How Toxic Is It to Bacteria?

The Interaction of the Components of Crude Oil with *Escherichia Coli* Bacteria

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experiment
bacteria
crude oil
thin layer chromatography
toxicity

Bacteria are the most ancient form of life on Earth, for which fossil records (called stromatoliths) of more than three billion years old exist. Due to their small size, they were discovered only around 350 years ago, and Antoni van Leeuwenhoek is credited for this scientific breakthrough. In 1882, Robert Koch discovered the mycobacteria as a cause of tuberculosis. In recent years, it has become clear that the human body is an open ecosystem containing many different bacteria in almost all tissues, with the largest numbers found in the colon (large intestine).[1] Taken together, the number of bacteria is comparable to the total number of cells in the human body.[2] The bacteria do not only help to digest the food we eat, but they also provide nutrients and vitamins to the cells in our large intestine. *Escherichia coli* bacteria (Figure 1) are found in the human intestine, so if they are found in water samples, this is a sign of fecal contamination. On the other hand, *E. coli* is the old workhorse for biochemistry and genetics. Here we describe the interaction of living *E. coli* bacteria with crude oil samples, a non-living material derived from ancient life.

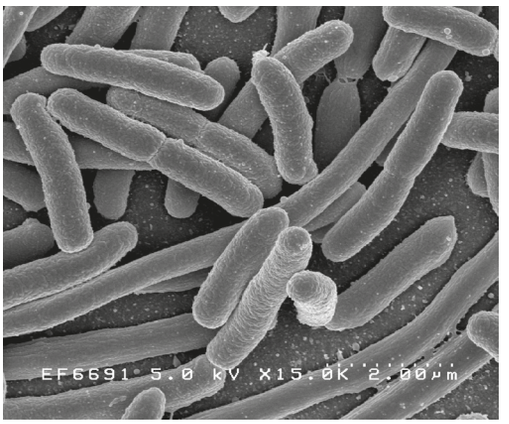

Figure 1
Electron microscopic image of *E. coli* bacteria (© National Institute of Allergy and Infectious Diseases NIAID).

Interaction of Bacteria with a Crude Oil Sample

Crude oil is a mixture of hundreds of components in widely different concentrations.[3]

How can we find out if some components are toxic to normal bacteria like *E. coli*?

In an altogether different field, extracts from *Stemona collinsae*, an Asian plant, were investigated for their toxic activity against the common fungus *Cladosporium herbarum*.[4] Plant extracts were separated by thin layer chromatography (TLC) and the fungal spores were directly sprayed onto the TLC plate. Areas of reduced fungal growth were tested for toxic components of the plant extract.

Figure 2
LB agar plate with *E. coli* bacterial colonies expressing the green fluorescent protein.

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E coli f1.
7.2.25

In a similar manner, we planned to test the interaction of *E. coli* bacteria with crude oil components. In order to generate clearly visible fluorescent bacteria, *E. coli* bacteria were transformed with a plasmid directing the synthesis of green fluorescent protein, according to the protocol from the supplier (Edvotek, Washington DC, USA). The fluorescent bacteria were streaked onto a suitable LB plate and photographed under UV light (Figure 2).

For the main experiment, we applied a sample of crude oil from the Beryl oil field, a major oil field in the UK sector of the North Sea, to a 10 x 15 cm TLC plate, separated the components with decane as a mobile phase and dried the plate. The bacteria were added to warm classical LB (lysogeny broth) medium containing 0.7% agar, then the mixture was poured onto the TLC plates and incubated at 37°C for 48 hours. The bacteria were then visualized with a UV lamp. Although the experiment was rather crude, the bacterial growth was seen all over the plate, no areas were detected without bacterial growth (Figure 3).

Conclusion

So, roughly, the components of crude oil were not highly toxic for *E. coli* bacteria. In a way, this was not altogether surprising as crude oil is transformed living matter, often originating from other bacteria.[5] On the other hand, human exposure to crude oil in oil drilling regions is known to cause cancer, liver damage, immunodeficiency, and neurological symptoms.[6] So, although this strange material is transformed living matter and drives our economy, it can also harm us directly and its combustion contributes to the drastic climate change that is currently being observed.

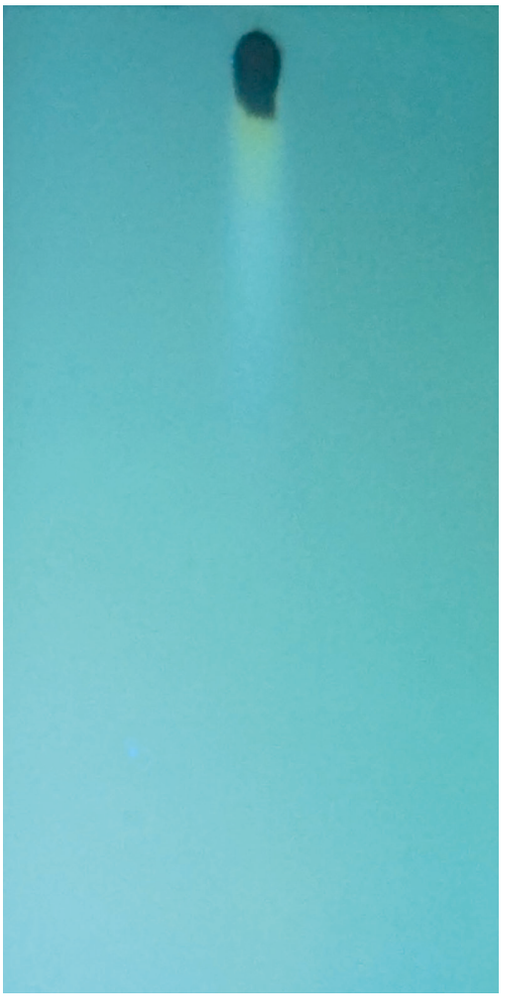

Figure 3
Bacterial growth on a TLC plate with a separated crude oil sample from the Beryl oil field. The large black spot in the top middle of the plate shows where the sample was loaded. The separated components are below the black spot.

The greenish-blue fluorescence of the bacteria (modified by the agar) is seen on the whole area and is not strongly inhibited by the crude oil components.

1 Ed Yong, *I Contain Multitudes: The Microbes Within Us and a Grander View of Life* (New York City: Ecco, 2016).

2 Ron Sender, Shai Fuchs, and Ron Milo, "Revised Estimates for the Number of Human and Bacteria Cells in the Body," in *PLoS Biol*, (August 19, 2016), https://doi.org/10.1371/journal.pbio.1002533.

3 Nagham Mahmood Aljamali and Nuha Salman Salih, "Review on Chemical Separation of Crude Oil and Analysis of Its Components," in *Journal of Petroleum Engineering and Technology* 11, no. 2 (2021), 35–49.

4 T. Pacher, C. Seger, D. Engelmeier, S. Vajrodaya, O. Hofer, H. Greger, "Antifungal Stilbenoids from *Stemona collinsae*," in *Journal of Natural Products* 65, no. 6 (May 11, 2002), 820–827.

5 Vasily Simanzhenkov and Raphael Idem, *Crude Oil Chemistry* (Boca Raton: CRC Press, 2003).

6 Jill E. Johnston, Esther Lim, and Hannah Roh, "Impact of upstream oil extraction and environmental public health: A review of the evidence," in *Science of The Total Environment* 657 (March 20, 2019), 187–199.

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