Azo Dye Toxicity: a measure of toxic effect metabolized azo dyes have on the body

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Abstract

Azo dyes represent the world's largest and most diverse group of synthetic dyes with uses spanning almost every industry (Pandey *et al.* 2007). Their significance to public health is great as these globally produced dyes have been proven to be carcinogenic upon biodegrading into their aromatic amines (Harmse 2013). But aside from their carcinogenic properties, azo dyes have another dangerous effect: their toxic ability to harm the intestinal bacteria that are most likely to digest them. I tested for azo dye metabolism in four dyes and three bacterial species and found all four azo dyes (Congo Red, Methyl Orange, Cibacron Red, Ponceau BS) were metabolized and created toxic metabolites by *Enterococcus faecium* and Suttonella indogenes, but *Rhodococcus rhodochrous* was only able to metabolize Congo Red and Methyl Orange. Despite this, the *R. rhodochrous* "metabolites" inserted into bacteria cultures, still incurred a toxic effect on bacterial growth. So, dye toxicity to bacterial growth was highest in those dyes metabolized the most, but also can be found in un-metabolized parent dyes. Therefore, the implication of our findings suggest the need for more research to help identify the toxicity of some 8000 azo dyes produced globally.

Keywords: Azo dyes, Metabolites, Toxicity, Microbiota

Introduction

Azo dyes are inexpensive synthetic colorants residing in 60-70% of all dyed products, and microbiota refers to the collective community of microorganisms in our body (Khehra *et al.* 2005). Azo dyes and our microbiota share two things in common: they are both about as ubiquitous as you can get and their effect on our health is largely underappreciated. The microbiota of our gut contributes to our health like another organ, providing, among many others, the following benefits: assistance to the digestive system, affecting vital human gene expression, enhancing immune system, and combating pathogenic microbes (Spanogiannopoulos 2016). Azo dyes also contribute to our health, but with opposite results. Toxicity from these dyes may derive from the chemical reduction and cleavage of the azo bonds performed by azoreductase in bacteria (Brown and DeVito 1993). Because some azo dyes are toxic to many intestinal bacteria that make up our microbiota, their presence can mean reduced functioning of this critical "organ."

This reduction and cleavage is usually only possible with exposure to anaerobic bacteria with the most common azo reducing bacteria making up the microbiota of our intestinal tract (Brown and DeVito 1993). The human microbiota contains many thousands of bacterial species, and many of these may break down azo dyes in different ways, producing different metabolites (Feng *et al.* 2012). The grand number of azo dyes further complicates this complexity, reaching higher than 8000, whose metabolites generated from azo reduction are not all known. The multiplicity of these variables, which cause confusion in the understanding of azo dyes toxicity, has caused a lack in understanding of the role azo dyes play in intestinal bacterial species like *Enterococcus faecium*. The complexity and sheer multitude of different molecularly structured dyes out there only worsens the azo dye induced health crisis because we just cannot know the effect every dye has on the body. Therein lies the problem which this study attempts to address: To help further the scientific body of knowledge concerning the effects different dyes have on different intestinal bacteria. To assist in the effort to reduce the number of unknown toxic dyes, I tested my hypothesis that some intestinal bacteria would be able to breakdown the tested azo dyes and produce toxic products from this metabolism.

Methods

Testing for Metabolism

I added 10 µL of each dye into tubes for a final dye concentration 20 µM containing 10 mL LB broth and bacteria. I measured absorption of dye

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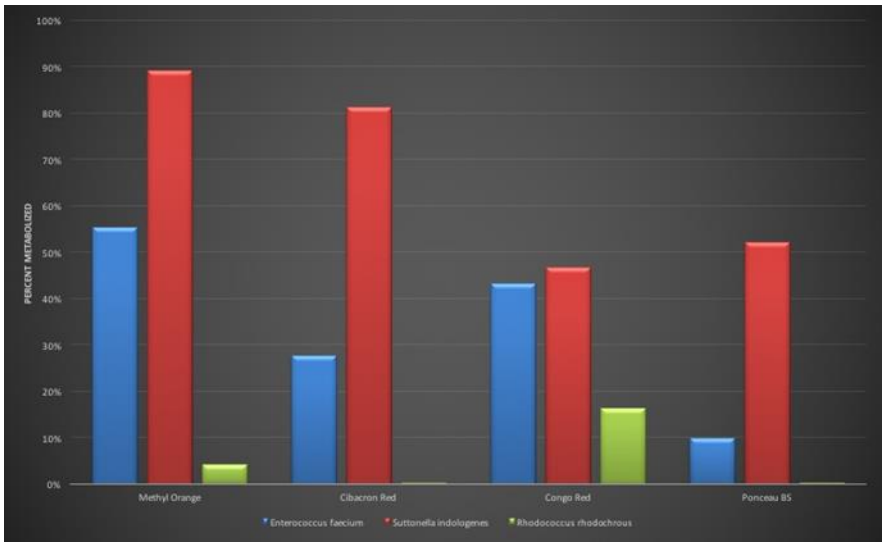

Figure 1 *- Percent dye metabolized by each bacteria*

metabolites, using a spectrophotometer at 1, 3, and 6 hrs. The supernatant (liquid phase containing dye metabolites) and cells from the culture were separated by centrifugation at each time period. After the first centrifugation, the tubes were incubated for one hour. The following bacteria were used in the study: *E. faecium*, *Suttonella indologenes*, and *Rhodococcus rhodochrous*. From these isolated metabolites, spanning increasing levels of digestion, I was able to determine the percent dye metabolized by each bacteria.

Testing for toxicity

I tested toxicity of the dye metabolites from the previous experiment by comparing the growth of treated bacteria (containing metabolite) with control groups (no metabolite). Over a 6-hour period, I recorded absorbance readings at the wavelength of bacteria cells (600 nm), which measures bacterial growth. From this, I determined the percent inhibition of treated groups compared with controlled groups.

Results

Evidence for metabolism

In Figure 1, the bacteria varied in their ability to breakdown the dyes, with *S. indologenes* being the strongest reducer, followed by *E. faecium*, then *R. rhodochrous* as the least able. In Figure 1, each dye tested was metabolized at levels. In order of most digested to least digested by the three tested

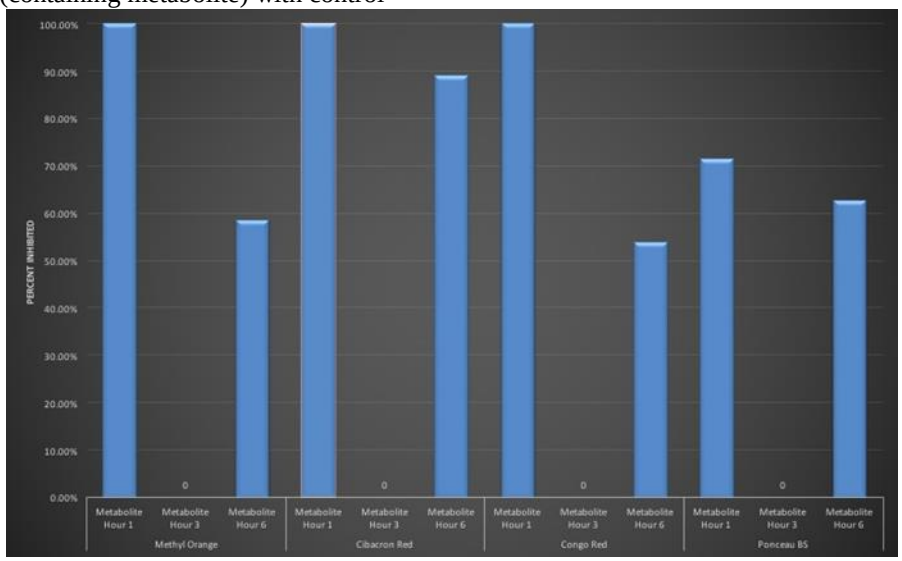

Figure 2 *- Percent inhibition of potential growth for* E. faecium *population*

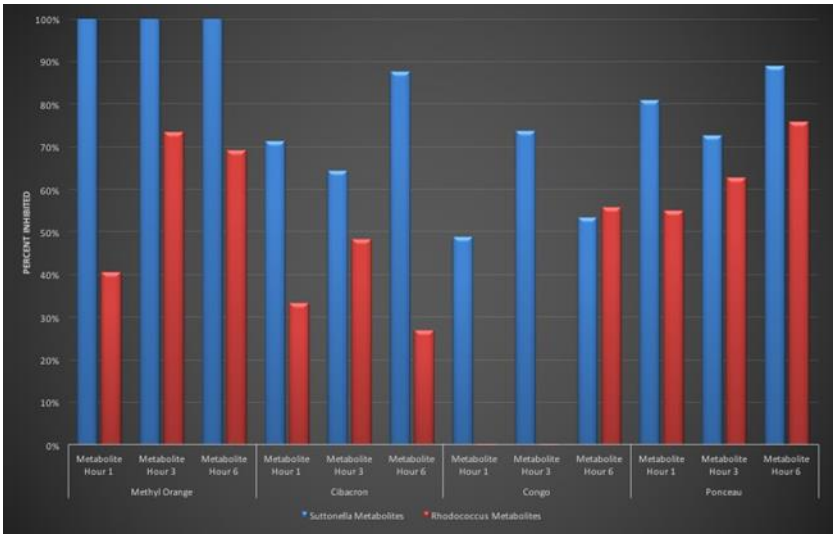

Figure 3 *- Percent inhibition of potential growth for* S. indologenes *population*

bacteria: Methyl Orange, Cibacron Red, Congo Red, Ponceau BS.

Evidence for toxicity

In Figure 1, All four azo dyes were metabolized by *E. faecium* and *S. indogenes*, but *R. rhodochrous* was only able to metabolize Congo Red and Methyl Orange. Despite this, the *R. rhodochrous* "metabolites" inserted into bacteria cultures, still incurred an inhibitory effect on bacterial growth (Figures 3 and 4). The apparent absence of trends in Figures 2 and 3 shows the complexity of chemical variance between dyes and their toxicity to various intestinal bacteria. Here, some dyes are more toxic in their parent dye form, not needing to be reduced to incur inhibitory effect on bacterial growth. In figure 4, the Congo Red data serves as a prototypic representation of the effect increasing levels of dye digestion have on the toxicity of that dye.

Discussion

My findings support the hypothesis of the microbiota's ability to breakdown azo dyes to produce toxic metabolites. The example of Congo Red in Figure 4 shows the relationship between amount of dye metabolized and toxicity. As the duration of metabolism increases, the toxicity also increases. Although 4 dyes were tested, not every intestinal bacteria can metabolize every dye, and it is evident from this research that in addition to those dyes that can be broken down, the indigestible dyes may still incur a toxic effect, as observed in the case of *R. rhodochrous*. My hypothesis concerning the

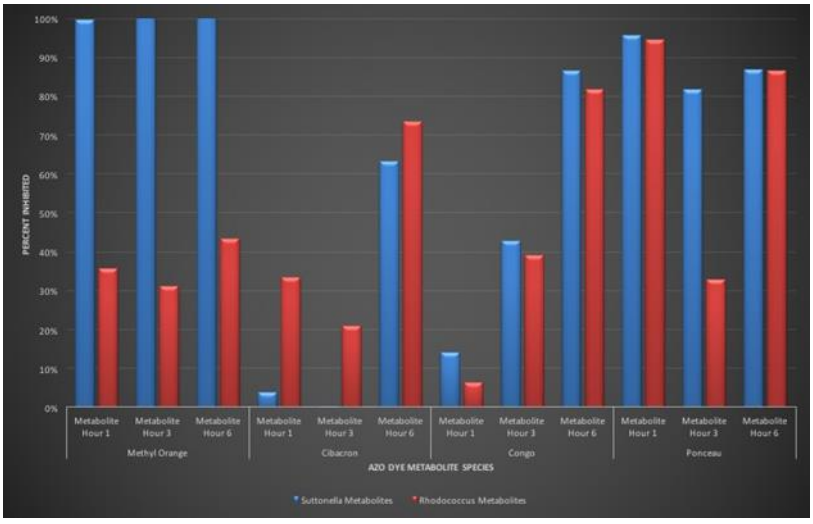

Figure 4 *- Percent inhibition of potential growth for Rhodococcus rhodochrous population*

persistent toxic effect of the undigested dyes in the *R. rhodochrous* culture, Ponceau BS and Cibacron Red, is that the parent dye (the pre-metabolism dye) may not need to be reduced in order to be toxic.

These results support the idea that azo dyes pose a potential health risk for people all over the globe. However, to combat this problem, we must learn more about the biochemical processes that lead to its genesis. Further research into the toxicity for each azo dye is needed to reduce the risk to our vital microbiota and its relationship with our health.

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