Soil bacteria \textit{Pseudomonas putida} and \textit{Alcaligenes xylosoxidans} subsp. \textit{denitrificans} inactivate triclosan in liquid and solid substrates

Maura J. Meade \textsuperscript{a,\*}, Rebecca L. Waddell \textsuperscript{a}, Terrence M. Callahan \textsuperscript{b,\dagger}

\textsuperscript{a} Allegheny College, Department of Biology, 520 N. Main St., Meadville, PA 16335, USA
\textsuperscript{b} Lake Erie College of Osteopathic Medicine, Erie, PA 16509, USA

Received 26 June 2001; received in revised form 31 July 2001; accepted 1 August 2001
First published online 14 September 2001

Abstract

Triclosan is a broad-spectrum antimicrobial agent that has been incorporated into many household and medical products. Bacteria with high levels of triclosan resistance were isolated from compost, water, and soil samples. Two of these bacteria, \textit{Pseudomonas putida} TriRY and \textit{Alcaligenes xylosoxidans} subsp. \textit{denitrificans} TR1, were able to use triclosan as a sole carbon source and clear particulate triclosan from agar. A decrease in triclosan concentration was measured by HPLC within 6 h of inoculation with strain TriRY and 24 h with strain TR1. Bioassays demonstrated that triclosan was inactivated in liquid cultures and/or embedded in plastic by the growth of strain TriRY and strain TR1, permitting the growth of triclosan-sensitive bacteria.

Keywords: Triclosan; Antibacterial resistance; Detoxification; \textit{Pseudomonas putida}; \textit{Alcaligenes xylosoxidans}

1. Introduction

Triclosan is a broad-spectrum antimicrobial agent that is incorporated into household and medical products as diverse as surgical drapes, antibacterial soaps, kitchenware, and paints. Widespread use of this compound has led to the detection of triclosan and its derivatives in the environment \cite{1-3}. Triclosan targets fatty acid biosynthesis through specific interference with the NADH binding site \cite{4,5} of the enoyl-ACP reductase FabI in \textit{Escherichia coli} \cite{6} and InhA in \textit{Mycobacterium smegmatis} \cite{7}. Alteration of this target enzyme provides low-level resistance but such bacteria remain susceptible to commercial triclosan concentrations. Bacteria that possess FabK, an enoyl-ACP reductase not affected by triclosan, instead of the susceptible FabI, are intrinsically resistant to higher levels of triclosan \cite{8}. Overexpression of a multidrug efflux pump locus also leads to low-level triclosan resistance in laboratory and clinical strains of \textit{E. coli} \cite{9}. The high-level intrinsic resistance of \textit{Pseudomonas aeruginosa} to triclosan is through active efflux of the compound \cite{10}. Triclosan is modified by industrial strains of the fungi \textit{Trametes versicolor} and \textit{Pycnoporus cinnabarinus} through conjugation of sugar moieties, leading to reduced toxicity \cite{11}. A consortium of bacteria has been reported to metabolize triclosan through cometabolism \cite{12}. In order to identify bacteria from environmental sources able to tolerate high levels of triclosan, samples from water, soil, and compost were collected. From these samples many different bacteria with moderate to high levels of triclosan resistance were isolated. The mechanism of triclosan resistance in two of these organisms was investigated through biochemical analysis and bioassays. The ability of one of the organisms to colonize triclosan-embedded plastic and form biofilms was also studied.

2. Materials and methods

2.1. Culture growth, identification, and characterization

Compost samples were collected from the Allegheny College Environmental Science Garden. Bacteria with high levels of triclosan resistance were isolated from compost samples by dilution plating on 1% triclosan test agar...
lated plastic and samples not treated with strain TR1

an overnight culture of

The cleaned plastic samples were then
in sterile distilled water and allowed to air-dry under ster-
surface-sterilized for 2 min as above then rinsed four times

A portion of the sample was
was washed in sterile distilled water and observed using

S. aureus

biofilm formation was observed after 10 days of growth at 24°C by phase contrast microscopy. A portion of the sample was
was washed in sterile distilled water and observed using

2gl

2.3. Chromatographic analysis of triclosan

Conical tubes containing 5 ml of 0.2% TTB (triclosan

Fig. 1. A clear zone is produced on 1% TTA plate inoculated with (A)
P. putida TriRY, and (B,C) A. xylosoxidans. Opacity of agar is due to the presence of crystalline triclosan.
triclosan as a sole carbon source in M9 minimal medium (data not shown) and no significant pH changes were recorded in the medium, supporting removal of triclosan as the probable clearing mechanism.

To determine if triclosan was being solubilized or degraded in the 1% TTA plates, *P. putida* TriRY and *A. xylosoxidans* TR1 were grown in 0.2% TTB. A laboratory strain of *P. aeruginosa* which is resistant to triclosan through active efflux [10] was used as a control. Lack of *E. coli* and *S. aureus* growth in the control tubes indicated that triclosan was not bound to the filter or bacterial cells. Both *E. coli* and *S. aureus* were able to grow in media that had initially been inoculated with *P. putida* TriRY and *A. xylosoxidans* TR1 (data not shown). This indicates that no active triclosan remained after triclosan-resistant bacteria were allowed to grow on media containing the antibiotic agent. *A. xylosoxidans* TR1 was able to colonize a triclosan-impregnated plastic (Fig. 2A) while triclosan-sensitive *S. aureus* did not form a biofilm after 10 days (Fig. 2B). *S. aureus* was able to colonize the plastic pretreated with *A. xylosoxidans* TR1 following removal of the TR1 biofilm (Fig. 2C). *S. aureus* colonization was confirmed through a positive reaction for catalase. This indicates that triclosan was removed from both liquid and solid substrates by *A. xylosoxidans* subsp. *denitrificans* TR1.

HPLC analysis demonstrated that triclosan decreased in the liquid growth medium within 6 h by *P. putida* TriRY and 12 h by *A. xylosoxidans* TR1. The concentration of triclosan decreased about 10-fold within the experimental period (Fig. 3). No decrease in triclosan was seen in uninoculated samples or those inoculated with *E. coli* (data not shown). Conjugated products of triclosan, as previously identified in triclosan detoxification by *T. versicolor* and *P. cinnabarinus* [11], were not observed in *P. putida* TR1 or *A. xylosoxidans* TR1 culture extracts at 280 nm, since there was a single peak with the identical retention time of triclosan. Combined with the growth of *P. putida* TriRY and *A. xylosoxidans* TR1 in medium containing triclosan as a sole carbon source and the ability of these isolates to detoxify triclosan-containing media and plastics, degradation appears to be the mechanism of triclosan detoxification by these bacteria.

---

Fig. 2. Biofilm formed on triclosan-impregnated plastics. (A) inoculated with *A. xylosoxidans* TR1. (B) inoculated with *S. aureus*; (C) plastic from A after biofilm removal and 10-day incubation with *S. aureus*. Panel C tested positive for catalase production (the other two were negative) indicating that the biofilm-forming organism was *S. aureus*.

Fig. 3. Concentration of triclosan in liquid culture inoculated with *P. putida* TriRY (●) and *A. xylosoxidans* TR1 (■) as determined by HPLC. Cultures were incubated at 24°C with shaking at 225 rpm and sampled as indicated.
The ability of *P. putida* and *A. xylosoxidans* to degrade halogenated phenolic compounds is well documented. *A. xylosoxidans* subsp. *denitrificans* dechlororates 2,4-dichlorobenzoate and uses the resulting product as a sole source of carbon and energy [15]. The production of dioxygenase by this organism enables it to degrade 1,3-dichlorobenzene [16]. *P. putida* can degrade trichlorobenzenes [17] and chlorocatechols through the ortho or meta pathways [18]. These studies indicate that strains related to TR1 and TriRY produce enzymes capable of triclosan degradation. The data presented in this paper support the hypothesis that triclosan resistance in *A. xylosoxidans* TR1 and *P. putida* TriRY is through degradation of the antibacterial agent.

Both *P. putida* and *A. xylosoxidans* are responsible for a steady rise in nosocomial infections since 1975, although neither is currently considered a major human pathogen [19–21]. *A. xylosoxidans* is resistant to the antiseptics chlorhexidine, benzenthonium chloride, and allyldiaminoethylglycine [21,22], and both organisms are resistant to benzalkonium chloride as well as multiple antibiotics [22,23]. Use of these compounds or triclosan in hospital disinfection may thus not be effective to combat nosocomial transfer of *A. xylosoxidans* or *P. putida*.

The fact that both *A. xylosoxidans* TR1 and *P. putida* TriRY form biofilms on antibacterial plastics has serious implications. Biofilms may be more persistent in environments where triclosan is used as a disinfectant and thus available to serve as foci of inoculation and infection. Also, if products that are exposed to the common soil bacterium *A. xylosoxidans* subsp. *denitrificans* do not retain their antibacterial properties, potentially harmful organisms such as *S. aureus* may grow on those surfaces. To our knowledge this is the first report of the removal of active triclosan from both liquid media and solid materials by pure bacterial cultures.

**Acknowledgements**

This work was supported by National Institute of Health Grant AI047854-01. We thank Margaret Nelson for her review of the manuscript.

**References**


