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An aqueous solution containing soluble substances from PVC char has no effect on the rate of transformation in E. coli cells.

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Summary

In transformation, bacterial cells can take in exogenous DNA molecules, or plasmids, some of which may contain genes for antibiotic resistance, or other factors which may aid in bacterial survival. These exogenous DNA sequences contribute to the evolution of antibiotic-resistant bacteria. Polyvinyl chloride (PVC) is a widely used plastic that poses many health hazards. Much research has been conducted on the harmfulness of PVC gas released when burned, yet relatively few studies have focused on the solid product, or char, of incinerating PVC. This study aimed to determine whether or not PVC char has an effect on bacterial transformation. It was hypothesized that PVC char would not increase transformation rates. In this experiment, *Escherichia coli* cells were attempted to be made competent to take up a pFluoroBlueTM plasmid, which contains IPTG (a fluorescent molecule) and ampicillin resistance. *E. coli* cells were treated with $CaCl_2$, PVC char solution, or a 50-50 mixture of $CaCl_2$/PVC char solution. The $CaCl_2$-treated cells produced between 1 and 10 fluorescing colonies, the 50-50 mixture produced one or no fluorescing colonies, and the PVC char solution produced no fluorescing colonies. PVC char alone did not cause the transformation of *E. coli* cells, yet it was observed that *E. coli* did grow on the agar plates containing Ampicillin. This finding is significant and warrants further investigation to determine whether or not PVC char decreases the effectiveness of the antibiotic Ampicillin, or whether PVC char induces mutations for antibiotic resistance without the acquisition of plasmids or foreign DNA from the environment.

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Introduction

Transformation is a mechanism of horizontal gene transfer, a process in which genetic information is transferred from one organism to another, whether or not this organism is an offspring of the parent cell. Genes conferring antibiotic resistance often accumulate on plasmids, which are small, exogenous DNA molecules (1). Because plasmids offer selective advantage to organisms that uptake and express them, they may spread rapidly among cells, contributing to the emergence and development of "superbugs", or multidrug-resistant bacteria (2).

Polyvinyl chloride (PVC) is a plastic polymer composed of vinyl chloride (C_2H_3Cl), a human carcinogen. In 2000, 27 million tons per year of PVC was produced worldwide (3). Due to the high chlorine content of PVC, toxic chemicals called dioxins are released upon the manufacturing and burning of PVC. The harmful effects of burning plastics are well-established (4, 5). Nevertheless, plastics are burned in waste incineration processes as well as accidental fires, which yield high concentrations of toxic organic compounds (6). It is known that metal ions, such as $CaCl_2$, $MgCl_2$, and $MnCl_2$, make bacterial cells competent such that they can take up exogenous DNA (9). However, it is difficult to ascertain whether the metal or the chloride component is responsible for this occurrence. As such, we decided to study PVC as a possible transformation compound because it contains chloride without the heavy metal. Since PVC is ubiquitous and transformation can significantly impact human health and the environment, it is important to study the effect of PVC char, or the solid product of burning PVC, on bacterial transformation.

In order to transform, cells must enter into a physiological state of competence (3). Competence, involving between 20 and 50 proteins, typically only occurs under certain conditions, such as altered growth conditions, cell density, and lack of nutrients, and lasts for a limited time. Because of the specificity of the requirements for competence development and transformation, we hypothesize that PVC char would have no effect on the rate of transformation in *E. coli*.

Results

In the experiment, we attempted to make *E. coli* cells competent to take up the pFluoroBlue™ plasmid, which contains genes coding for ampicillin resistance and fluorescence when in the presence of IPTG and UV lighting. A $CaCl_2$ solution, used in the standard protocol for creating artificially competent cells, was the control. We treated another set of cells with only a PVC char solution, and another with a 50-50 mixture of $CaCl_2$/PVC char solutions.

In this experiment, we assayed for *E. coli* cell competence by its susceptibility to transformation by the pFluoroBlue™ system. Successfully transformed cells take up the pFluoroBlueTM plasmid, which confers ampicillin resistance and fluorescence in the presence

$CaCl_2$					
Trial	(a) –DNA	(b) –DNA/+Amp	(c) +DNA/+Amp	(d) +DNA/+Amp/+IPTG	
1	growth	no growth	50 CFU	70 CFU	7 fluorescent
2	growth	no growth	34 CFU	187 CFU	10 fluorescent
3	growth	no growth	225 CFU	8 CFU	1 fluorescent

Table 1. *E. coli* cells made competent using $CaCl_2$. *E. coli* was grown on (a) LB agar without any additives, (b) LB agar containing Ampicillin, (c) LB agar containing Ampicillin and in the presence of the pFluoroBlue™ plasmid, and (d) LB agar containing Ampicillin, the pFluoroBlue™ plasmid, and IPTG, a fluorescent molecule that would indicate if E. coli did indeed acquire the plasmid through the transformation process.

PVC char solution					
Trial	(a) –DNA	(b) –DNA/+Amp	(c) +DNA/+Amp	(d) +DNA/+Amp/+IPTG	
1	growth	16 CFU	1 CFU	no growth	none
2	growth	6 CFU	no growth	2 CFU	none
3	growth	8 CFU	18 CFU	no growth	none

Table 2. *E. coli* cells made competent using PVC char solution. *E. coli* was grown on (a) LB agar without any additives, (b) LB agar containing Ampicillin, (c) LB agar containing Ampicillin and in the presence of the pFluoroBlue™ plasmid, and (d) LB agar containing Ampicillin, the pFluoroBlue™ plasmid, and IPTG, a fluorescent molecule that would indicate if E. coli did indeed acquire the plasmid through the transformation process.

50/50 Mixture of $CaCl_2$/PVC char solution					
Trial	(a) –DNA	(b) –DNA/+Amp	(c) +DNA/+Amp	(d) +DNA/+Amp/+IPTG	
1	growth	1 CFU	5 CFU	2 CFU	1 fluorescent
2	growth	no growth	1 CFU	5 CFU	none
3	growth	3 CFU	100 CFU	25 CFU	1 fluorescent

Table 3. *E. coli* cells made competent using a 50/50 mixture of CaCl2//PVC char solution. *E. coli* was grown on (a) LB agar without any additives, (b) LB agar containing Ampicillin, (c) LB agar containing Ampicillin and in the presence of the pFluoroBlue™ plasmid, and (d) LB agar containing Ampicillin, the pFluoroBlue™ plasmid, and IPTG, a fluorescent molecule that would indicate if E. coli did indeed acquire the plasmid through the transformation process.

of IPTG. To determine whether PVC char renders *E. coli* cells competent, we treated *E. coli* cells with PVC char solution before subjecting it to transformation by the pFluoroBlue™ plasmid. We also included a positive control for transformation by $CaCl_2$ treatment, as well as a 50-50 mixture of $CaCl_2$/PVC char solution treatment group for reference.

In all trials, there was *E. coli* colony formation on the -DNA plates, which were negative controls containing non-selective media and bacteria subjected to transformation without plasmid material. The $CaCl_2$, or positive control, plates had no growth on the -DNA/+Amp plates (**Table 1**), while the PVC solution resulted in between 6 and 16 colony-forming units (CFUs) (**Table 2**), and the mix formed between 1 and 3 CFU (**Table 3**). On the +DNA/+Amp plates, between 34 and 225 CFU formed from the $CaCl_2$-treated cells (**Table 1**). The PVC and mix plates grew fewer colonies, with 0 to 2 CFUs (**Table 2**) and 2 to 25 CFUs (**Table 3**), respectively. On the plates containing IPTG in addition to ampicillin, cells treated with $CaCl_2$ grew between 1 and 10 fluorescing colonies (**Table 1**). PVC-treated cells did not fluoresce (**Table 2**), and only 1 CFU treated with the 50/50 mix grew and exhibited fluorescence (**Table 3**). Based on the number of fluorescing colonies, the transformation efficiency of $CaCl_2$-treated cells across three trials

was 2.8 x 10^2, 4 x 10^2, and 4 x 10^1 transformants per µg of DNA. The mix-treated cells had transformation efficiencies between 0 and 4 x 10^1 transformants/µg of DNA. In the experiment, it was found that PVC char did not aid in the transformation of *E. coli* cells, thus supporting the null hypothesis.

Discussion

PVC is a plastic that poses many health hazards (4, 5). Though the intentional incineration of plastic is strictly prohibited by law, products containing PVC are still burned in accidental fires. The harmful properties of the gases released upon the burning of PVC have been extensively researched, but relatively few studies have focused on the solid product, or char, of burning PVC. Since transformation can potentially impact human health and the environment, this study sought to determine the effect of PVC char on bacterial transformation.

The ability of cells to fluoresce when in the presence of IPTG and a long wave UV light is an indicator that the cells successfully took up the pFluoroBlue™ plasmid. The last column of **Table 2** showed that the PVC char did not cause any cells to transform and, in turn, fluoresce. The mix (**Table 3**), compared to the control (**Table 1**), transformed relatively few. A comparison between the control and the mix, both of which contained $CaCl_2$, showed that the transformation rate was decreased. However, since the $CaCl_2$ solution was part of a kit and we needed to mix this with PVC char, the concentration of $CaCl_2$ was reduced by half when mixed with the PVC char solution. It cannot be determined from the current study if the reduction was due to the presence of PVC char solution, or the reduced amount of $CaCl_2$ used. Since PVC char did not induce transformation, it is likely that the lowered rate was due to the decreased amount of $CaCl_2$. Nevertheless, future experiment will include a control with the same concentration of $CaCl_2$ in the negative control as is mixed with PVC char. Additionally, we will include a control group with no $CaCl_2$ and no PVC char in order to determine the full effect either have on transformation efficiency.

As seen in **Table 2**, no colonies formed on the control -DNA/+Amp plates for the *E. coli* cells that were exposed to $CaCl_2$ alone, but there was growth on the corresponding PVC char and the 50/50 mixture plates. Moreover, the colony count is higher in PVC char than in the 50/50 mixture plates. Although it is concluded that the PVC char solution did not cause cells to take up the plasmid, colonies still formed on the PVC and mixture plates containing Ampicillin. Therefore, it is possible that PVC char contains substances that reduced the antibacterial effect of Ampicillin. Since incorporating plastics with antiseptic and antimicrobial compounds in medical catheters and food packaging to combat contamination is increasingly common (7, 8), these results warrant further investigation. An alternative explanation for the aforementioned growth is that the PVC char samples may have been contaminated with other Ampicillin-resistant bacteria, or, although highly unlikely, that the PVC char induced a mutation in the Ampicillin binding site of the cells, thereby conferring resistance. These are also avenues for future exploration.

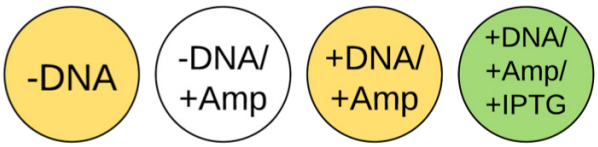

Figure 1. Plates containing LB agar with additives. This image shows the growth conditions of *E. coli.* –DNA indicates no pFluoroBlue™ plasmid present, +AMP indicates that Ampicillin was added to the medium, +DNA indicates the pFluoroBlue™ plasmid is present, and +IPTG indicates that if the organism grows on this plate, it will fluoresce when put under a UV light.

There were several limitations inherent in this study. Materials and procedures from Edvotek® Kit #222 were used to conduct this experiment, with appropriate substitutions using the PVC char and 50/50 mixture. The strain of *E. coli* used was unknown, and therefore, its original susceptibility to Ampicillin as well as the other reagents is not known. Additionally, the concentration of $CaCl_2$ in the $CaCl_2$ solution was not disclosed; therefore, the concentrations of $CaCl_2$ and PVC char solution in the mix solution are not exact, but relative, composed of 50% of each. It is possible that we did not observe any effect of PVC char on bacterial transformation because the concentration was too low. This could have been because our method of burning PVC was not very effective or that PVC char does not dissolve well in distilled water. Future experiment will attempt to quantify PVC char concentrations to better control this variable in different trials and to test increasing concentrations on transformation efficacy. Another limitation was that the PVC char was not sterilized before being immersed into distilled water. The char may have contained other strains of bacteria. While contamination is a possibility, this is more representative of the state of PVC char when naturally released into the environment. There was variation in the masses of the pieces of char, as well as the extent to which each sample was burned. Finally, the plates were incubated for nearly 48 hours (from Saturday to Monday), resulting in probable breakthrough growth. This is most problematic in interpreting the colony counts on the Ampicillin plates, as it cannot be said if the colonies counted were indeed resistant to Ampicillin or had acclimated to its presence. Despite these limitations, it can be said that PVC char does not aid in transformation in *E. coli*.

Methods

Vinyl floor tiles containing PVC were cut by an Olfa multi-purpose cutter knife into 3 pieces measuring 1 x 4 cm. The pieces were held using metal tongs and burned with a propane torch under a fume hood. They were then individually placed into sealable bottles. The PVC char solution was prepared by adding 2 mL of distilled water into each bottle, followed by overnight incubation at 42° C in a water bath.

Materials from Edvotek® Kit #222 were used for the experimentation. To prepare the petri dishes, the ReadyPour™ LB Agar was melted and cooled to 60° C. Twenty small (60 x 15 mm) plates were marked with a single stripe, ten with two stripes, and the remaining ten unlabeled. 10 mL of agar was poured into each of the 5 large petri plates using a 10-mL pipet and pipet pump. After the entire amount of Growth Additive was mixed into the agar bottle, a fresh 10-mL pipet was used to pour 5 mL of the agar into the ten unlabeled plates. After all the Ampicillin was mixed into the agar bottle, a fresh 10-mL pipet was used to pour 5 mL of the LB/Amp medium into the ten unlabeled plates. After all the Growth Additive was mixed into the agar bottle, a fresh 10-mL pipet was used to pour 5 mL of the LB/Amp/IPTG into the ten unlabeled plates. The plates were covered and left for at least twenty minutes to solidify.

For each of the five large *E. coli* source plates, a single Bactobead™ was transferred onto the edge of a large petri dish using a sterile inoculating loop. 10 µL of sterile water was added in order to dissolve the bead. After the bacteria were streaked, the plates were covered, inverted, and incubated at 37° C overnight.

Three microcentrifuge tubes were labeled "+DNA" and three "-DNA". With a sterile 1-mL pipet, 500 µL of ice-cold $CaCl_2$ solution was transferred into a "-DNA" tube. Fresh pipets were used to transfer 500 µL of PVC char solution into another "-DNA" tube, and to transfer 250 µL of $CaCl_2$ solution and 250 µL of PVC char solution into the third "-DNA" tube. With a toothpick, fifteen well-isolated colonies were transferred from the *E. coli* source plates to each "-DNA" tube. 250 µL of the cell suspensions were transferred into the corresponding "+DNA" tube. 10 µL of pFluoroGreen™ was added to each of the "+DNA" tubes, which were then incubated on ice for 2 minutes. With a 1-mL pipet, 250 µL of Recovery Broth was transferred into every tube. All the tubes were incubated for 30 minutes in a 37º C water bath. This was repeated for cultures using PVC solution and a 50/50 PVC/$CaCl_2$ mixture (substituted for $CaCl_2$), for a total of 9 microcentrifuge tubes (3 per condition).

While the cells were being incubated, 3 petri dishes were labeled "-DNA", 3 "-DNA/+Amp", 3 "+DNA/+Amp", and 3 "+DNA/+Amp/+IPTG". These 12 plates were for $CaCl_2$ (**Figure 1**). In the same way, 12 were labeled for PVC and 12 for the 50/50 mixture. With a sterile 1-mL pipet, the recovered cells were transferred from the tube to their corresponding plate. An inoculating loop, changed every time to avoid cross-contamination, was used to spread the cells over the whole plate. The plates were covered and given 5 minutes so that the cell suspension could diffuse across the agar. The plates were then stacked and placed inverted in a 37º C incubator for 48 hours. Afterwards, the number of colony-forming units on each plate were observed, counted, and recorded. The plates containing IPTG in the media were visualized using a long-wave UV light, and the number of fluorescing colonies were recorded.

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