

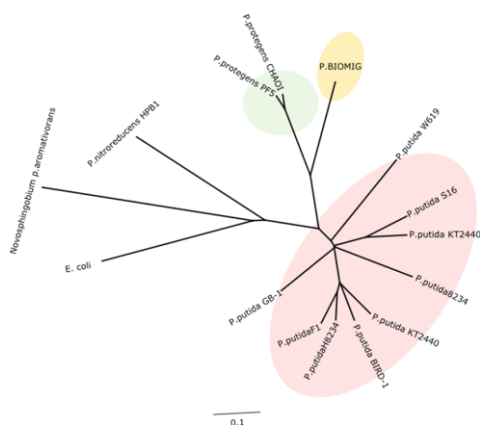
ROLE OF BIOTRANSFORMATION ON THE DYNAMICS OF ANTIMICROBIAL RESISTANCE

FINAL REPORT

We all scare of “microbes” that make us sick. Therefore we use disinfectants to get rid of them and preserve our living environment free of disease causing microbes. However, our hygiene obsession has created a bigger problem; we taught microbes how to tackle with disinfectants. The talent that they have developed due to our unintended use of disinfectants is “antimicrobial resistance”. Many antimicrobial resistance mechanisms are also used against antibiotics which makes the problem a significant threat to human health. Recently, it is discovered that microbes can consume disinfectants and most of the antibiotics as their carbon and energy source. Are we in trouble?

In order to answer this very important question, ROBODAR was launched in the Biotransformations and Microbial Genetics Lab of Institute of Environmental Sciences at Bogazici University in September 2011. The overall objective of the ROBODAR is to systematically evaluate if biotransformation is a threat or a control mechanism for the evolution and dissemination of antimicrobial resistance in the environment. In particular, we focus to assess: (a) the role of biotransformation of benzalkonium chlorides (BACs), an active ingredient of many domestic, industrial and medical disinfectants, on the BAC resistance of a microorganism; and (b) the dynamics of the survival of microorganisms and development of BAC resistance in a community having microorganisms with different tolerance and biotransformation capacity for BACs.

Discovery of a new *Bacterium*

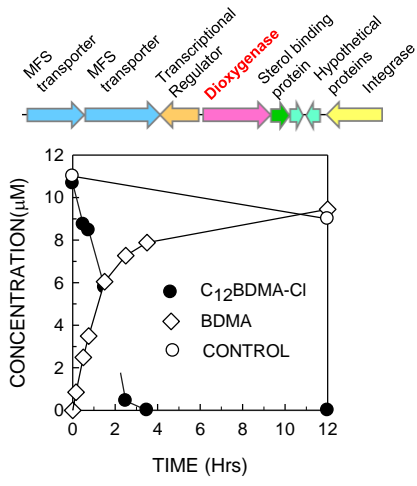


We have developed five microbial communities from domestic wastewater, activated sludge, pristine soil, sea sediment, house floor that actively degrade BACs. We isolated a BAC degrading *Pseudomonas* sp. (strain BIOMIG1, figure on the left) which is common and the most predominant species in each community. Strain BIOMIG1 is an oxidase and catalase positive *Bacterium* which tolerates BACs up to 1000 mg/L and mineralizes it within 8 hours at 25 mg/L concentration. We also isolated *E. coli*'s and *Serratia marcescens* species that are non-BAC degraders and oxidase negative either in the inocula or in the communities. These microbes will serve as model organisms to elucidate the role of disinfectant biotransformation on the evolution and the dissemination of antimicrobial resistance in the environments with temporal

disinfectant concentration gradient.

Discovery of a novel enzyme

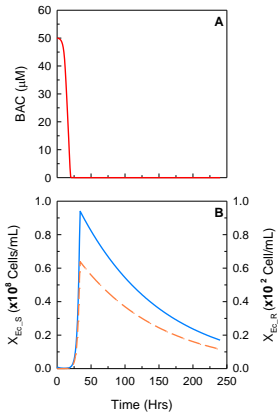
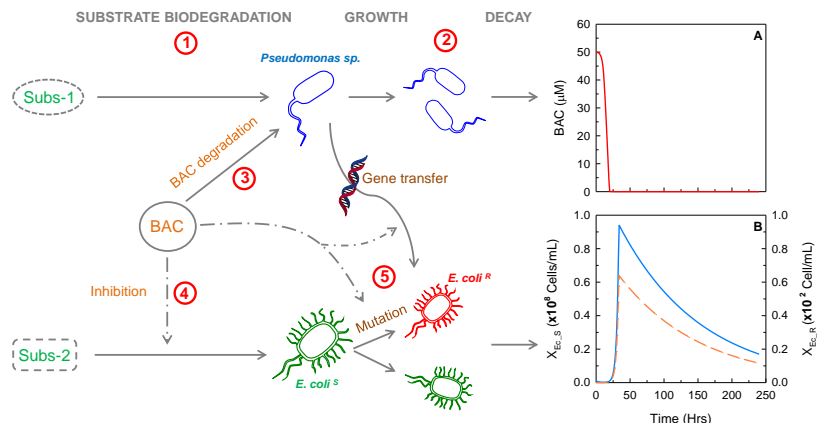
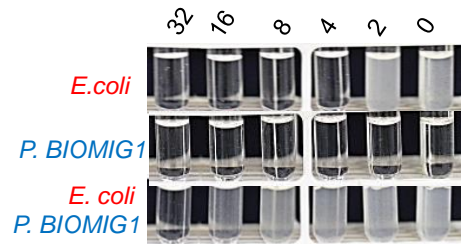
BIOMIG1 is a new bacterium that can degrade BACs. It has an about 7.5 Mbp genome length. In order to identify the genes that play role in the BAC biotransformation, we sequenced and then compared the genomes



of four BIOMIG1 phenotypes that do different levels of BAC biotransformation. The comparative genomics revealed that a gene array (figure on the left-top) containing two Major Facilitator Superfamily transporters, a transcriptional regulator, a dioxigenase, a sterol binding protein, two hypothetical proteins and an integrase is present only in BAC transforming phenotypes therefore the genes within this array are candidate genes for BAC biotransformation. The only catabolic gene in the array is a gene synthesizing a dioxigenase. This gene was cloned in an expression plasmid and inserted into an *E. coli*. *E. coli* having the plasmid containing the dioxigenase successfully converted BACs to BDMA (figure on the left-bottom). As a result, a dioxigenase, which has not been reported previously, is discovered as the key enzyme responsible for the transformation of BACs to BDMA.

Biotransformation protects the susceptible microorganisms and facilitates antimicrobial resistance

In order to understand how biotransformation effects the survival of susceptible bacteria in a community containing a BAC-degrader, a co-culture susceptibility experiment was performed. *E. coli* species susceptible to BACs above 2 mg/L was used as a model susceptible microorganism. *E. coli* was incubated in 1000 mg/L maltose at BAC concentration ranging between 2 to 1024 mg BAC/L with and without BIOMIG1 which utilizes BACs but not maltose. *E. coli* could not grow in maltose above 2 mg BAC/L when BIOMIG1 was not present (Figure on the right). On the contrary, it survived and grew above 16 mg BAC/L when BIOMIG1 was present. When BAC concentration in the tubes were measured after 4 days of incubation, we reported that BAC was completely utilized in the tubes inoculated with BIOMIG1 which suggests that biotransformation protects the susceptible *E. coli* and let it grow at initial BAC concentrations that are inhibitory to *E. coli* in absence of a BAC-degrader. We also showed that recombinant *E. coli* having plasmid containing the dioxigenase that converts BACs to BDMA survives at BAC concentrations above that it cannot survive when the dioxigenase is not present.



We also developed a growth model simulating the dynamics of susceptible bacteria and evolution of resistant mutants in a co-culture transforming BACs based on our observations and results (Figure on the left). Model suggests that a BAC-degrader present in a microbial community decreases the BAC concentration below subinhibitory level which facilitates the survival of the susceptible microorganisms and development of BAC resistant mutants of the same microorganism.

In conclusion, ROBODAR project tested and proved all of its hypotheses. The outcomes suggest that biotransformation reduces the efficacy of disinfectants and results in the survival of target microorganisms and development of antimicrobial resistance in a microbial community.