria alternata*, *and A. consortiale*. No presence of *Seimitosporium hypericinum* has been found. This particular species [Zimowska 2004a] poses a considerable threat for plantations of St. John's wort and strongly reduces the quality of the herbal material. Its presence on the aerial parts of the plant in manifested by red-amber spots. Zimowska [2004a] found that some of the moulds found in the phyllosphere of St. John's wort efficiently inhibited the growth of the pathogen. These included *Trichoderma* sp. and *Gliocladium roseum* (*Clonostachys rosea*) [Zimowska 2004b]. The antagonism of epiphytes towards plant pathogens may be a very advantageous property from the viewpoint of the host. The literature contains many examples of the practical use of this activity in biocontrol. Studies on the antifungal activity of bacteria inhabiting the phyllosphere of St. John's wort involved moulds of the genera: *Aspergillus*, *Fusarium*, *and Alternaria* were selected. Moreover, *Stachybotrys chartarum*, *Byssochlamys* sp., *Trichoderma viride*, *Penicillium terrestre* and *Botrytis cinerea* were also included in the studies. The broadest spectrum of antifungal activity was demonstrated by strains of *P. polymyxa*, *P. agglomerans* and *A. baumannii*. *P. polymyxa* is a species that can be employed in biocontrol. The antifungal properties of *P. polymyxa* were also described in other studies [Bunt and Rovera 1955, Nielsen and Sørensen 1996, Beatty and Jansen 2002, Cho et al. 2007, Haggag 2008, Mageshwaran et al. 2012]. Its activity against bacterial pathogens has also been observed [Li et al. 2011]. The *P. polymyxa* isolate from the phyllosphere of St. John's wort was active against *Botrytis cinerea*, known as the gray mould, which is the causative agent of many plant diseases and causes the spoiling of plant products. According to Zimowska and Machowicz-Stefaniak [2004] *Botrytis cinerea* plays a significant role in causing diseases of St. John's wort and is a frequent cause of reduced quality of the herbal material. The epiphyte of St. John's wort *P. polymyxa* was also active against three test moulds of the genus *Fusarium*, which are plant pathogens too. One of these species, *F. oxysporum*, is mentioned as the cause of diseases of St. John's wort. One of the isolates belonging to the species *Pantoea agglomerans* L15 also demonstrated antagonistic activity towards

B. cinerea and the test strains of *Fusarium* sp. It is interesting that in the case of the two other isolates from this species, i.e. isolates 2 and 20, no antagonism towards gray mould was observed. The third strain demonstrating high antifungal activity is *Acinetobacter baumannii*. The literature shows that under laboratory conditions the species produces substances inhibiting the growth of some plant pathogens [Liu et al. 2007].

CONCLUSIONS

1. *Hypericum perforatum* phyllosphere is settled by different microbial communities of moulds, yeasts and bacteria. The oligotrophic and copiotrophic bacteria were isolated. Gram-negative bacteria were dominant in tested phyllosphere.

2. Plant pathogens (*Alternaria alternata*) and their antagonists (*Pseudomonas putida*) were isolated from *H. perforatum* phyllosphere.

3. From two *H. perforatum* epiphytes identified as *Pantoea agglomerans* (L15, L20), only one strain, L15, had strong antifungal properties so these is a strain-dependent properties.

4. The evaluation of potential to use of the tested endophytic bacteria as a biocontrol agents needs further researches.

5. The above ground parts of *H. perforatum*, phyllosphere and endosphere, are settled by human pathogens, like: *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Alcaligenes faecalis*. Therefore, commercial use of these herb should be preceded by hygienization treatment.

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BIORÓŻNORODNOŚĆ HODOWALNYCH MIKROORGANIZMÓW ZASIEDLAJĄCYCH NADZIEMNE CZĘŚCI *Hypericum perforatum*

Streszczenie. W przeprowadzonych badaniach oceniono występowanie bakterii endofitycznych w tkankach części nadziemnych *Hypericum perforatum* oraz poznania bioróżnorodności wspólnoty mikroorganizmów zasiedlających fyllosferę dziurawca. Rośliny zebrano na terenie Polski trzykrotnie w sezonie wegetacyjnym. W ich częściach nadziemnych oznaczono obecność bakterii endofitycznych oraz liczebność wybranych grup drobnoustrojów zasiedlających fyllosferę. Wyizolowane endofity oraz dominujące epifity poddano badaniom w celu oznaczenia ich przynależności taksonomicznej, a następnie oceniono ich aktywność antagonistyczną w kierunku wybranych pleśni. Z endosfery dziurawca wyizolowano szczepy należące do dwóch gatunków: *Alcaligenes faecalis* i *Bacillus licheniformis*. W fyllosferze dziurawca liczebność *Azotobakter* sp. była bardzo niska, natomiast najliczniejszą grupą były kopiotrofy, nieco mniej liczną bakterie oligotroficzne, bakterie wyrosłe na podłożach Bunta i Roviry i Kinga B oraz pleśnie i drożdże. Wśród pleśni dominujących stwierdzono *Cladosporium herbarum*, *C. cladosporioides* oraz *Alternaria consortialis*, *A. alternata*, *Clonostachys rosea f. catenulata* (*Gliocladium catenulatum*), *Scopulariopsis brevicaulis i Penicillium terrestre*. Wśród fyllobakterii dominowały w fyllosferze: *Burkholderia cepacia*, *Klebsiella pneumoniae*, *Acinetobacter baumannie*, *Pseudomonas fluorescens*, *P. putida Pantoea agglomerans*, *Paenibacillus polymyxa*, *Bacillus cereus*, *Rhodococcus* sp. *R. erythropolis Cellulosimicrobium cellulans*. Najszersze spektrum aktywności przeciwgrzybowej wobec testowanych pleśni stwierdzono dla szczepów *Paenibacillus polymyxa*, *Pseudomonas putida* oraz *Pantoea agglomerans*. *P. polymyxa* ograniczał wzrost ponad 82% badanych pleśni, *P. agglomerans* ponad 77%, a *P. putida* ponad 73%.

Słowa kluczowe: mikroorganizmy zasiedlające zioła, dziurawiec, endofity, epifity, właściwości fungistatyczne

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