SELECTION OF CHLORINE TOLERANT *E. coli* O157:H7

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ABSTRACT

Enterohemorragic *E. coli* O157:H7 strains have been recognized as a cause of serious gastro-intestinal infections in humans, acquired by ingestion of contaminated food and water. *E. coli* O157 has been recovered from municipal water supplies, suggesting some chlorine tolerance mechanism. Chlorine- resistant clones of the genomic strain Sakai of *E. coli* O157: H7 were selected during six passages of chlorine exposure. Clones with stable resistance levels greater than 5 times than the wild- type were obtained. Results of a panel of twenty-eight biochemical tests were unchanged in the resistant clones. The resistance mechanism appears to be a reduction of cell wall permeability, as shown by a lowering of fluorescence of nucleic acid stain SYTO-16 and propidium iodide with the majority of selected clones. Data suggesting also that the increased chlorine tolerance appears to be multifactorial, including increases in catalase activity and potentially other changes to counteract the oxidative damage generated by chlorine. Chlorine applies selective pressure on the vast number of prokaryotic organisms in our municipal water systems, the effects of which are still unclear.

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INTRODUCTION

Escherichia coli (E. coli) are gram-negative enterobacteria that are normal flora in the intestines of animals and humans. Presence of *E. coli* and others species of bacteria within our intestines is necessary to remain healthy. The majority of the *E. coli* are beneficial. Even though *E. coli* inhabits the intestinal tract as a beneficial microorganism, it has been associated with a wide range of diseases and infections, including meningeal, urinary tract, wound and bacteremic infections in all age groups. Also *E. coli* may cause five types of diarrheal illnesses, because the strains that produce these kinds of infections are known to produce toxins. The five different types of toxins each have a different mechanism of action (Mahon 2002 and Moat 2002).

The Centers for Disease Control and Prevention (CDC), the preeminent public health arm in America, is particularly concerned of the danger presented by Enterohemoragic *E. coli* O157:H7, because it has been associated with disease outbreaks.(CDC web site). Enterohemoragic *E. coli* (EHEC) strains have been recognized as a cause of serious disease for a number of years. Enterohemorrhagic is defined as bacteria that are capable of causing an outbreak of hemorrhagic diarrhea, colitis and Hemolytic Uremic Syndrome (HUS). HUS is a serious and potentially lethal invasion from the intestine into the bladder and kidneys. In the EHEC group of strains the predominant serotype is O157: H7. O refers to the serotype due to the arrangement and character of O chain sugars on the lipopolysaccharide (LPS) and H refers to the flagellar protein serotype. Infection with O157:H7 causes acute abdominal pain and bloody diarrhea. In less than 10% of patients, this condition worsens cute renal failure can result. This potentially fatal complication is more frequent in children younger than five years and elderly patients (Salyers 1998 and Lysle 1998), presumably because of a weaker immune system. For example, in 1992 more than 600 people were struck with severe diarrhea and intestinal cramps

after eating undercooked hamburgers in a fast-food restaurant. Forty-five out of 600 persons exhibited HUS and three toddlers died (Levy 2003).

The infection usually results from ingestion of undercooked grinder meat contaminated with intestinal contents during the animal slaughter. However outbreaks associated with unpasteurized milk, apple cider and municipal water supplies have been reported (Salyers 1998, Rice 1995, Mahon 2002). The stress tolerance of O157:H7 [i.e., starvation, hydrogen peroxide and chlorine] (Lysle 1998, Arana 1999) results in exposure to humans.

Chlorine

Chlorine is a strong oxidizer agent, commonly used to decontaminate our municipal water supplies. In that role, it has many advantages: it is inexpensive, a broad-spectrum germicide against bacteria, fungi, protozoa, and viruses, and it has residual disinfect ion capacity downstream of its point of use. However, it also has disadvantages; the chlorine residuals even at low concentrations are toxic to aquatic life. Certain forms of chlorinated compounds are corrosive to the water distribution system. Chlorine can react with amines in water, generating chloramines. Some evidence suggests that chloramines can cause cancer in animals.

The forms of chlorine in drinking water are molecular chlorine (Cl_2), hypochlorous acid (HOCl), and hypochlorite ions (OCl^-). The proportion between these forms depends on water pH levels and temperature (Dunkan 1996). The proportions are the same for any set conditions whether the chlorine is introduced as chlorine gas or as hypochlorite (Johnson 1975). HOCl is favored at a pH of 4-7 (expected for the protonated form), while OCl^- dominates in a pH above 9 (McDonnell 1999) and Cl_2 tends to be formed at a pH below 2. Hypochlorous acid has slightly stronger bactericidal action than Cl_2 and OCl^-. Le Dantec observed mycobacterium inactivation

by chlorination is more efficient at higher temperatures (Le Dantec 2002). Free chlorine is a term used to indicate the combined concentrations of hypochlorous acid and hypochlorite ion in a solution, as these are the most reactive of longer-lived forms. Combined chlorine is the measurement of chlorinated ammonia or organic nitrogen compounds. Total chlorine is the additive of both free and combined chlorine. Free chlorine has a stronger virucidal and bactericidal effect than combined chlorine (Johnson 1975). However, it can oxidize certain types of organic matter in wastewater creating more hazardous compounds, called disinfectant byproducts (DBPs), like trihalomethanes, haloacetic acid, haloacetonitriles, and haloketones. DBPs have been shown to cause cancer in laboratory animals. Chlorine can also increase the level of dissolved solids.

Chlorine is the most widely used disinfectant for municipal water and wastewater, sanitization of equipment and surfaces, and external treatment of food such as poultry, seafood, and vegetables. The five major groups of chlorine sanitizing compounds include Cl_2, hypochlorite (commonly called bleach), inorganic chloramines, organic chloramines and chlorine dioxide (Solomon 1998 and Russell 1999). Despite its widespread usage for over a century, the exact molecular mechanism of action of chlorine against microbes is unknown. Many hypotheses have been developed to explain the germicidal effect of chlorine and its compounds. Some of the proposed mechanisms based on *in vitro* data are:

Oxidation. Chlorine is an oxidant that takes away electrons from substances. It is thought that it either acts as or generates oxidative free radicals in a nonspecific manner. Free radicals are capable of causing damage by three predominant pathways in cells

(Contran 1999):

a) Cell wall damage.

Lipid peroxidation, which compromises membrane permeability and stability, modification of cell wall permeability. It may destroy the cell wall permeability and as a result important solutes and nutrients diffuse out of the cell. Hydrolysis. Chlorine hydrolyses the cell wall polysaccharides causing the weakness of the cell wall and can dehydrate the cell.

b) Protein Damage.

Oxidative modification of amino acid in proteins, inhibiting protein activity and chlorine precipitates protein thus causing inactivation and/or aggregation.

c) DNA Damage.

Oxidative modification of nucleic acids leading to lesions in DNA (McDonnell 1999) and denaturation of DNA. The reaction between hypochlorous acid and the bases in the DNA leads to a loss of hydrogen bonds, which destroy the double strand, resulting in single strands of DNA, which can be easily degraded.

It has been also suggested (Barrette 1989) that hypochlorous acid produces cellular death due to interference with ATP production. Adenylate energy charges decrease quickly in several species (*P. aureuginosa, E. coli and S. lactis*) following chlorine exposure. Exposure to HOCl generated energy-depleted states where these bacteria were incapable of attaining normal adenylate energy charge values necessary for biosynthesis and growth, despite the presence of external nutrient energy sources. The disruption of ATP production may be a consequence of inhibition of inner membrane bound systems responsible for these processes.

Thus, the interactive and temporal relationship between the above mechanisms, as well as the relevance in cells, is still unclear. Which mechanism is most toxic or most relevant to cell death and which are merely secondary consequences, is also unclear. Most studies in the past in