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Bacteria isolated from injuries to growing spruce trees *(Picea abies* (L.) Karst.)

Kasvavien kuusien vaurioista eristetyt bakteerit

Tauno Kallio

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BACTERIA ISOLATED FROM INJURIES TO GROWING SPRUCE TREES (PICEA ABIES (L.) KARST.)

TAUNO KALLIO

SELOSTE:

KASVAVIEN KUUSIEN VAURIOISTA ERISTETYT BAKTEERIT

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INTRODUCTION AND STUDY OUTLINE

It has long been known that bacteria participate in the decomposition of wood material. Not until recently has any cooperation between bacteria and fungi in wood decomposition been shown (McCreary et al. 1965). Most studies of wood decay use substrates not favouring bacterial growth. For this reason the role of bacteria in the decomposition of wood is easily underestimated. Fungi decompose wood material more quickly when its nitrogen content is high (Cowling and Merrill 1966). The role of bacteria in the decay of living trees has acquired new importance since it was shown that some of the bacteria isolated from injuries in *Abies concolor* (Gord.) Engelm. were able to bind atmospheric nitrogen (Seidler et al. 1972).

The present work was the continuation of a study reported on earlier (Kallio 1973), in which fungi and bacteria were isolated from one-year old injuries to growing spruce trees. One of the substrates used in the said study had been specifically developed for bacteria (Taylor 1951). The number of bacterial populations isolated was unexpectedly high. For this reason it was considered useful to investigate the properties of these bacteria, especially since only bacteria could be isolated from a few injuries.

The purpose of the present study was to describe the bacteria, by means of bacteriological tests, infecting growing spruce injuries, and to ascertain, using laboratory tests, the possible antagonism of bacteria that had grown alone in spruce injuries, to three spruce-decaying fungi.

MATERIAL AND METHOD

The material comprised 91 bacterial colonies isolated from injuries in growing spruce trees. Four of these, one year after infliction of the injury, were growing alone at the site of the injury. Several others were accompanied by fungal growth.

The bacteria were spread onto TGE agar on dishes (SKERMAN 1969) using a 10^{-3} dilution. After a few days, macroscopically different bacterial colonies were collected and re-spread in the same way as above. This was repeated 3—6 times until pure colonies were obtained. They were then gram-stained. The staining revealed that some of the colonies were still mixed. Attempts to respread them failed: they resulted either in the same mixed colonies or else the bacteria died.

After gram-staining, the fermentative or oxidative metabolism of the bacterial populations was tested (HUGH and LEIFSON 1953). The bacterial capacity for cellulose utilisation was tested with cellulose agar (EGGINS and PUGH 1962), and also with various other substrates containing cellulose (BASU and CHOSE 1960, SKINNER 1960), with the nitrogen of the substrate either in organic or inorganic form. When the substrate contained inorganic nitrogen, a trace element solution containing manganese, boron, copper, zinc and cobalt was added.

RESULTS

Bacteria were found from 44 injuries, 26 per cent of the total. Infection was most common in March—April and in June. No infection occurred in January, and it was very infrequent also in December and February. Bacterial infection was most frequent in sapwood injuries of roots above soil level (for a description of injuries, see KALLIO 1973, p. 5). Twenty-four (c. 55 per cent) of the bacterial colonies collected had been caused by root damage. Twelve colonies (27 per cent) were isolated from injuries made with increment borer at breast height and extending to heartwood. Seven colonies (16 per cent) infected injuries made at breast height in sapwood, and one (c.2 per cent) infected an injury made by increment borer at stump height and extending to heartwood.

A total of 91 bacterial populations were isolated from the injuries. They were stored for 6—12 months in refrigerator on storage agar, which was similar to the TGE agar except that its agar content was 3 g/l. Eighty-three of these colonies grew. In connection with purification they were spread, whereby 162 bacterial colonies were obtained. Of these, 89 (55 per cent) were gram-positive rods, 47 (29 per cent) gram-negative rods, 8 (5 per cent) gram-variable rods, 10 (6 per cent) gram-positive cocci, 5 (3 per cent) gram-negative cocci, and 3 (2 per cent) gram-variable cocci.

In 106 (65 per cent) of the bacterial colonies the metabolism was fermentative, in 22 (14 per cent) slowly fermentative, in 11 (7 per cent) oxidative, in 13 (8 per cent) slowly oxidative and in 10 (6 per cent) alkalizing.

The bacteria utilizing cellulose made up a total of 30 colonies (19 per cent). Of these, 24 (15 per cent) required the presence of inorganic nitrogen, 5 (3 per cent) the presence of organic nitrogen, and c. 1 per cent the presence of either form of nitrogen, to be capable of cellulose utilization. The above figures include all bacteria with a capacity of cellulose utilization, regardless of how weak it was.

ANTAGONISM

There were four injuries to growing spruce trees from which, one year after the damage, only bacteria were isolated: 1. an increment borer injury made non-aseptically on April 14, 1971, at stump height in a control spruce of the dominant crown layer, 2. a root damage infected with *Peniophora gigantea* (Fr.) Massee in a spruce of the dominant crown layer on April 14, 1971, 3. a root damage made on June 14, 1971, in a control spruce of the dominant crown layer, and 4. a trunk sapwood injury made on June 14, 1971, in a spruce of the suppressed crown layer infected with *P. gigantea*.

Antagonism tests on malt agar to *Fomes annosus* (Fr.) Cooke, *Stereum sanguinolentum* (A & S.) Fr., and *P. gigantea* showed that the bacterial colony of Item 4. was highly antagonistic to all these fungi (Fig. 1). This antagonism on malt agar was relatively much stronger than e.g. the antagonism of *P. gigantea* to *F. annosus* (cf. KALLIO 1971).

The antagonistic bacterial strain formed a colony containing solely gram-negative

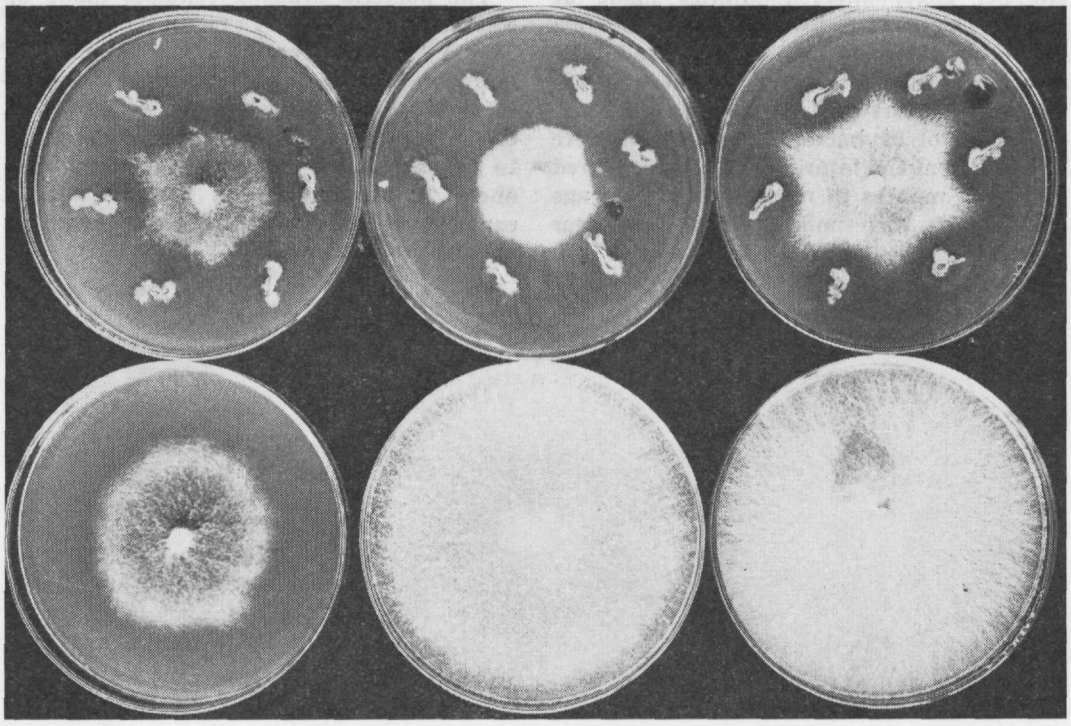

Fig. 1. Antagonism of bacteria 10 days after inoculation. Below, controls, above, the fungi inoculated into the centre of bacterial colonies. From the left *S. sanguinolentum*, *P. gigantea*, and *F. annosus*.

Kuva 1. Bakteerikannan antagonistisuus 10 vrk kuluttua ymppäämisestä. Alhaalla kontrollit, yläpuolella sienet ympättynä bakteerikasvustojen keskelle. Vasemmalta S. sanguinolentum, P. gigantea ja F. annosus.

rods (Fig. 2). It was slowly oxidative and unable to utilize cellulose in the presence of either organic or inorganic nitrogen. Bacterial culture tests revealed that this bacterial strain showed pleomorphism reminiscent of the *Corynebacterium* family (Fig. 3).

Fig. 2. Antagonistic rod bacteria, magn. c. ~~2000~~ 1500 x.

Kuva 2. Antagonistisia sauvamaisia bakteereita. Suurennus n. 1500 x.

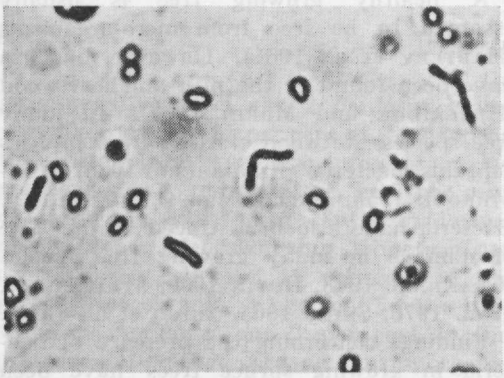

Fig. 3. Pleomorphic forms of the antagonistic bacterium, magn. c. 1100 x.

Kuva 3. Antagonistisen bakteerin pleomorfisia muotoja. Suurennus n. 1100 x.

DISCUSSION

A healthy growing tree is usually assumed to be free from micro-organisms (HARTLEY et al. 1961). However, bacteria have been found in the pith and heartwood (ETHERIDGE and MORIN 1967). In many tree species, wetwood starting from injuries contains bacteria (LAGERBERG 1935, HORNIBROOK 1950, WILCOX and OLDHAM 1972). Bacteria have also been traced at the site of damage in many growing tree species (GRANDALL 1943, BUTIN 1960, STRIVASTAVA et al. 1970, SHIGO 1965, SHIGO et al. 1971).

Findings concerning the presence of bacteria in growing spruce trees have been reported also from Scandinavia (LAGERBERG 1935, ROLL-HANSEN 1970, KALLIO 1971, LUNDEBERG 1972). However, no detailed data on the bacterial species involved are available. Nor was any determination of the bacterial species attempted in the present study. The fact that bacteria were isolated from practically every fourth damage indicates, however, the high frequency of bacteria in spruce injuries.

A large proportion of the bacteria infected sapwood injuries close to soil level. There may be several reasons for this, e.g. the structure of wood in the root, environmental factors (temperature, humidity, daylight), or nearness of a strong source of contamination. A large proportion of all bacteria had a fermentative metabolism. Only a total of 15 per cent were oxidative or slowly oxidative. On this basis it is easy to understand that bacteria are often isolated from the tips of discoloration starting from the injuries and extending deep into the wood material (cf. ISOMÄKI and KALLIO 1974).

The role of bacteria in the wood decomposition process is probably connected both with the primary decomposition (LIESE and KARNOP 1968, GREAVES 1971) and with the complicated ecological processes in which a microbial population decomposes wood material (SHIGO 1965, MALOY and ROBINSON 1968). A large number of the present bacteria had no capacity for cellulose utilization, and a fermentative metabolism. The main role of these bacteria is probably to destroy the metabolic products of other microbes in wood.

The antagonism noted in the present study between bacteria and other microbes is no new phenomenon. Several reports have been published earlier concerning the inhibitory effect of bacteria on wood-inhabiting fungi. Bacteria have been found to inhibit the infection of the roots of pine species by *Phytophthora cinnamom* Rands (MARX and BRYAN 1969). Bacteria are also known to inhibit decomposition of cellulose (HENIS et al. 1961). *Cytophaga* bacteria have been used to control the damping-off and root disease in seedling nurseries (HOCKING and COOK 1972).

The role of bacterial populations in the wood decomposition process has come under new light after the presence of *Clostridium* spp. in the decomposition process of growing trees has been shown (SHIGO et al. 1971). Many *Clostridium* species are able to bind atmospheric nitrogen. It has later been shown that wood-inhabiting bacteria really do bind nitrogen from the air (SEIDLER et al. 1972). Bacteria, therefore, can increase the amount of nitrogen available to fungi. On the other hand, bacteria can effectively compete with fungi for the available nitrogen. They can also produce biostatic substances in their neighbourhood.

Bacteria antagonistic to the most important decay fungi are relatively easy to isolate from e.g. the wood material and phloem of damaged spruce trees. However, antagonism in laboratory conditions does not guarantee antagonism in nature. A bacterial strain isolated in the present study remained as the only microbe in the same injury for a whole year. The injury was infected with *Peniophora gigantea* which, however, failed to start growing, nor could any other fungus be isolated from the injury in question. However, the bacterial strain involved requires field tests before its use can be started e.g. to provide protection against fungal infection for injuries caused by mechanical timber harvesting.

SUMMARY

Infection of living spruce trees by bacteria, and the properties of these bacteria were studied. Bacterial antagonism to three spruce-decaying fungi was also studied in laboratory conditions.

Bacteria occurred in 26 per cent of the total number of spruce injuries. Bacterial infection was most frequent in injuries made in March—April and June, and least frequent in December—February. Bacteria infected most often sapwood injuries in spruce roots above soil level, and 55 per cent of the bacterial colonies were isolated from these injuries. 27 per cent of the colonies were isolated from injuries made by increment borer at breast height and extending to heartwood, 16 per cent from sapwood injuries at breast height, and 2 per cent from injuries at stump height made by increment borer and extending to heartwood.

Fifty-five per cent of the bacteria were gram-positive rods, 29 per cent gram-negative rods, 5 per cent gram-variable rods, 6 per cent gram-positive cocci, 3 per cent gram-negative cocci and 2 per cent gram-variable cocci. In 65 per cent of the bacteria the metabolism was fermentative, in 14 per cent slowly fermentative, in 7 per cent oxidative, in 8 per cent slowly oxidative, and in 6 per cent alkalizing. Nineteen per cent utilized cellulose, 15 per cent in the presence of organic, 3 per cent in the presence of inorganic, and 1 per cent in the presence of either organic or inorganic nitrogen.

One bacterial strain, isolated from a sapwood injury at breast height in a spruce of the suppressed crown layer was the only micro-organism growing in this injury a year after the damage, although the injury had been infected with *Peniophora gigantea*. In experiments on malt agar in the laboratory, this bacterial strain proved to be antagonistic to *Fomes annosus*, *Stereum sanguinolentum* and *P. gigantea*. This was a rod bacterium, gram-negative and slowly oxidative. It had no capacity for cellulose utilization. Pleomorphism reminiscent of the *Corynebacterium* family was observable.

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Seloste:

KASVAVIEN KUUSIEN VAURIOISTA ERISTETYT BAKTEERIT

Tämä tutkimus liittyy jatkona aikaisemmin ilmestyneeseen tutkimukseen (KALLIO 1973). Viimeksi mainitussa tutkimuksessa eristettiin kasvavien kuusien vuoden vanhoista vaurioista sieniä ja bakteereita. Bakteerien eristämiseksi käytettiin yhtenä kasvualustana erityisesti bakteereita varten kehitettyä kasvualustaa (TAYLOR 1951).

Tutkimuksen tarkoituksena oli bakteriologisia testejä käyttäen kuvata kasvavien kuusien vuoden vanhoista vaurioista eristettyjä bakteereita sekä selvittää laboratoriokokein yksinään kuusen vaurioissa kasvaneiden bakteerien mahdollinen angatonismi muutamia tärkeimpiä kuusen lahottajasieniä kohtaan.

Tutkimuksen materiaali käsitti 91 kasvavien kuusien vaurioista eristettyä bakteerikasvustoa. Näistä oli neljä sellaista, jotka vuoden kuluttua vaurioittamisesta kasvoivat yksinään vaurioitumakohdassa. Useiden muiden seuralaisina kasvoi sieniä.

Bakteereita tavattiin kaikkiaan 44 eri vauriosta eli 26 %:ssa vaurioiden lukumäärästä. Tavallisinta iskeytyminen oli maalis—huhtikuussa ja kesäkuussa. Tammikuussa tehtyihin vaurioihin bakteerit eivät iskeytyneet lainkaan, ja iskeytyminen oli harvinaista myös joulu- ja helmikuussa. Tavallisimmin bakteereita iskeytyi juureen maanpinnan yläpuolelle tehtyyn mantopuuvaurioon (vaurioiden selitykset ks. KALLIO 1973 s. 5). Saaduista bakteeriviljelmistä 55 % oli peräisin juurivaurioista, 27 % rinnankorkeudelle kasvukairalla tehdyistä sydänpuuhun ulottuneista vaurioista, 16 % rinnankorkeudelle tehdyistä mantopuuvaurioista ja 2 % kannon korkeudelle kasvukairalla tehdyistä sydänpuuhun ulottuneista vaurioista.

Bakteereista oli gram-positiivisia sauvoja 55 %, gram-negatiivisia sauvoja 29 %, gram-variabelisauvoja 5 %, gram-positiivisia kokkeja 6 %, gram-negatiivisia kokkeja 3 % ja gram-variaabeli-kokkeja 2 %. Aineenvaihdunnaltaan fermentatiivisia oli bakteereista 65 %, hitaasti fermentatiivisia 14 %, oksidatiivisia 7 %, hitaasti oksidatiivisia 8 % ja alkalisoivia 6 %.

Selluloosaa pystyi käyttämään 19 % bakteerikannoista. Bakteerikannoista 15 % käytti selluloosaa epäorgaanisen typen läsnäollessa ja 3 % orgaanisen typen läsnäollessa. Yksi prosentti bakteerikannoista oli sellaisia, jotka käyttivät selluloosaa sekä epäorgaanisen että orgaanisen typen läsnäollessa. Edellisissä luvuissa on otettu huomioon kaikki, myös erittäin heikosti selluloosaa käyttäneet bakteerit.

Sellaisia kasvavien kuusien vaurioita, joista vuoden kuluttua vaurioittamisesta onnistuttiin eristämään ainoastaan bakteereita, oli kaikkiaan neljä. Laboratoriossa tehdyissä antagonistisuustesteissä näistä osoittautui tehokkaimmaksi antagonistiksi vallitun latvuskerroksen kuusen rungon mantopuuvauriosta eristetty bakteeri. Mallasagarilla suoritetuissa kokeissa se oli erittäin antagonistinen *Fomes annosus*ta (Fr.) Cooke, *Stereum sanguinolentum*ia (A. & S.) Fr. ja *Peniophora giganteaa* (Fr.) Massee kohtaan (kuva 1).

Antagonistinen bakteerikanta oli gram-negatiivisia sauvoja sisältävä kasvusto (kuva 2). Se oli hitaasti oksidatiivinen eikä pystynyt käyttämään selluloosaa sen paremmin orgaanisen kuin epäorgaanisenkaan typen läsnäollessa. Kasvatuskokeissa kävi ilmi, että tällä bakteerikannalla esiintyi *Corynebacterium*-sukuun viittaavaa pleomorfismia (kuva 3). Antagonismi laboratoriokokeissa ei kuitenkaan takaa antagonismia luonnossa. Kokeet luonnossa ovat välttämättömiä, ennen kuin tätä bakteerikantaa ehkä voidaan ryhtyä käyttämään esim. koneellisessa puunkorjuussa syntyvien vaurioiden suojaamiseksi ilmateitse tapahtuvalta lahottajasienien infektiolta.

KALLIO, TAUNO O.D.C. 172.3: 461

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Bacteria were isolated from injuries to growing spruce, and their properties were tested in the laboratory. Bacteria were present in 26 per cent of all injuries to spruce. Bacterial infection was most frequent in injuries made in March—April and June, and least frequent in December—February. The bacteria most often infected sapwood injuries of the root above soil level. The majority of the bacteria were gram-positive rods, with fermentative metabolism. Nineteen per cent had the capacity for cellulose utilization. One bacterium was the only micro-organism isolated from a sapwood injury at breast height. In laboratory tests this bacterium proved to be antagonistic to *P. gigantea*, *S. sanguinolentum* and *F. annosus*. It was a rod bacterium, gram-negative and slowly oxidative, without the capacity for cellulose utilization. Pleomorphism suggestive of the *Corynebacterium* family could be observed.

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ACTA FORESTALIA FENNICA

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Vol. 127, 1972. Kalervo Salonen.
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