Biofilms

for agricultural, horticultural and related applications

Evaluation and Licensing Opportunities

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Patent Literature

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The addition of a synthetic polymer to microorganism cultures enables the formation of stable Biofilms even in microorganisms that usually do not form them

Biofilms can be used as seed coatings for more vigorous and effective microorganisms in symbiosis with crop plants

Biofilms are formed when individual cells of microorganisms adhere to each other and often also to an adjacent surface. The biofilms contain extracellular polymeric substances (EPSs), components produced by microorganism, which are a mixture of polysaccharides, proteins and lipids. Forming a biofilm affords protection from environmental stresses, such as desiccation, pathogens and antibiotics and, in addition, the microorganisms become more metabolically efficient. While many microorganism species naturally form biofilms, some will only do so under certain conditions or only form weak and less stable biofilms.

Now Dr Timothy Overton and Dr Francisco Fernandez-Trillo at the University of Birmingham have discovered molecules and methods that aid in the formation of biofilms to enable microorganisms to form strong and stable biofilms while also making this process fully reversible. The use of novel synthetic polymers promotes biofilm formation in a wide spectrum of bacteria. This approach will for example enable the use of biofilms in seed coating to make the microorganisms more resilient and productive and thereby provide more benefits to crop plants by enhancing the symbiotic relationship between microorganisms and plants.

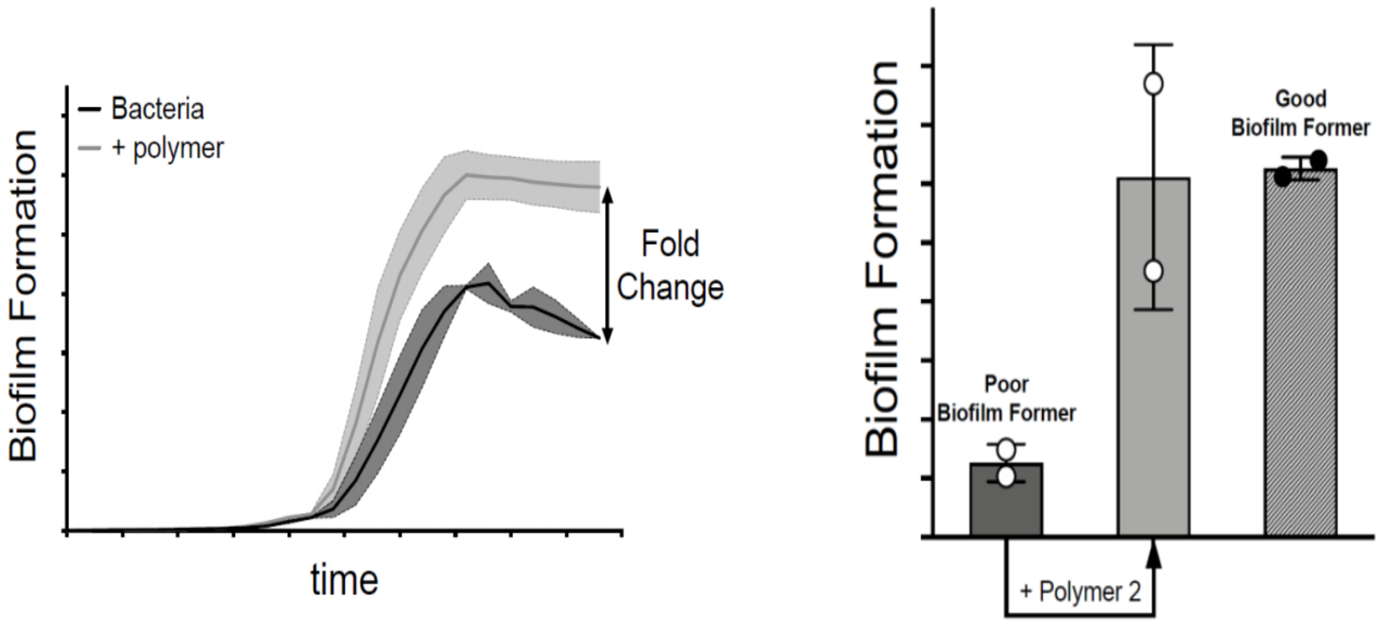

Figure 1: In the graph on the left, the addition of the **polymer doubles microorganism density**. The addition of the novel polymers leads to a significant increase in fluorescence of GFP in cultures over a 48 hours' incubation period with or without a polymer pAH-2AP. On the right the graph shows the limited biofilm produced by a "poor biofilm former" compared with the extensive biofilm of a "good biofilm former". Upon addition of the novel polymer the "poor biofilm former" will **aggregate into stable biofilms** similar to the "good biofilm formers".

The method to produce biofilms involves growing the microorganism in a liquid medium and then adding the polymer (see Figure 2 below for examples of polymers tested, a full list is available in the patent application WO 2021/209765). It is also possible to separate the microorganism-polymer from the aqueous medium. The microorganism-polymer complex can then be dried and stored for later use. For agricultural or horticultural applications, the Biofilm complex may be **coated onto seeds** or other materials to be inserted in the soil or alternatively the complex may be topically applied onto crops. The inventors are currently exploring Biofilm complexes for the plant growth-promoting microorganisms ***Bacillus subtilis*** (Gram positive) and ***Pseudomonas fluorescens*** (Gram negative).

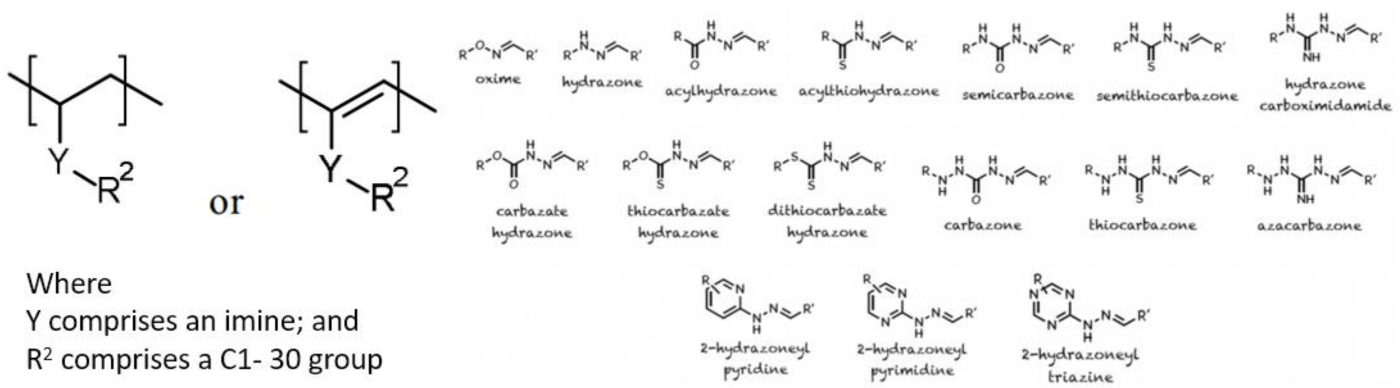

Figure 2: On the left the two possible polymer backbone structures and on the right a selection of the imine - containing functional groups that are used as part of the linker group Y.

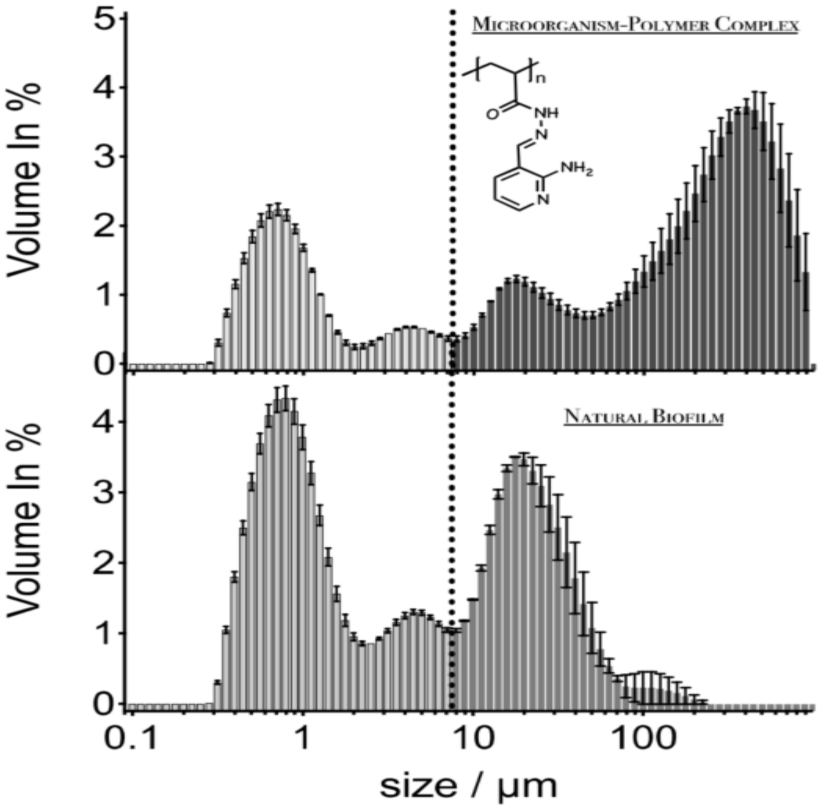

Figure 3: The graphs show the results of an analysis comparing the size distribution (by % volume) of complexes induced by the polymer pAH-2AFP (molecular structure shown in graph) and biofilms formed without the polymer:

After 48 hours of culturing; for E. coli that was not mixed with the polymer (bottom graph) the culture retained a large proportion of planktonic cells. There were some small clusters of natural biofilm, having a peak size of about 20µm. There were very few clusters greater in size than 200µm and no clusters greater in size than 300 µm.

The E. coli that was mixed with pAH- 2AFP (top graph) had a lower proportion of planktonic cells and had a **higher proportion of clusters of high diameter**, up to 1,000µm in size. This indicates that the **polymer formed complexes** with the cells of the microorganism.

In summary the inventors have demonstrated that using **novel polymers** microorganism can **form strong and stable biofilms**. Such biofilm complexes can be used in **crop applications**, either directly as **seed treatments** or alternatively as **sprays** or on other material or structures to be deployed in soil. The complexed microorganisms are more **protected from environmental stresses** and are more productive, enhancing the symbiotic effect on plants.