# Suppressiveness of Root Pathogens in Closed Cultivation Systems

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**Keywords:** glasshouse horticulture, root pathogens, rockwool, disease suppressive soilless substrate, recirculated nutrient solution, beneficial microflora, antagonists

**Abstract**

**The trend in glasshouse horticulture has always been to keep culture systems as clean as possible by using new substrates, by applying disinfection techniques, pathogen-free propagation material, etc. However, several root diseases cause problems under these conditions. Especially, zoospore-producing organisms such as *Pythium* and *Phytophthora* spp. are well adapted to aquatic life and flourish in soilless substrates. The present paper shows the importance of the microflora in suppressing root pathogens in soilless systems, either by introducing selected antagonists or by stimulation of a beneficial microflora. The limited volume of substrate and a "biological vacuum" in soilless systems, facilitate inoculation and establishment of antagonists. Examples of soilless systems that contain a suppressive microflora are described. Moreover, the impact of recirculation and disinfection of the nutrient solution on pathogen spread and disease suppression is discussed. Increased knowledge on the beneficial microflora, its ecology, and treatments that influence its composition, will stimulate the exploitation of microbially balanced and optimised soilless systems.**

## INTRODUCTION

Soilless culture systems have proven to be a successful alternative to the use of methyl bromide and other soil fumigants for economic production of several crops. Other advantages of soilless systems are a higher production, energy conservation, better control of growth, and independence of soil quality (van Os, 1999). In general, the strategy in glasshouse horticulture has been to keep the growing systems as "clean" as possible. Problems, e.g. with survival of soil-borne pathogens such as *Fusarium oxysporum* in deeper soil layers, can be avoided by using soilless substrates which are regularly renewed. However, other root diseases have been shown to cause problems in these new systems. Zoospore-producing organisms, such as *Pythium* and *Phytophthora* spp., are well adapted to aquatic life and flourish in soilless substrates such as rockwool that exhibit a high water retention capacity (Stangellini and Rasmussen, 1994). Zoospores can actively swim to their hosts. Infection occurs within minutes and multiplication of these pathogens is explosive under favourable conditions. Closed systems, in which the nutrient solution is recirculated to avoid pollution of ground and surface water by fertilisers, have an increased risk of disease spread.

To avoid the use of chemical pesticides, alternative strategies of disease control are needed. Over the last 5 years several examples of suppression of root diseases in soilless culture systems with beneficial organisms have been described (Paulitz and Bélanger, 2001; Postma et al., 2000; Rattink and Postma, 1996). This suppression may be the result of introduced micro-organisms or due to stimulation of the natural microflora. In soil systems, the occurrence of disease suppression is generally accepted. Here, studies on the underlying mechanisms have resulted in the detection of new types of antagonists (Alabouvette et al., 1979; Jager et al., 1979; Lifshitz et al., 1984).

Disease suppression in soilless culture systems is a new research area. Not much is known yet about the circumstances under which this phenomenon occurs or which the causative agents and mechanisms are. However, the examples mentioned in this paper

Proc. IS on Growing Media
Eds.: Alsanius, Jensén & Asp
Acta Hort 644, ISHS 2004

show the potential of micro-organisms to suppress root diseases, even in the soilless systems at which they have traditionally been neglected. This paper mainly focuses on rockwool as an example of an important soilless substrate. A fundamental difference with soil is that rockwool starts without an organic fraction, which, in soil, is an important source of nutrients and promotes the survival of micro-organisms.

## BIOLOGICAL CONTROL OF ROOT DISEASES

Biocontrol agents can play an important role in suppressing root pathogens in soilless systems. The limited volume of the matrix around the roots in these systems facilitates introduction of the antagonist in the root environment and allows a good interaction between host, pathogen and antagonist. Also, the establishment of antagonists will be easier in soilless systems with a new substrate having a “biological vacuum”, than in a microbiologically buffered system such as soil. Many antagonists of root pathogens are known and have been tested in greenhouse cropping systems. However, limited numbers of antagonists are available as commercial products. Excellent reviews of this subject are available about the commercial products (Fravel, www.barc.usda.gov/psi/bpdl/bioprod.htm) and the possibilities in greenhouse systems (Paulitz and Bélanger, 2001; Whipps, 1992). Antagonists can be active through several mechanisms, such as (myco)parasitism, antibiosis or other inhibitory substances, competition for nutrients or space, induced resistance or a general increase of the plant condition. Examples of antagonists active through these different mechanisms have been described for two important root pathogens in soilless systems, i.e. Fusarium wilt and Pythium root rot (Postma, 1996). A biocontrol mechanism newly described which is interesting for soilless systems with a high water retention capacity, is the application of micro-organisms that produce biosurfactants which cause lysis of zoospores. One example is a *Pseudomonas aeruginosa* isolate which produces rhamnolipids and could successfully control or delay Phytophthora root rot in pepper when a suitable carbon source was added to the system (Stanghellini and Miller, 1997).

Nevertheless, biocontrol of root diseases often shows variable results (Postma et al., 2001). This can be due to a lack of survival or activity of the biocontrol agents, or to insufficient colonization by these of the infection sites. Especially if the antagonist was originally isolated from soil or from a crop different from the one to which it is applied, it may not be fully adapted to soilless systems. For effective biocontrol, the activities of the antagonist and pathogen should be synchronized in time and in space. That the effect of the antagonist is influenced by its location in the system in relation to the location of the pathogen, is illustrated by the failure of suppression of Fusarium wilt in carnation in a recirculation system, when the antagonist was added on top of the rockwool blocks, while the pathogen was introduced via the nutrient solution (Rattink and Postma, 1996). However, when introduced via the nutrient solution like the pathogen, the antagonist proved to be extremely effective.

Problems of insufficient or variable biocontrol might be solved by selecting organisms that are better adapted to soilless systems and synchronized to the pathogens occurring in these systems. A specific group of micro-organisms which has received little attention yet, are endophytes, i.e. organisms present inside the plant tissue (Hallmann et al., 1997). They have the advantage of being protected from environmental stresses and might be able to protect the plant in a soilless system from propagation material up to end of the crop. Interesting are micro-organisms that protect the plant through systemic induced resistance (e.g. Chen et al., 1999). This protection is not necessarily limited to the location where the antagonist is present. However, not much is known about the importance of this mechanism for an entire crop, since most research on systemic induced resistance of root pathogens has been done with individual young plants using a split root system.

## DISEASE DECLINE

Disease decline is a well known phenomenon in natural soil systems. Famous is the “Take-all decline” in wheat, for which continuous cropping of wheat first showed an increase of the disease caused by the fungus *Gaeumannomyces graminis*, followed by a decline after 4 to 5 years of continuous wheat monoculture. Root-associated fluorescent *Pseudomonas* spp. producing the antibiotic 2,4-diacetylphloroglucinol were identified as the key components of this natural biological control which develops in response to the disease and continuous cropping (Raaijmakers and Weller, 1998).

Two cases of disease decline have been detected in soilless systems. In a multi-year experiment, where the nutrient solution from a nutrient film technique (NFT) system was not refreshed between the crops, the incidence of Fusarium wilt decreased from 98% in the first carnation crop, to 60% in the second and 34% in the third crop. When the pathogen was introduced in the fourth crop in a completely disinfected unit, disease incidence was again increased to 70%, compared to 45% in the non-disinfected crop (Rattink and Postma, 1996). This result indicates a gradual development of suppressiveness in a non-renewed nutrient solution, which was (partially) caused by the microflora since it was destroyed by sterilisation.

The second experience with disease decline in soilless systems was with *Pythium aphanidermatum* in cucumber grown on rockwool (Table 1) (Rattink, personal communication). Each crop, on new or on previously used rockwool slabs, was inoculated with the pathogen. The large variations in disease incidence between the five crops are caused by natural differences in temperature. Nevertheless, the disease incidence in the used slabs containing an established microflora was clearly less during the fourth and fifth crop than that in the new slabs. In another multi-year experiment, new slabs showed 53% diseased plants compared to only 15% in used slabs re-used after three previously healthy crops (i.e. the re-used rockwool was only inoculated in the fourth crop). However, the used slabs that had been inoculated with the pathogen each crop, showed similar disease percentages as the new rockwool. Repetition of such multi-year experiments is complicated due to the length of the experiment and due to variations in the weather conditions. Moreover, growers do not re-use their substrate after a heavy disease attack, which makes the study of naturally-occurring disease decline impossible. Nevertheless, these experiments show that Pythium disease suppression can develop in rockwool slabs after consecutive cucumber crops. The necessity of the presence of the pathogen in the disease decline is not clear.

## SUPPRESSIVENESS OF THE SOILLESS SUBSTRATE

Whereas in soil and peat the existence and the potential of a suppressive microflora towards root pathogens is generally accepted, suppressiveness in soilless systems has only been recently demonstrated (Postma et al., 2000, 2001). Suppression of *Pythium aphanidermatum* disease in cucumber in re-used rockwool was proven to be the result of the microflora present in this substrate, since suppressiveness of sterilised rockwool was recovered after its recolonisation with the original microflora. Suppressiveness was restored to 50 to 100%, either through contact between the sterilised and non-sterilised slabs or through the nutrient solution taken from the slab, indicating that the suppressive microflora is easily translocated. In contrast to the application of singly-introduced antagonists, the indigenous microflora caused reproducible suppression of Pythium crown and root rot in cucumber. Without exception, all rockwool slabs without Pythium symptoms in the previous crop, were suppressive. Re-used rockwool slabs with Pythium symptoms in the previous crop showed variable but mainly high disease percentages and are not advised to be re-used. It is not yet known which organisms are responsible for the pathogen suppression.

In general, substrates are selected on the basis of their horticultural productivity and not for disease suppressive properties. Therefore, not many substrates have been compared properly as regards to their influence on disease development. A complicating factor is that substrates can vary in physical and chemical properties, besides differences in biological characteristics. Volcanic scoria (Tuff) was found to be more suppressive to *Pythium* than rockwool (Kritzman et al., 2000). However, it is unclear if this is due to the presence of a suppressive microflora, or to the physical and chemical properties being less favourable for *Pythium*.

A different composition of the microflora in root samples of two types of substrate has been described for tomato (Khalil and Alsanius, 2001). Lower numbers of total aerobic bacteria and pseudomonads, but higher numbers of filamentous actinomycetes and fungi were present in peat compared to rockwool slabs. However, it is not known if these differences in composition of the microflora reflect differences in suppressiveness.

## THE ROLE OF RECIRCULATION AND DISINFECTION IN DISEASE SUPPRESSION

Recirculation of the plant nutrient solution is needed to avoid loss of nutrients to the environment. However, this increases the risk of spreading root diseases in soilless systems, especially in case of pathogens producing zoospores. Nevertheless, two papers described closed systems that showed less disease than run-to-waste systems. In an NFT system with *Phytophthora cryptogea* in tomato, closed systems showed less disease than parallel run-to-waste systems (McPherson, 1998). It was suggested that a beneficial microflora in the solution led to the suppression of the root disease, but this was not proven. Also, in a rockwool system with *Pythium* spp. in tomato, closed systems showed less disease than run-to-waste systems (Tu et al., 1999).

To avoid the spread of pathogens through the recirculated nutrient solution, different disinfection methods can be used (Runia, 1996; van Os, 1999). Total disinfection such as achieved by UV and heat treatment, aim at eliminating >99.9% of the microflora, and this may include the beneficial micro-organisms. Such a drastic elimination of micro-organisms might cause a microbiologically unbalanced system, in which regrowth of the microflora (beneficial, neutral or pathogenic) will occur. Unluckily, pathogenic *Pythium* spp. are pioneer organisms, which can multiply extremely fast in situations with a microbiological vacuum. Slow (sand) filtration, however, provides a way to eliminate pathogens (Wohanka, 1995), but to keep alive a large part of the microflora. After slow sand filtration, over 90% of the total aerobic bacterial population was still present (van Os and Postma, 2000). However a shift in the population between influent and effluent of the filters occurred, since the numbers of fluorescent pseudomonads, filamentous actinomycetes and fungi decreased more drastic than the total numbers of bacteria. Also the potential of the microflora to utilise different carbon sources and the genetic profile of the bacterial population changed.

The hypothesis that different degrees of disinfection (“total” versus “partial” or “active” versus “passive”) affect the microbial community structure in the soilless system and consequently disease suppressiveness, is examined in the European project “MIOPRODIS” (van Os et al., 2001; several other papers in this issue). The ultimate answer, however, is the effect of disinfection treatments on disease development, as a consequence of the changes in the microflora.

## ECOLOGY OF THE MICROFLORA IN SOILLESS SUBSTRATE

Agricultural soils contain $10^7$ to $10^9$ colony forming units (CFU) per g soil, whereas nutrient solutions in soilless systems (i.e. in rockwool slabs) contained only about $10^6$ CFU $ml^{-1}$ (van Os and Postma, 2000), indicating that substrate systems constitute a rather poor medium for micro-organisms. In soil and soilless systems, a substantial part of the nutrients for the microflora is derived from the plant roots. The rhizosphere is defined as the volume of soil (or substrate) that is adjacent to and influenced by the plant root (Bolton et al., 1992). Roots release organic compounds such as exudates, mucilage, lysates, dead cell material, which is in total estimated to be as much as 15 to 20% of the total carbon fixed by the plant. The nature of plant-derived compounds found in the rhizosphere is dependent on plant species, growth conditions, rooting medium, and stage of plant development. The nature and abundance of organic compounds probably has a major influence on the types of micro-organisms that colonise the rhizosphere. The rhizosphere microflora is likely to be altered as a function of time, because of changes that occur in the exudation patterns of roots as plants age.

In soil, where water transport is minor as compared to soilless substrate, the rhizosphere can extend up to 2 mm from the root surface (Bolton et al., 1992). In soilless substrate, a flow of nutrient solution and diffusion are transporting exudates more easily away from the roots as compared to soil. The micro-organisms rapidly metabolise the available carbon leaking from the root. Therefore, the surface of the root (rhizoplane) and not the volume around it might be the most important niche for the microflora in soilless systems. Consequently, attachment properties will be important.

The microflora on the surface of roots was visualized by scanning electron microscopy (SEM). On cucumber roots grown in a nutrient solution, micro-colonies of bacteria were present (Fig. 1 and 2) which are also known to exist on the surface of roots grown in soil. On cucumber roots in a rockwool system mainly separate cells were present (Fig. 3) Remarkable were cells which were attached with their short side to the roots (Fig. 1 and 3). This might be the most efficient way of catching nutrients which leak from roots.

## DISCUSSION AND CONCLUSION

From the examples given in the present paper, it is clear that soilless systems can contain suppressive microbial populations. In soil systems, the occurrence of disease suppression is fully accepted and has led to the isolation of new antagonistic species. In soilless systems it is not known yet, which part or what properties of the microflora are important for disease suppression. Important factors influencing the disease suppression might be: (1) the activity of the total microflora, (2) diversity of the microbial population in relation to niche occupancy, and (3) the presence of specific types of antagonists. Further research should give an answer on this. Hopefully, antagonists that are better adapted to soilless systems will be found by analyzing the microbial composition of suppressive soilless systems. Nevertheless, single antagonists might be less robust in their biocontrol activity than combinations of organisms. Less variable results can probably be achieved with mixtures of antagonists combining different ecological traits and biocontrol mechanisms.

In contrast to the application of antagonists, the indigenous microflora showed a very reproducible suppression of Pythium crown and root rot in cucumber. The exploitation of the indigenous microflora to suppress disease in soilless systems might be a new trend, which is breaking with the “sterility” concept commonly used in soilless systems. Where biocontrol agents of pathogens have to be registered according to the pesticide law, this is not the case for natural occurring microbial populations. This gives the exploitation of natural populations a big financial advantage compared to biocontrol agents.

For practical applications, it is important to understand how growers or soilless substrate suppliers can enhance the abundance or activity of a suppressive microflora. Probably the suppressiveness in soilless systems can be stimulated through specific amendments. In soil systems, the addition of compost or compost extract is commonly used to enhance disease suppressiveness. Amendments with the purpose to stimulate the disease suppression of soilless substrates, will lead to a new generation of substrates, optimising the physical, chemical, as well as the biological properties. If soilless systems can be created with disease suppressiveness comparable to natural soils, they will combine the advantages of soil systems and soilless substrates, i.e. a microbiologically well buffered culture system, as well as the possibility to have a pathogen free start by renewing the substrate.

## ACKNOWLEDGMENTS

J.D. van Elsas and E.A. van Os are acknowledged for fruitful discussions and critically reading the manuscript.

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**Tables**

Table 1. Percentage diseased cucumber plants during five consecutive crops on new and re-used rockwool after inoculation with *Pythium aphanidermatum* during a multi-year experiment at the Research Station for Floriculture and Glasshouse Vegetables (data obtained from H. Rattink).

| | crop 1 | crop 2 | crop 3 | crop 4 | crop 5 |
|---|---|---|---|---|---|
| new rockwool for each crop | 60 | nd | 8 | 40 | 78 |
| continuously re-used rockwool | nd | 100 | 3 | 0 | 25 |
| % reduction | nd | nd | 60 | 100 | 68 |

nd = not determined.

**Figures**

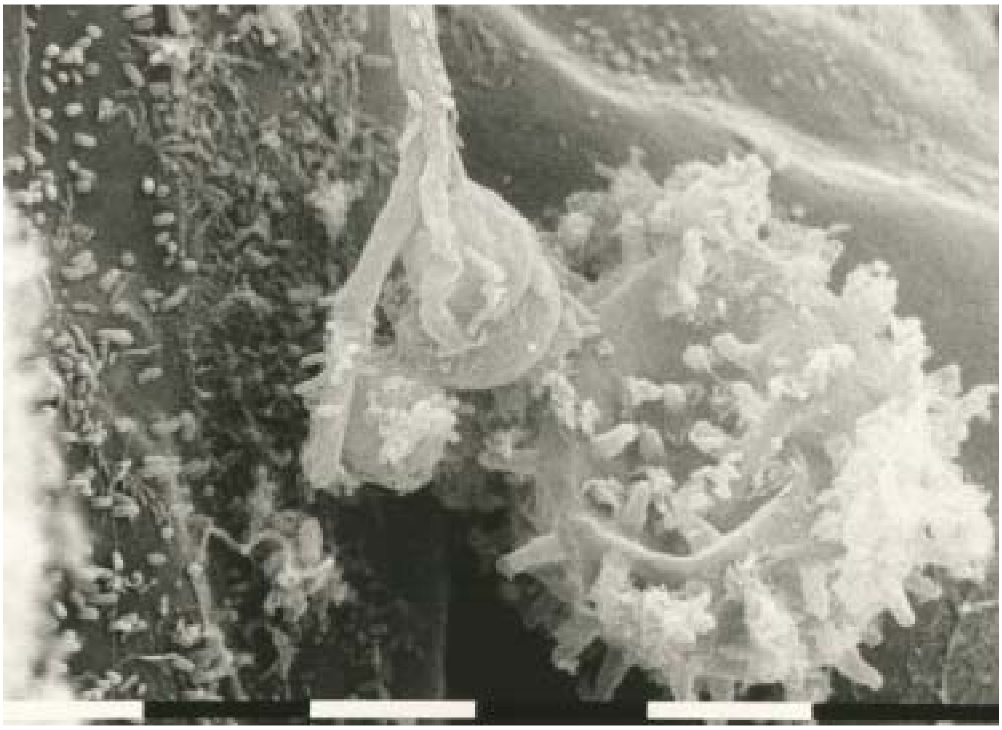

Fig. 1. Bacterial population, *Pythium aphanidermatum* (PA) and *P. oligandrum* (PO) on a cucumber root grown for 3 weeks in nutrient solution (bar = 10 µm). Arrow points at a micro-colony. (Photo by Anke Clerkx).

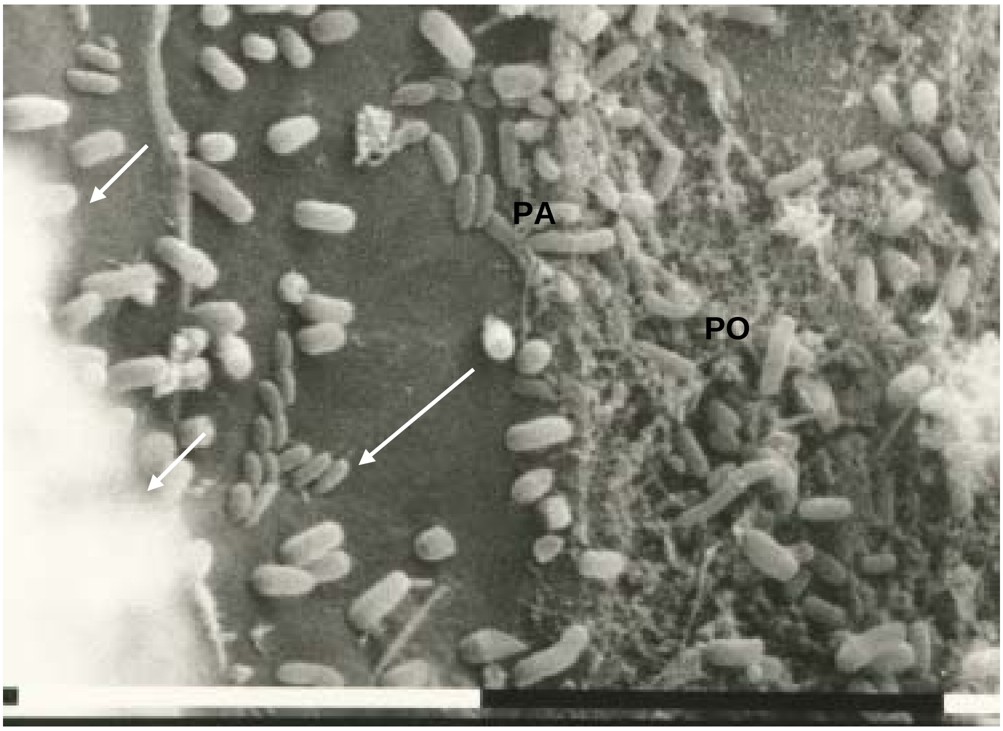


Fig. 2. Bacterial populations on a cucumber root grown in nutrient solution (bar = 10 µm). Arrow points at a micro-colony (detail of Fig. 1). (Photo by Anke Clerkx).

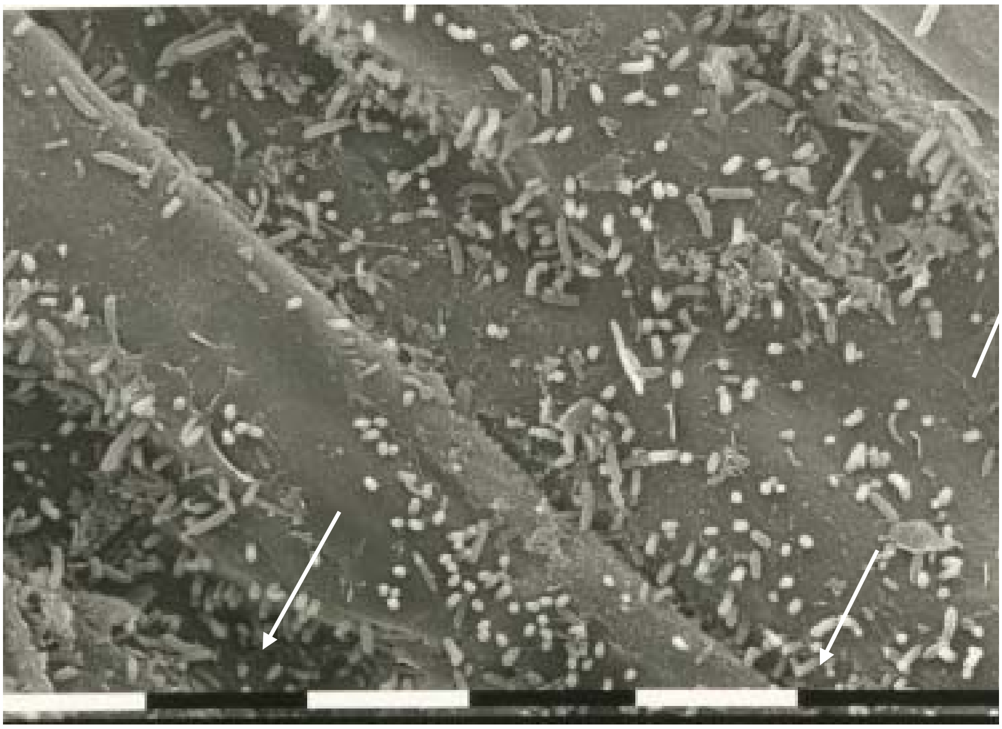

Fig. 3. Bacterial population on a three-week old cucumber root grown in rockwool (bar = 10 µm). Arrows point at bacterial cells attached at their short side. (Photo by Anke Clerkx).