ISOLATION OF ORGANOTIN DEGRADING BACTERIA UNDER AEROBIC AND ANAEROBIC CONDITIONS

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Tributyltin (TBT) is used as a biocide in antifouling paints on ship hulls and in the materials protection, and triphenyltin (TPT) is employed as a pesticide in agriculture. In anoxic sediments TBT is preserved for long periods of time, whereas TPT may undergo degradation. In this study, an attempt has been made to isolate microorganisms under aerobic and anaerobic conditions able to use these organotin compounds as the sole source of carbon and energy.

Surface sediment samples from Eel Pond were incubated under aerobic conditions in presence or absence of about 1 mM TBT chloride and 1 mM TPT chloride. Two liquid enrichments were made, and microorganisms from the first enrichment were streaked out on agar plates containing either no organic carbon source (controls), or about 1 mM of TBT or TPT as the carbon source. In the original flasks with sediment, in the enrichment cultures, and on agar plates a slow growth of fast moving rods, motile spirilli, non-motile rods, bent rods, and cocci were identified in the presence of TPT. In presence of TBT, motile and non-motile rods and non-motile cocci were observed. On the agar plates, small grey and transparent colonies developed in the presence of TPT and TBT, whereas in controls they were only very small, transparent, and slow growing. Non-motile small cocci and rods were identified in control plates. Streaking out of single colonies yielded very small new colonies after two days of incubation in presence of TBT and TPT, but not in controls.

Attempts to isolate sulfate reducing bacteria under anaerobic conditions from Eel Pond and Quissett harbor sediments able to use TPT and TBT as a sole carbon source were unsuccessful.

This experiment indicates that the colonies in the aerobic enrichment may be able to use TBT and TPT as a sole source of carbon and energy. Further isolation steps must be undertaken to isolate the different species and to assess their ability to growth on organotins. Only chemical analysis can prove, however, if true TPT and TBT degrading bacteria have been enriched.
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Tributyltin (TBT) is used as a biocide in antifouling paints on ship hulls and in the materials protection, and triphenyltin (TPT) is employed as a pesticide in agriculture. In anoxic sediments we have shown that TBT is preserved for long periods of time, whereas TPT may undergo degradation. In this study, an attempt has been made to isolate microorganisms under aerobic and anaerobic conditions able to use these very toxic organotin compounds as the sole source of carbon and energy.

Aerobic conditions

Surface sediment from Eel Pond was incubated at 37°C in a rotatory shaker with mineral salts medium plus trace elements and vitamins (6 vitamins + vitamin B₁₂). Flasks were incubated with about 1 mM TBT chloride and 1 mM TPT chloride, whereas control flasks contained no oxydizable organic carbon source. After nine days, an inoculum was transferred to a new Erlenmeyer flask containing the same mineral salts medium, and a second transfer was made after additional six days. After four days of incubation microorganisms from the first enrichment were streaked out on agar plates containing mineral salts medium, trace elements, vitamins, and either no organic carbon source (controls), or about 1 mM of TBT or TPT as the carbon source. Observations of bacteria and growth were made by microscopy, and for identification, a fluorescence in situ hybridization technique with rRNA probes was applied.

In all but the TBT containing flask, a green color developed with time. The absorption spectrum of this pigment peaked at 671 nm and was identified as chlorophyl a. The color likely originated from an unidentified microalgae or eukaryote, rather than from cyanobacteria which could not be detected as judged by fluorescence microscopy. The lack of chlorophyl a in presence of TBT was likely due to the toxic effect of this compound on these organisms. Chlorophyl a was also absent in the liquid enrichment cultures and on plates.

In the original flasks with sediment, and in the enrichment cultures under light and dark conditions, a slow growth of a fast moving rod could be observed in the presence of TPT, but not in the controls. In addition motile spirilli, non-motile rods, bent rods, and cocci were
identified. In the presence of TBT, only a few fast moving rods were observed besides non-motile cocci, but they were not detected in the control.

On the agar plates, growth of colonies could be detected after four days of incubation. In the presence of TPT and TBT small grey and transparent colonies developed, whereas in controls only very small, transparent, slow growing colonies occurred. Bacteria on control plates were identified as non-motile small cocci and rods. Streaking out of single colonies yielded very small new colonies after two days of incubation in presence of TBT and TPT, but not in controls. The bacteria observed in presence of TPT were motile and non-motile rods, motile spirilli, non-motile cocci, and non-motile bent rods. Attempts to identify these bacteria by an situ hybridization technique failed. In presence of TBT, only non-motile and motile rods and non-motile cocci were observed.

This experiment indicates that the enriched colonies may be able to use TBT and TPT as a sole source of carbon and energy. Further isolation steps must be undertaken to isolate the different species and to assess their ability to growth on organotins. Only chemical analysis can prove, however, if true TPT and TBT degrading bacteria have been enriched.

Anaerobic conditions

An attempt has been made to isolate sulfate reducing bacteria from harbor sediments able to use TPT and TBT as the sole carbon source. Black iron reduced sediment both from Eel Pond and Quissett harbor were incubated in the presence or absence of about 1 mM TBT or TPT at 320°C in the dark under anaerobic conditions. Mineral salts media containing sulfate, trace elements and vitamins were used. During the incubation period of 20 days, no bacteria could be isolated. Some turbidity was only observed after incubation in the TBT containing flask with Quissett sediment, but it did not increase with time.

This experiment indicates either that no sulfate reducing bacteria were present able to use organotin compounds as the sole carbon source, or more likely, that the incubation period was too short for observing growth. Similar studies with other toxic and persistent organic chemicals have shown that growth of bacteria can only be expected after relatively long periods of incubation.