

Effects of some terricolous lichens [*Cladonia rangiformis* Hoffm., *Peltigera neckerii* Hepp ex Müll. Arg., *Peltigera rufescens* (Weiss) Humb.] on soil bacteria in natural conditions

A.U. Akpınar¹, S. Oztürk¹, M. Sinirtas²

¹Department of Biology, Faculty of Art and Sciences, University of Uludağ,
Gorukle-Bursa, Turkey

²Department of Microbiology, Faculty of Medicine, University of Uludağ, Gorukle-Bursa,
Turkey

ABSTRACT

This paper is aimed to investigate the effects of some terricolous lichens on soil bacteria's growth in natural conditions. It is focused on species of bacteria and also on numbers of colony of soil specimens that were taken from substrates of three different terricolous lichen species. *Peltigera rufescens* (Weiss) Humb., which has not secondary metabolites, did not show an inhibition effect on soil bacteria. However, *Peltigera neckerii* Hepp ex Müll. Arg., which has secondary metabolites, has a negative effect on soil bacteria's growth. Besides, it was observed that *Cladonia rangiformis* Hoffm., which has many kinds of secondary metabolites, has the highest inhibition effect among the studied species. For this reason, we think that in the next researches, it is convenient to investigate elaborately by soil analysis the effect of lichen's secondary metabolites that have an effect on soil mineralization, on soil bacteria's growth.

Keywords: lichens; secondary metabolites; soil bacteria

Lichens are formed through symbiosis between fungi and algae or cyanobacteria. Lichens commonly grow on rocks, barks, soil and synthesize numerous metabolites, 'lichen substances' including aliphatic, cycloaliphatic, aromatic and terpenic components. Numerous and various biological activities of lichens and their metabolites are known, such as: antiviral, antibiotic, antitumoural, antiallergic, antiherbivorous and they inhibit growth of plants as well as various enzymes (Rankovic et al. 2007). Some lichens can accumulate high concentrations of secondary metabolites that appear to form constitutive defenses against both grazers (Giez et al. 1995) and pathogens (Lawrey 1997).

Lichens can become physically damaged in many ways. Perhaps the most common being as a result of herbivore feeding, for example by slugs, springtails, oribatid mites and larger animals such as reindeer. In addition to the direct loss of tissues caused by injury, opportunistic plant pathogens

may gain entry to lichen tissues through wound sites (Beckett and Minibayeva 2003). Certain obligate fungivorous animals and fungal parasites (Lawrey 1997) consume lichens, which suggest that tolerance to certain lichen compounds may play a role in the ecology of these organisms. Indeed, there is some evidence that lichen parasites are generally more tolerant of lichen compounds than nonlichenicolous fungi (Lawrey 1997). Nonetheless, various studies have demonstrated that extracts and lichenic acids play various biological roles and appear to function as allelopathic agents in nature. Lawrey (1977a, b) showed that lichen extracts and lichen products inhibit spore germination of several mosses *in vitro*. A study carried out by Gardner and Mueller (1981) showed that eight tested lichenic acids inhibited spore germination and sporeling growth of *Funaria hygrometrica*. Similarly, toxic effects of *Cladonia foliacea* on the growth of bryophytes were observed. Vartia

(1950) tested nine crystalline lichenic acids on eleven species of bacteria and observed antibiotic properties against Gram positive bacteria. Vartia (1973) reviewed studies on the antibiotic effects of lichen substances; i.e. usnic acids, the lichesterinic acid group, orcinol-type depsides and depsidones demonstrating to be the most effective substances to reduce bacterial growth. Halama and Haluwin (2003) studied the antibacterial activity of nine lichens and observed activity mainly against Gram positive bacteria, but also observed some activity against Gram negative bacteria in the case of three lichens.

In Rancovic's paper (2007), the highest antibacterial activity was found in usnic acid, comparable with the one given by the streptomycin standard. It inhibited all the tested bacteria in extremely low concentrations. Usnic acid also demonstrated a good antifungal effect, but in a higher concentration compared with the antibacterial one. In the same paper, the results showed that the tested lichen substances demonstrated a strong antimicrobial effect against the tested microorganisms (Rankovic et al. 2007).

Accordingly, the purpose of the presented work was to investigate effect of secondary metabolites that transferred from thalli of lichens to soil in natural conditions. We also investigated antibacterial effect of some lichens (*Peltigera* sp. and *Cladonia* sp.); we dealt with lichen genera that are located in soil and we also observed whether these genera have secondary metabolites or not.

MATERIAL AND METHODS

Soil samples collection. Lichen thalli which were tested in this paper, were collected in two different locations: on 16th March, 2008 in the Sadag Canyon; altitude: 205 m, N: 40°11'36'', E: 29°04'40''; on 21st April, 2008, in the Campus of Uludag University; altitude: 150 m, N: 40°15'00''E: 28°52'49''.

Peltigera rufescens and *Peltigera neckerii* were collected from the Sadag Canyon; *Cladonia rangi-*

formis was taken from *Quercus* spp. forest in the University of Uludag.

In field experiment, we measured the diameter of lichens thalli. First of all, we took soil samples from underneath of thalli (Control or Zone 0). Then, soil samples were taken from directions of south, north, east and west in the same distances [10 cm (Zone 1); 30 cm (Zone 2); 50 cm (Zone 3)] from thalli of lichen. Therefore, we collected totally 13 soil samples for each specimen.

Preparation of soil solutions and identifications of bacteria. Soil solutions were prepared in laboratory conditions to determine bacteria colonies in soil samples taken from the defined zones. To prepare a soil solution in 10⁻² density, first, 0.5 g soil sample was taken and distilled water was added to it. Thus, we obtained totally 50 ml soil solution in 10⁻² density (the first solution).

Numbers of bacteria colonies were however too high in the first solution to be counted. Therefore, we decided to prepare a soil solution in 10⁻⁴ density by decreasing density of the first solution. For this reason, distilled water was added to 500 µl of the first solution to obtain totally 50 ml soil solution in 10⁻⁴ density (the last solution).

1000 µl of the last solution was used to inseminate Petri dishes with diameter of 9 cm. Then, each Petri dish was incubated at 36°C, for 24 hours. After incubation, morphologically different colonies were counted. According to Gram negative or Gram positive properties, each of these bacteria was identified by painting. Finally, the bacteria species were closely analysed by using the crystalline methods in the Department of Microbiology, University of Uludag.

RESULTS

In Table 1, it is shown that species of *Bacillus* sp., *Burkholderia gladioli* and *Corynebacterium bovis* are present in soil of all three lichen thalli. Besides, it was determined that *Aerococcus* sp. existed in the soil around *Cladonia rangiformis* and similarly, species of *Dermaococcus nishinomiyaensis* were

Table 1. Bacteria determined in the soil of lichen thallus

Lichen species			Bacteria species		
<i>Cladonia rangiformis</i>	<i>Bacillus</i> sp.	<i>Burkholderia gladioli</i>	<i>Corynebacterium bovis</i>	<i>Aerococcus</i> sp.	
<i>Peltigera neckerii</i>	<i>Bacillus</i> sp.	<i>Burkholderia gladioli</i>	<i>Corynebacterium bovis</i>	<i>Dermaococcus nishinomiyaensis</i>	
<i>Peltigera rufescens</i>	<i>Bacillus</i> sp.	<i>Burkholderia gladioli</i>	<i>Corynebacterium bovis</i>	–	

Table 2. Bacteria colony amounts in soil of around *Cladonia rangiformis*

Species of bacteria	<i>Bacillus</i> sp.	<i>Burkholderia gladioli</i>	<i>Corynebacterium bovis</i>	<i>Aerococcus</i> sp.	Total amounts of colony
Control	–	34	8	1	43
Zone 1	1	52	16	–	69
Zone 2	1	86	14	4	105
Zone 3	6	84	27	–	117

recorded in soil around *Peltigera neckerii* thalli. Consequently, 5 bacteria species were evaluated in the observation of effect of lichen substances on soil (Table 1).

As for bacteria density in the soil samples taken from *Cladonia rangiformis* thalli, we found that density of *Corynebacterium bovis* was lower than *Burkholderia gladioli* and both colony amounts increased with distance. *Bacillus* sp. was very low in this soil, with no colony in Control 0, and only one colony was found in Zone 1 and Zone 2; however, *Bacillus* sp. had six colonies in Zone 3. Another bacteria reported in soil samples around *Cladonia rangiformis* was *Aerococcus* sp. Its colony amounts increased little between Control 0 and Zone 2 compared with other zones. The results related to density of bacteria colony around *Cladonia rangiformis* showed generally (not important whether it is Gram positive or Gram negative) that lichen metabolites, revealed from thalli to soil, were of changeable ratios of inhibition effect on soil bacteria, according to distance from lichen thalli. This result was the same also in bacteria colony amounts and bacteria diversity (Table 2).

Table 3 shows density of bacteria belonging to soil around *Peltigera neckerii*. In this table, it is shown that there are four bacteria in soil samples around thalli. Colony density of *Burkholderia gladioli*, Gram negative bacteria, was higher in Zone 1 and 2 than in Control 0. Similarly, colonies of *Bacillus* sp. and *Corynebacterium bovis* were little in Control 0 and increased in Zone 1. *Dermacoccus nishinomiyaensis* was reported in

soil samples around thalli as well. However, colony amounts of these bacteria did not show any changes between Control 0 and other zones, as other bacteria species. We reported one colony of these bacteria in Zone 2.

In the field experiments, thalli of *P. neckerii* were close to each other and thus, we were careful to take soil samples. As shown in Table 3, inhibition in Zone 2 decreased and at the same time, inhibition effect appeared in Zone 3 and the amounts of colony decreased again. It was shown that these results were the same for all soil microorganisms around lichen thalli.

Findings on bacteria density in soil samples around *Peltigera rufescens* thalli showed that control results and zones were not much different in species and total density areas (Table 4).

DISCUSSION

Although it is known that phenolic secondary metabolites in lichens have an antimicrobial effect on soil microorganisms, there are not many researches which have done observation under natural conditions in this field (Stark et al. 2007).

In literature, it is indicated that there are many metabolites in *Cladonia foliacea* and usnic acid is the most frequent in the metabolites of *Cladonia* sp. (Stark et al. 2007). Usnic acid, one of the most common lichen compounds, is widely found in many lichen genera, e.g. *Cladonia*, *Ramalina*, *Usnea* and others. The biological activities of usnic acid

Table 3. Bacteria colony amounts in soil of around *Peltigera neckerii*

Species of bacteria	<i>Bacillus</i> sp.	<i>Burkholderia gladioli</i>	<i>Corynebacterium bovis</i>	<i>Dermacoccus nishinomiyaensis</i>	Total amounts of colony
Control	6	24	12	–	42
Zone 1	8	47	18	–	75
Zone 2	6	212	14	1	234
Zone 3	4	38	16	–	60

Table 4. Bacteria colony amounts in soil of around *Peltigera rufescens*

Species of bacteria	<i>Bacillus</i> sp.	<i>Burkholderia gladioli</i>	<i>Corynebacterium bovis</i>	Total amounts of colony
Control	2	263	10	275
Zone 1	4	273	10	287
Zone 2	3	273	12	288
Zone 3	2	268	8	278

such as antiviral (Scirpa et al. 1999), antifungal (Lauterwein et al. 1995, Lawrey 1995, Cocchietto et al. 2002), antiprotozoal (Fournet et al. 1997), antiproliferative, antiherbivoral (Huneck 1999), analgesic (Vijayakumar et al. 2000), hepatotoxic (Pramyothin et al. 2004) have been reviewed by Ingolfssdottir (2002). Rankovic et al. (2007) determined that usnic acid has the strongest antimicrobial activity. Otherwise, it was determined that usnic acid has also an insecticidal effect against the larvae of house mosquito, *Culex pipiens* L. (Cetin et al. 2008). According to the present paper, it was observed that *Cladonia foliacea* was the most effective between the other lichens against soil bacteria. We thus suppose that inhibition effect of *Cladonia foliacea* on soil bacteria is due to having large amounts secondary metabolites.

Rowe et al. (1989) studied the antibacterial activity of nine lichens and observed activity mainly against Gram positive bacteria. However, it was not proved that these phenolic secondary compounds in lichens are also effective against Gram negative bacteria. It is known that depsides, depsidones (Lawrey 1986) and usnic acid are active against Gram positive microorganisms (Huneck and Yoshimura 1996). However, Halama and Haluwin (2003) observed that three lichens have some inhibition effects against Gram negative bacteria as well. Similarly, Rankovic et al. (2008) showed that the secondary metabolites of *Physcia aipolia*, *Umbilicaria polyphylla*, *Parmelia caperata* and *Hypogymnia physodes* have antimicrobial effect on some of Gram negative and Gram positive bacteria. Our findings showed that lichen substances have an effective inhibition on Gram negative bacteria's grown in soil.

According to the results presented in this paper, we think that inhibition effect on soil bacteria around thalli of lichen species (*Peltigera neckerii* and *Peltigera rufescens*) is comparatively changeable with having secondary metabolites amounts and variety. Brodo et al. (2001) observed that *P. rufescens* has not secondary metabolites. In our

findings, it was shown that *Peltigera rufescens*, which has no secondary metabolites, did not show an inhibition effect on soil bacteria.

Gardner and Mueller (1981) pointed out that usnic acid has not a phytotoxic effect in lichen-dominated areas. Also, it was emphasized that pH of soil is an effective factor in activity. Nevertheless, we observed that soil bacteria *P. neckerii* showed higher inhibition effect than *P. rufescens*, although *P. neckerii*, that has secondary metabolites, and *P. rufescens*, that has not secondary metabolites, were collected from the same location.

When we look at Table 3, inhibition effect in Zone 2 decreased. Following to this, inhibition effect appeared in Zone 3 and amounts of colony decreased again. We think that inhibition between Zone 2 and 3 occurs with effect of other closed thalli for the same species.

This study is a pre-assay to investigate the effect of favored lichen thalli on soil bacteria in the natural conditions. We think that it will be benefit to investigate microbial effect on soil around different lichen thalli in natural conditions and effect on other different bacteria of the lichen with secondary metabolites which were extracted from lichen thalli.

Acknowledgements

I would like to thank everyone who has helped me in the research, and experiment stages. I also want to thank Dr. Atilla Akpinar for proof reading of the paper and Aras. Gör. Fatma Tugba Dinc for helping me in laboratory. I also thank my precious family for their support.

REFERENCES

- Beckett R.P., Minibayeva F.V. (2003): Wounding induces a burst of extracellular superoxide production in *Peltigera canina*. *Lichenologist*, 35: 87–89.

- Brodo I.M., Sharnoff S.D., Sharnoff S. (2001): Lichens of North America. Yale University Press, London.
- Cetin H., Tufan O., Ozdemir T.A., Tay T., Candan M., Yanikoğlu A., Sümbül H. (2008): Insecticidal activity of major lichen compounds, (–) and (+) usnic acid, against the larvae of house mosquito, *Culex pipiens* L. *Parasitology Research*, 102: 1277–1279.
- Cocchietto M., Skert N., Nimis P.L., Sava G. (2002): A review on usnic acid, an interesting natural compound. *Naturwissenschaften*, 89: 137–146.
- Fournet A., Ferreira M.E., Rojas de Arias A., Torres de Ortiz S., Inchausti A., Yaluff G., Quilhot W., Fernandez E., Hidalgo M.E. (1997): Activity of compounds isolated from Chilean lichens against experimental cutaneous leishmaniasis. *Comparative Biochemistry and Physiology B*, 111: 69–74.
- Gardner C.R., Mueller D.M.J. (1981): Factors affecting the toxicity of several lichen: effect of pH and lichen acid concentration. *American Journal of Botany*, 68: 87–95.
- Giez I., Lange O.L., Proksch P. (1995): Growth retarding activity of lichen substances against the polyphagous herbivorous insect *Spodoptera littoralis*. *Biochemical Systematics and Ecology*, 22: 113–120.
- Halama P., Haluwin C. van (2003): Antifungal activity of lichen extracts and lichenic acids. *BioControl*, 49: 95–107.
- Huneck S. (1999): The significance of lichens and their metabolites. *Naturwissenschaften*, 86: 559–570.
- Huneck S., Yoshimura I. (1996): Identification of Lichen Substances. Springer-Verlag, Berlin.
- Ingolfssdottir K. (2002): Usnic acid. *Phytochemistry*, 61: 729–736.
- Lauterwein M., Oethinger M., Belsner K., Peters T., Marre R. (1995): In vitro activities of the lichen secondary metabolites vulpinic acid, (+)-usnic acid and (–)-usnic acid against aerobic and anaerobic microorganisms. *Antimicrobial Agents and Chemotherapy*, 39: 2541–2543.
- Lawrey J.D. (1986): Biological role of lichen substances. *The Bryologist*, 89: 111–122.
- Lawrey J.D. (1977a): Adaptive significance of O-methylated lichen depsides and depsidones. *Lichenologist*, 9: 137–142.
- Lawrey J.D. (1977b): Inhibition of moss spore germination by acetone extracts of terricolous *Cladonia* species. *Bulletin of the Torrey Botanical Club*, 194: 49–52.
- Lawrey J.D. (1995): The chemical ecology of lichen mycoparasites: a review. *Canadian Journal of Botany*, 73: S603–S608.
- Lawrey J.D. (1997): Isolation, culture and degradative behavior of the lichen parasite *Hobsonia santessonii*. *Symbiosis*, 23: 107–116.
- Pramyothin P., Janthasoot W., Pongnimitprasert N., Phrukdom S., Ruangrunsi N. (2004): Hepatotoxic effect of (+) usnic acid from *Usnea siamensis* Wainio in rats, isolated rat hepatocytes and isolated rat liver mitochondria. *Journal of Ethnopharmacology*, 90: 381–387.
- Ranković B., Misić M. (2007): Antifungal activity of extract of the lichens *Alectoria sarmentosa* and *Cladonia rangiferina*. *Mikologija i Fitopatologija*, 41: 276–281.
- Ranković B., Misić M., Sukdolak S. (2008): The antimicrobial activity of substances derived from the lichens *Physcia aipolia*, *Umbilicaria polyphylla*, *Parmelia caperata* and *Hypogymnia physodes*. *World Journal of Microbiology and Biotechnology*, 24: 1239–1242.
- Rowe J.G., Saez M.T., Garcia M.D. (1989): Contribution à l'étude de l'activité antibactérienne de quelques lichens du sud de l'Espagne. *Annales pharmaceutiques françaises*, 47: 89–94.
- Scirpa P., Scambia G., Masciullo V., Battaglia F., Foti E., Lopez R., Villa P., Malecore M., Mancuso S. (1999): A zinc sulphate and usnic acid preparation used as post-surgical adjuvant therapy in genital lesion by human papillomavirus. *Minerva ginecologica*, 51: 255–260.
- Stark S., Kytöviita M.M., Neumann A.B. (2007): The phenolic compounds in *Cladonia* lichens are not antimicrobial in soils. *Oecologia*, 152: 299–306.
- Vartia K.O. (1950): Antibiotics in lichens. II. *Annals of Medical Biology Fennicae*, 28: 7–19.
- Vartia K.O. (1973): Antibiotics in lichens. In: Ahmadijan V., Hale M.E. (eds): *The Lichens*. Academic Press, New York, 547–561.
- Vijayakumar C.S., Viswanathan S., Kannappa M., Parvathavarthini S., Kundu A.B., Sukumar E. (2000): Anti-inflammatory activity of (+) usnic acid. *Fitoterapia*, 71: 564–566.

Received on November 24, 2008

Corresponding author:

Aysegül Ugur Akpınar, University of Uludağ, Faculty of Art and Sciences, Department of Biology, Gorukle-Bursa, Turkey
e-mail: augur@uludag.edu.tr
