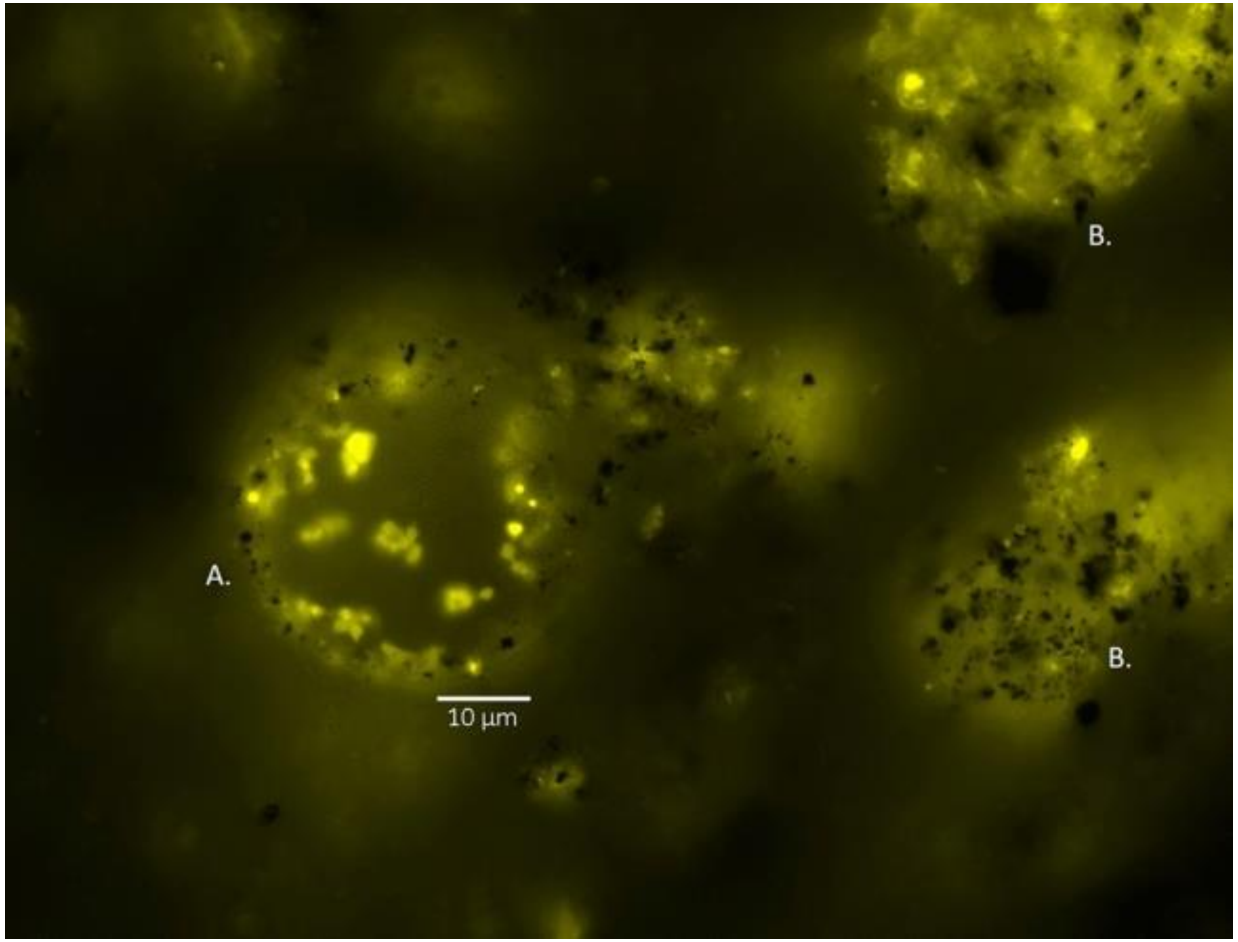
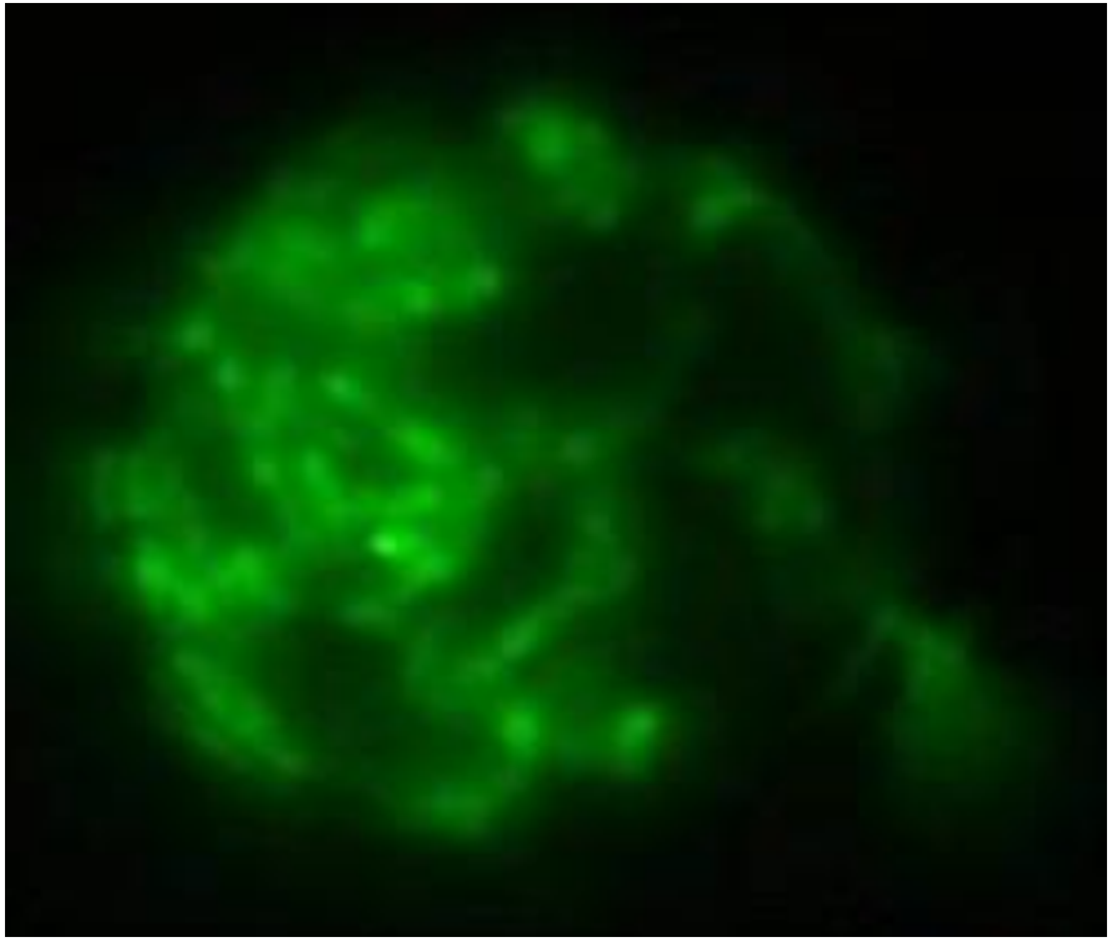
**Viewing the Mechanisms of Hydrocarbon-degrading Bacteria**

The RPI Newsletter typically presents information about our products and projects. This month I'll present some observations from a couple of journal articles by Katherine French. The main article focuses on horizontal gene transfer, but I want you to see the physical relationship between the bacteria and petroleum hydrocarbon. French et al. show bacteria using three strategies for degrading crude oil: biofilm formation, direct adherence to oil droplets, and vesicle encapsulation of oil. The pictures teach lessons on their own. Of course, I'll comment as I present them, but the pictures teach! I have cited the French et al. articles at the end of the presentation.

In the main article, French et al. constructed an E. coli bacterium that overexpressed hydrocarbon-degrading enzymes. For our purposes, we don't need to know the details about the enzymes. We just need to know that they are well-characterized, hydrocarbon-degrading enzymes and tagged with a fluorophore so you can see them. To avoid confusion, three enzymes are used in the pictures taken from French's work: p450cam, alkB, almA, and xylE. The enzymes were expressed in an E. coli construct. Two wild-type hydrocarbon-degrading bacteria are pictured, A. Cupriavidus sp. OPK and Pseudomonas sp. Strain BSS.

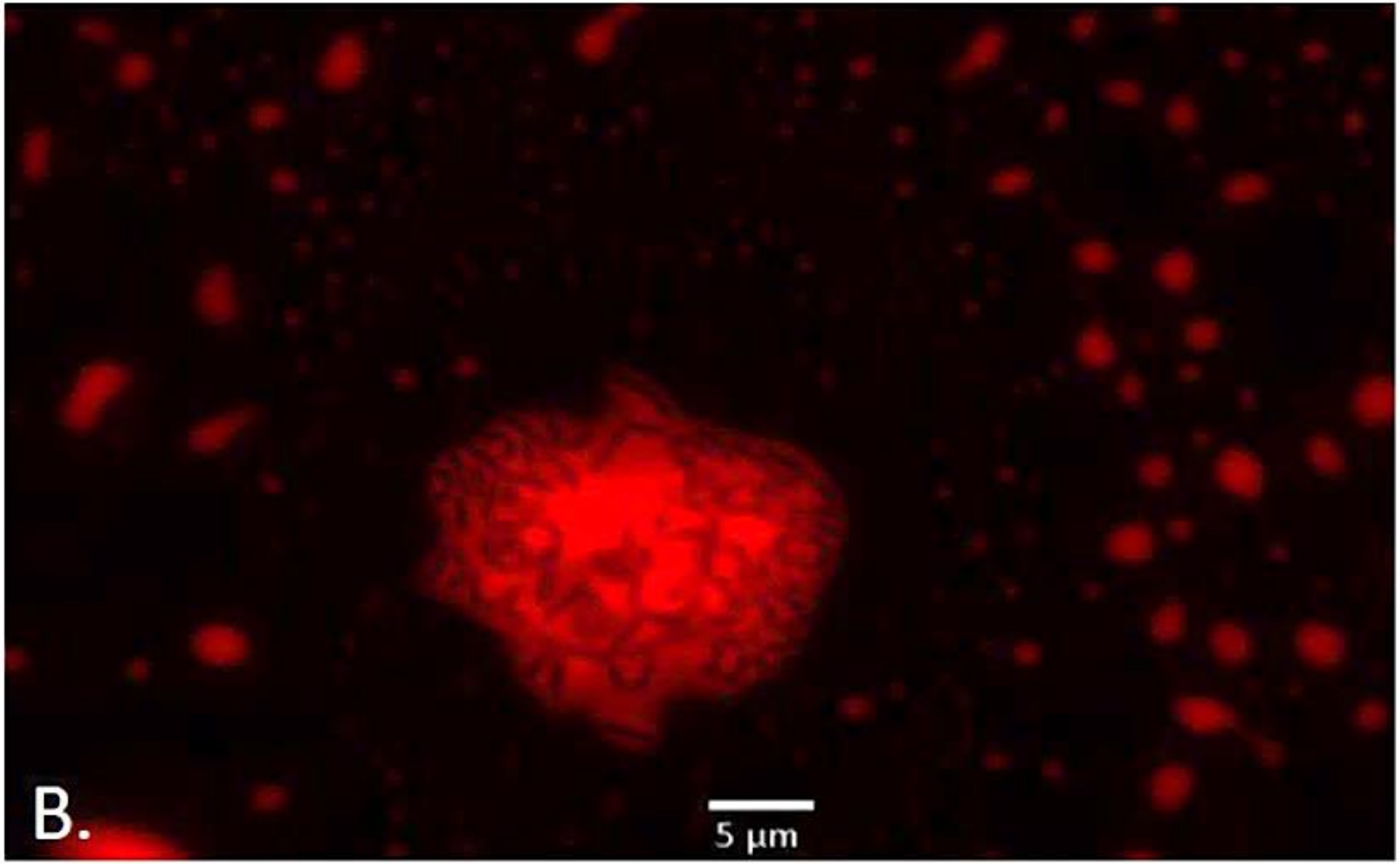



Microbial communities expressing the enzyme p450cam in sediment from a former oil refinery. The bacteria embedded in the biofilm are colored yellow. The biofilm forms thin nets over large soil particles, while small soil particles can be seen as black 'dots' on the biofilm surface.



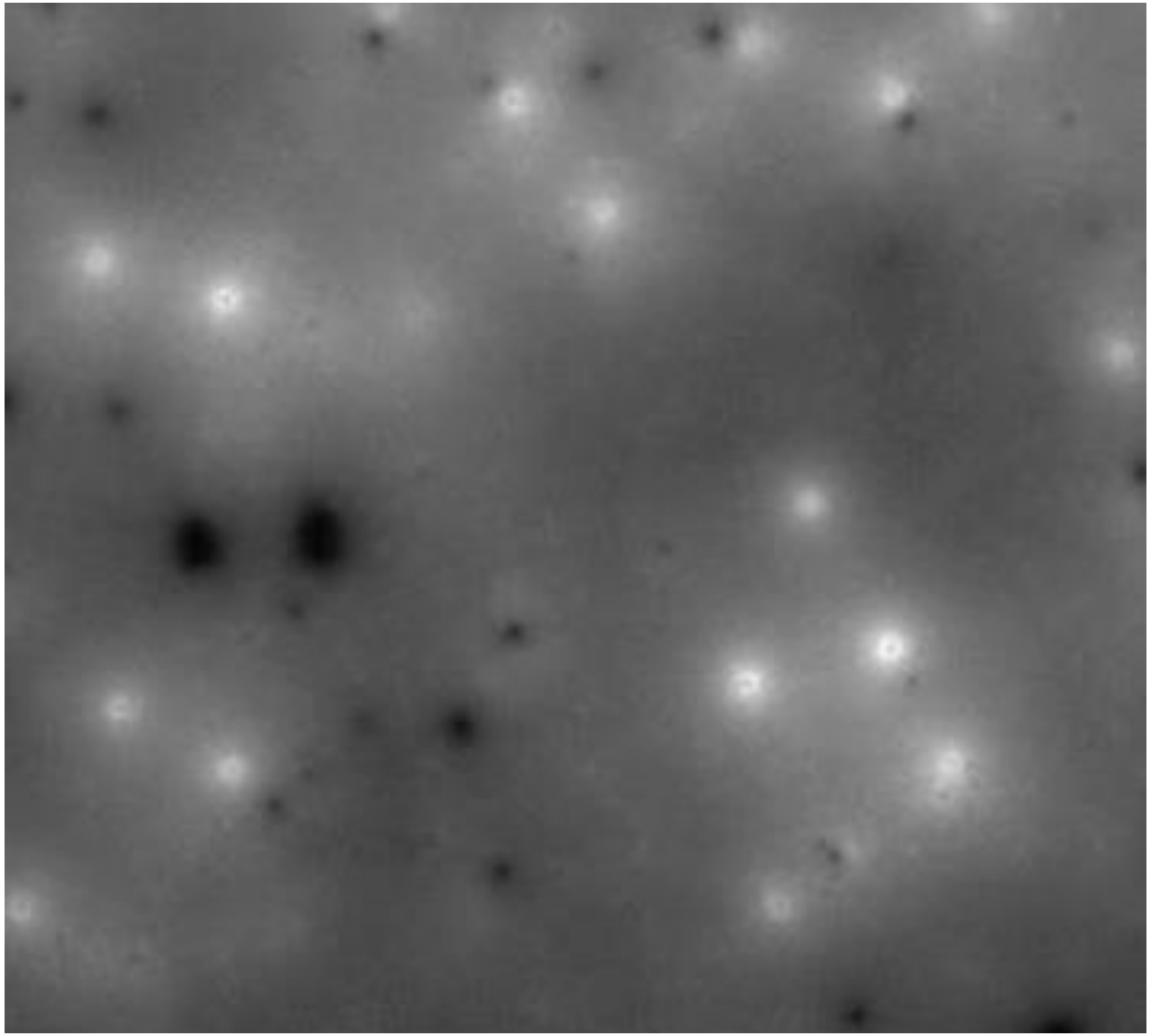
Bacteria expressing alkB were found clinging to spheres containing crude oil. Many images have been published demonstrating certain bacterial species locate to the surface of petroleum hydrocarbons.

These pictures indicate that the biofilm supports bacterial degradation of hydrocarbons by locating the bacteria advantageously to the hydrocarbon source. In these two cases, different hydrocarbon-degrading bacteria are shown located on hydrocarbon droplets.



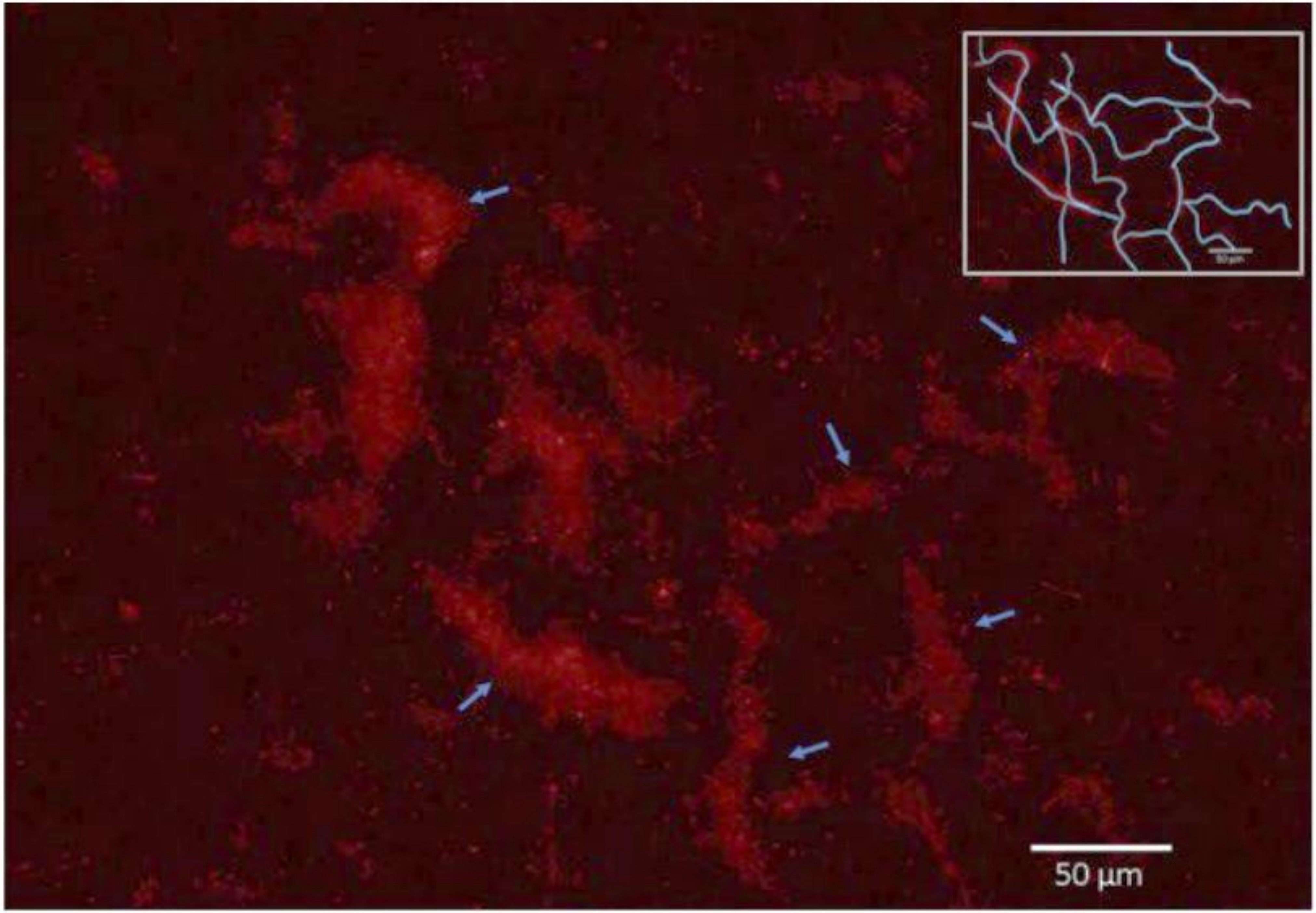

Rhodococcus erythropolis strain OSPS1 (black rods) is also known to adhere to the surface of crude oil droplets. The oil droplet depicted ≈21 µm in diameter. This expression is similar for alkB and P450cam.

This picture provides the same information as the two pictures above, with the addition of small vesicles. I like the color.



The hydrocarbon-degrading enzymes xylE were tagged and appeared as bright white donuts. XylE was found around small pores (ca. 500 nm) within the Extracellular Polymeric Substance (EPS) matrix. The positioning at pores in the EPS is advantageous to contacting petroleum hydrocarbon.  Three enzymes, alkB, xylE, and p450cam, were embedded within the EPS matrix. Both alkB and xylE were concentrated around the pores. Only xylE is shown.

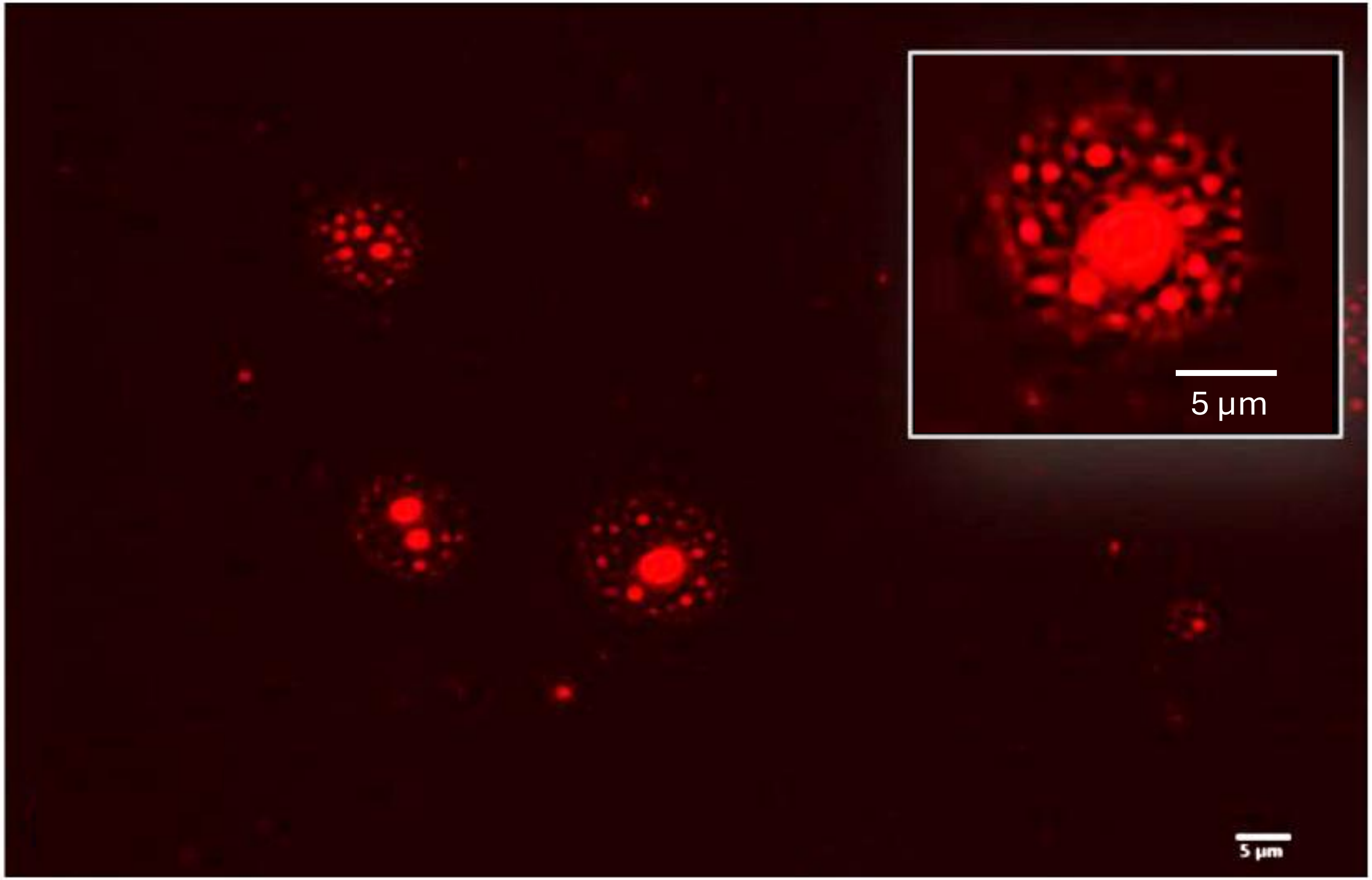
This visually confirms that hydrocarbon-degrading enzymes can embed into the EPS.




A bacterial species, A. Cupriavidus sp. OPK, needs direct contact with the petroleum for degradation. To facilitate contact, it transports crude oil through a biofilm network. The blue arrows point to channels within the network; the inset box shows a rough network tracing for clarity.

The biofilm network allows for the efficient diffusion of petroleum hydrocarbons across highly specialized membranes embedded with hydrocarbon-degrading enzymes.

This illustrates that the biofilm network contributes to hydrocarbon distribution and degradation.




The bacteria, Pseudomonas sp. strain BSS produced biosurfactants that spontaneously formed vesicles when exposed to crude oil. The single vesicle depicted in the inset is 11.31 µm in diameter and contains small oil droplets ranging in size from 790 nm - 1.33 µm in diameter. Note that the outside parameter of the vesicles is easier to identify in the larger picture than in the inset.

In the E. coli construct, three enzymes, alkB, almA, and p450cam, were found in extracellular vesicles.

The vesicles may support the degradation of high hydrocarbon concentrations that are not advantageous to live bacteria. The vesicle enzymes may degrade the hydrocarbon into “bite-sized” fractions.

These pictures indicate that hydrocarbon-degrading bacteria can excrete an extracellular polymeric substance that forms a biofilm. The biofilm structure can be used to locate the bacteria advantageously in relation to the hydrocarbon source. The biofilm supports adherence to hydrocarbon droplets. It can become a transport mechanism to bring hydrocarbon to the degrading bacteria, or it can provide support for hydrocarbon-degrading enzymes operating outside the bacteria. Finally, the hydrocarbon-degrading bacteria can release vesicles that contain enzymes. These vesicles can degrade hydrocarbons, presumably in locations that are less conducive to bacterial life.

French, K.E., Zhou, Z. and Terry, N., 2020. Horizontal ‘gene drives’ harness indigenous bacteria for bioremediation. *Scientific Reports*, *10*(1), p.15091.

French, K.E. and Terry, N., 2019. A high-throughput fluorescence-based assay for rapid identification of petroleum-degrading bacteria. *Frontiers in Microbiology*, *10*, p.1318.