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Survival and control of *Escherichia coli* O157:H7 in foods, beverages, soil and water

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Escherichia coli O157:H7 is a significant human pathogen that has mostly foodborne and waterborne modes of transmission. Although capable of infecting several hosts, the main source of this bacterium is cattle. In humans, it mainly causes hemorrhagic colitis, bloody diarrhea and hemolytic uremic syndrome. This bacterial pathogen is fairly resistant to various stresses and can survive for significant periods of time in the environment outside of a host. Some of the factors impacting its survival include the indigenous microbial communities, its ability to attach to food contact surfaces and form biofilms, temperature and dehydration. To address the public health concerns associated with this pathogen, several disinfection and sanitization procedures and technologies have been developed in recent years. Synergies between different procedures have been evaluated as well. This review addresses recent developments regarding the survival and disinfection of *E. coli* O157:H7.

Introduction

Escherichia coli O157:H7 is a Gram-negative rod-shaped enteric bacterium (**Fig. 1**), which produces Shiga toxins 1 and 2 (Stx1 and Stx 2) as important virulence factors. Cattle are the most significant source of this bacterium.[1-3] But this serotype has also been isolated in fecal samples from horses, dogs, birds, sheep and flies.[2,4,5] It can also be found in rivers and streams subjected to fecal contamination run-offs.[6] This bacterium is a significant public health problem. In humans, *E. coli* O157:H7 is typically associated with hemorrhagic colitis, bloody diarrhea and hemolytic uremic syndrome (HUS) with renal tubular damage.[7,8] HUS can lead to kidney failure and possible death, especially in children and in the elderly.[9-11] Obrig[12] showed that the Shiga toxins bind to small vessel endothelial cells of the lamina propria of the intestines and to small vessel endothelia in the kidneys, where they cause apoptosis by inhibition of protein synthesis. The infectious dose in humans appears to be very low at less than 50 cells in one study or approximately 700 in another study.[13,14]

Since *Escherichia coli* O157:H7 emerged in the early 1980s, infections in humans have been most commonly associated with foodborne, person-to-person and waterborne transmission.[11,14,15] According to Rangel et al. the majority of outbreaks and cases in the United States involved a foodborne transmission, often associated with communities, restaurants and schools. In the United States, it is estimated that there are approximately 73,000 foodborne cases per year with a case fatality rate of 0.0083.[16] In the United Kingdom between 1999 and 2008, the mean annual incidence rate was 4.4 cases per 100,000 people.[17] In Scotland, the human infection rate ranged from 2.9 to 5.6 cases per 100,000 between 1999 and 2008 and about 20% of cattle farms are positive for this bacterium.[18] Moreover, approximately 3% of all laboratory-confirmed cases of food poisoning in Scotland are related to *E. coli* O157:H7, a figure that is much higher than in other parts of the United Kingdom.[4]

Cattle and cattle farms are often implicated with the presence of this organism. In a recent study of Scottish farms, it was estimated that, although about 20% of farms may harbor this bacterium at any given time, more than 80% may harbor it at some point during the year.[19] In a study of 180 farms in Belgium, it was discovered that *E. coli* O157:H7 was present in 37.8% of the farms.[20] The highest prevalence was associated with dairy cattle farms (61.2%), whereas beef farms had a prevalence of 22.7%. In neighboring Netherlands, a study found a high prevalence of these bacteria in organic and conventional Dutch dairy farms.[21] In an extensive study of hundreds of cattle samples in the United States, Reinstein et al. found the prevalence of this bacterium was 14.8 and 14.2% for organically- and naturally-raised cows, respectively. Finally, cattle fed a diet of wet distillers grains with solubles from corn were found to be twice as likely to harbor *E. coli* O157:H7 as animals fed on a control diet.[23]

Foodborne outbreaks of *E. coli* O157:H7 have been reported with a variety of foods including ground beef, spinach, lettuce, radishes, sprouts, fermented sausages, unpasteurized fruit juices, apple cider and raw milk.[24-27] Beef carcasses can become contaminated by fecal contamination during slaughtering and processing and each step must include sanitary procedures.[28] Recent studies suggest that cattle hides are important source of transmission of *E. coli* O157:H7.[29,30] *E. coli* O157:H7 outbreaks have also been reported in association with contaminated drinking water[15,31] and recreational water.[32,33] More recently, additional serotypes of Shiga toxin-producing *E. coli* have been associated with foodborne infections in humans. These include various serogroups such as O8, O26, O45, O91, O103, O104 (including O104:H4), O113, O121 and O145.[34-36] This diversity highlights the need for more research in this area, especially since the O104:H4 serotype

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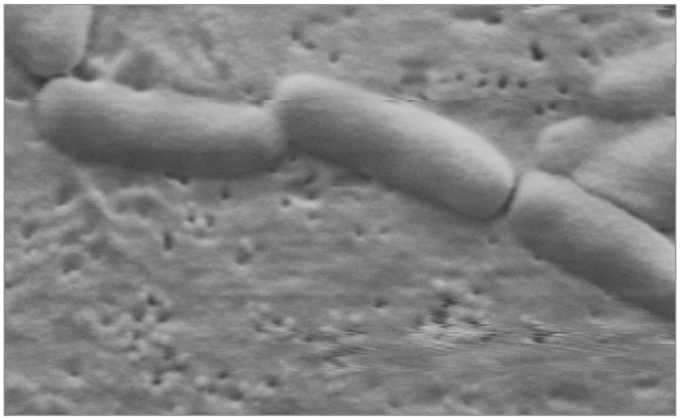

Figure 1. *Escherichia coli* O157:H7 by scanning electron microscopy.

was associated with the 2011 outbreak in Germany. This review will focus on the survival of the *E. coli* O157:H7 serotype in soil, in manure, food, water and on abiotic surfaces as well as potential techniques for inactivation, disinfection and control of this microorganism.

Survival of *E. coli* O157:H7

Survival of *E. coli* O157:H7 has been studied under various conditions and in different environments. This bacterium is generally considered to survive very well in the environment. Its survival, however, can be impacted by various factors such as the indigenous microbial communities, temperature, dehydration, moisture and aerobic conditions. *E. coli* O157:H7 is capable of producing stress response mechanisms that facilitate its adaptation. Of significance is its acid tolerance, which provides more resistance in acidic environments.[37] Acidic foods would include some fruits, apple cider, mayonnaise and ground beef. Expression under acidic conditions of the alternate sigma factor (*rpoS*) is associated with increased acid resistance and *E. coli* O157:H7 can be considered an intrinsically acid-resistant bacterium.[37] The *rpoS* gene controls the expression of more than 50 proteins that are mostly involved in the general stress response.[38] Moreover, heat shock (e.g., *dnaK*) and cold shock (e.g., *cspA*, *yfiA*) genes have also been shown to have a high level of expression in stressed *E. coli* O157:H7, thus increasing its survival.[38] These genes generally protect cells during exposure to sublethal environmental temperatures. A recent study in which the gene expression of *E. coli* O157:H7 was evaluated using microarrays suggests that this bacterium may express many different genes during transport through the environment.[38] Some of these expressed genes include antibiotic resistance genes and virulence genes. Duffitt et al. concluded that survival may lead to increased stress-associated disinfection resistance, increased virulence, and some level of resistance to antibiotics. Thus, a good understanding of the survival and fate of this pathogen in the environment (including in various foods) is critical to minimizing its risk to public health.

Survival in soil and manure. Several studies have established that *E. coli* O157:H7 is capable of significant survival in soils and manure. For example, Duffitt et al. demonstrated that cells of this organism survived for up to 179 d at 15°C in a sterile fox silt loam soil. Similarly, *E. coli* O157:H7 survived for at least 245 d in the sediments of microcosms simulating contaminated cattle water troughs.[39] This survival ability is a significant factor in increasing the risk of crop contamination.[40] van Overbeek et al.[40] studied the effect of indigenous soil bacterial communities on survival in 36 different soil types. These researchers observed that "soils that are under constant intensive pressure by farming procedures [are] more likely display unpredictable behavior of microbial communities" and accordingly survival of *E. coli* O157:H7 is more difficult to predict in such environments. Ibekwe et al. pointed out that *E. coli* O157:H7 survival was greatest in soils with reduced microbial diversity. Similar results were found by Vidovic et al. who demonstrated that dehydration and inhibition by indigenous soil microorganisms were two significant factors affecting survival in the silty clay soils that they studied. In another study using two different agricultural soils (a sandy loam and a silty clay), *E. coli* O157:H7 survived for up to 90 d.[43] Even in manure-amended tropical agricultural soils (fresh loamy soil from Uganda), with temperatures fluctuating between 16 and 42°C, cells of this organism survived for two to three months.[44] Finally, in a study of 36 Dutch soils, Franz et al.[45] found that the range of survival times was 54 to 105 d. In general, finer-textured soils (such as the ones rich in clay) resulted in greater survival rates than coarser-textured soils (sandy soils). These researchers speculated that the higher availability of protective spaces against predators (e.g., feeding protozoa) was responsible for the greater survival in clay-rich soils.[45]

Survival in undiluted manure is also very complex. For example, survival of *E. coli* O157:H7 at 16°C in anaerobic manure (approximately 6 mo) was greater than in aerobic manure (approximately two weeks).[46] These differences appear to be due mostly to changes in the indigenous microbial community since it is known that various protozoa graze and grow on *E. coli* O157:H7.[47] In an earlier study, Semenov et al.[48] established that the survival of this bacterium in untreated manure decreases with increasing temperatures. These researchers also demonstrated that *E. coli* O157:H7 can grow in sterilized manure, indicating once again that indigenous microbial communities play an important role on the survival.[48]

Survival in foods. As seen from above, *E. coli* O157:H7 cells can survive for some times in soil and manure, potentially leading to contamination of fruits and vegetables. Additionally, meat, especially ground beef, can become contaminated during slaughtering and processing. This leads to the next questions: How long can this bacterium survive in foods and what are the factors impacting its survival in various products?

E. coli O157:H7 has become a significant problem with leafy vegetables such as lettuce and spinach in recent years.[49] Several studies have shown that *E. coli* O157:H7 cells can survive very well for 10 to 14 d on lettuce leaves, with highest survival on traumatically injured leaves.[50,51] Under refrigerated conditions at 4°C, no significant changes in bacterial concentrations were observed after 14 d.[49] Aruscavage et al. concluded, regarding leafy vegetables, that "maintaining healthy plants and minimizing physical damage around the time of harvest might improve the safety of fresh produce." In a field study, irrigation water containing various levels of *E. coli* O157:H7 was used to spray lettuce and spinach leaves.[52] The plants were field-grown under sunny or shaded conditions, and *E. coli* O157:H7 cells were found on leaf surfaces for up to 27 d post-spraying. In some treatments, the bacterial cells were also found internally in lettuce leaves.[52] However, Zhang et al.[53] reported that heat stress (up to 36°C during the day) did not promote internalization of *E. coli* O157:H7 in lettuce leaves. It appears that both the presence of flagella and the type III secretion system enhance colonization of spinach and lettuce leaves and ensure the survival of these bacteria.[27] Storage temperature was also shown to have a significant impact on the fate of *E. coli* O157:H7 on lettuce and spinach, with very little growth below 8°C, but moderate growth above 8°C and significant growth above 12°C.[54,55] In addition to survival, virulence must be carefully assessed. A significant finding in a recent study was the fact that the *stx1* and *stx2* toxin genes were upregulated in *E. coli* O157:H7 cells contaminating Romaine lettuce and incubated at 4° or 15°C for up to nine days.[56] This suggests that survival on lettuce leaves causes a selective pressure for bacterial virulence to increase.

On various fruits, *E. coli* O157:H7 is capable of survival and growth. For example, approximately 2 to 3 log of growth was produced after two days of incubation on peaches at either 20 or 25°C.[57] At lower temperatures, there was little or no growth, but survival took place for at least 14 d. Very similar results were found with mangos and papayas, in which growth and survival of *E. coli* O157:H7 was observed.[58] Survival on refrigerated mangos, apples, papayas was observed for at least one month, whereas survival on cut and frozen fruits was seen for at least 180 d.[58,59] Finally, survival on refrigerated cantaloupe was noted for up to 7 d.[60] Therefore, these results suggest that various fruits can serve as vehicles in disease transmission.

The survival and growth of *E. coli* O157:H7 in meats and carcasses has also been documented. In ground beef, for example, very little growth was observed at 5 and 10°C.[61] At temperatures above 15°C, growth followed a sigmoid curve. Differences in survival of *E. coli* O157:H7 were observed during storage at 10°C based on beef sites, with bacterial growth on the neck, but no growth on the briskets and rump.[62] Survival of up to 21 d has been observed in untreated ground beef patties, with little decline in numbers.[63] Autoinducer (AI) cell-to-cell signaling molecules, such as AI-2, have been shown to regulate gene expression and enhance *E. coli* O157:H7 survival and virulence.[64] However, some compounds in ground beef can interfere with these signaling molecules.[65] These researchers postulated that cell-to-cell signaling (and their inhibitors) must be accounted for when studying survival and virulence of these foodborne bacteria.

Survival in beverages and water. It is well established that *E. coli* O157:H7 can survive relatively well in acidic beverages. For example, *E. coli* O157:H7 has been shown to survive in apple cider (pH of approximately 4) for up to 31 d at 8°C (which simulates storage conditions), suggesting that refrigerated apple cider can serve as a route of transmission.[24] These results were confirmed in a recent study in which some *E. coli* O157:H7 cells were found to survive for up to three weeks in apple juice at 4°C.[66] Survival declined more rapidly at 23°C. Yuk et al.[67] found that cells stored at 7°C in calcium lactate-supplemented orange, carrot or apple juices dramatically reduced resistance to simulated gastric fluids (and thus survival). On the other hand, *E. coli* O157:H7 cells stored in tomato juice with pulp had the greatest acid resistance.[67] Ukuku et al. found that a decline of at least 5 log in survival rates in tomato juice over a 24 h period. Finally, Makutu et al.[69] noted that some cells of this organism can survive very well for at least 120 h in various fresh tropical fruit juices under both room temperature and refrigeration conditions (4°C).

Outbreaks of *E. coli* O157:H7 infections have also been related to consumption of unpasteurized raw milk and cheese products.[70-73] In cheddar cheese made from unpasteurized milk and spiked with *E. coli* O157:H7, survival was observed for at least 120 d at 7°C.[74] In another study, cells could be detected in Cheddar and Gouda made from contaminated milk for up to 270 d.[75] Because of acid tolerance, this bacterium is also capable of surviving at 7°C in fermented goat milk Amasi, a traditional food product in South Africa.[76]

In chlorinated drinking water, *E. coli* O157:H7, as most Gram-negative bacteria, is not very resistant and its inactivation by chlorine is very rapid.[77] The inactivation of this bacterium by monochloramine is also relatively rapid.[77] In dechlorinated tap water, however, viable cells of *E. coli* O157:H7 were still detected after seven days of incubation at 15°C, emphasizing the critical importance of water disinfection to remove this pathogen.[77] This was especially true in Walkerton, Canada in May 2000, when 2,300 people became infected (and at least six died) with *E. coli* O157:H7 through waterborne exposure.[78] The failure of the chlorine disinfection equipment used for the Walkerton drinking water supply was identified as one of the major causes of these tragic events.[79] Similarly, Wang and Doyle[80] showed that *E. coli* O157:H7 could survive for several weeks in autoclaved filtered municipal water, reservoir water and lake water. As expected, survival was greatest in cold water (8°C) than in warm water (25°C). Their results also indicate that this bacterium may enter a viable but non-culturable state in water. These findings are similar to those of Duffitt et al. who found that some *E. coli* O157:H7 cells survived in autoclaved stream water at 15°C for up to 234 d. Another problem is that chlorine easily reacts with organic material in drinking water, thus reducing its efficacy.[77]

Survival on abiotic surfaces. Contamination of food-processing surfaces, equipment and facilities with pathogenic bacteria can lead to disease transmission. Several bacteria, including *E. coli*, Salmonella and Listeria, can attach to abiotic surfaces and survive for extended time periods in this way.[81] *E. coli* O157:H7 is capable of adhering to stainless steel surfaces.[82] Although early studies suggested very little biofilm growth on stainless steel, more recent studies have shown that *E. coli* O157:H7 can form a biofilm, especially under conditions that favor the production of extracellular polysaccharides (EPS).[83,84] These bacteria can survive on stainless surfaces, in a desiccated state, for up to 28 d.[85] Moreover, stainless steel biofilms of mutant strains of *E. coli* O157:H7 that overproduce EPS and produce curli were especially resistant to chlorine disinfectant.[86] Even biofilms developed on stainless steel material with non-mutant strains showed a relative resistance to chlorine.[83,86] All of these results are significant issues since stainless steel is a commonly used material for a variety of surfaces in hospitals, restaurants, slaughterhouses and food processing facilities. However, copper-containing alloys (e.g., copper nickels and copper silver) have been shown to dramatically reduce the survival of *E. coli* O157:H7.[85] A recent study has shown that a combination of reuterin and nisin or NaOCl had a significant disinfecting impact against biofilms of *E. coli* O157:H7 on stainless steel.[87] In addition, new emerging technologies, such as pulsed electric field and low-temperature plasma, appear promising at controlling these bacteria.[88]

Procedures for Reduction of *E. coli* O157:H7

The hardy survival exhibited by *E. coli* O157:H7 under most conditions highlights the importance of having appropriate disinfection and sanitation methods to deal with it and similar bacteria. The public health significance of this pathogen and its relative prevalence has triggered a large amount of research directed specifically at finding novel ways of eliminating it. Manufacturers have looked at single steps and combination of processes to significantly reduce bacterial populations. The following sections describe recent advances and developments with technologies aimed at controlling this bacterial pathogen.

Heat treatment. In milk, pasteurization at 72°C eliminates these pathogens and the application of heat remains an important technology to kill this bacterium.[89] Lower temperatures, such as 55°C, can be lethal if used in combination with acidification and lactoperoxidase.[89] Additionally, Avery et al. showed that treatment at 60°C for 10 min completely eliminated these bacteria from organic wastes from abattoirs. In clear apple juice, a D value of 4.43 min at 55°C was obtained for *E. coli* O157:H7.[91]

Chemical treatment. A large amount of research has been conducted to evaluate chemical treatment options for *E. coli* O157:H7. Many protocols and procedures use chemicals such as free chlorine, chlorine dioxide, calcinated water, and a variety of organic acids.[92,93] With fruits for example, chlorine solutions (50–200 ppm) are widely used in commercial facilities. However, since some chlorine compounds can react with organic molecules and form potential toxic disinfection byproducts, alternative sanitizers are being sought.[94] Some of these chemicals cause cancer in lab animals and they are suspected carcinogens in humans as well,[94] explaining why some European countries have banned the use of low-level chlorine wash in poultry for example.

Chlorine dioxide (ClO_2) has been widely studied as a disinfectant for a variety of uses including drinking water.[94] Chlorine dioxide is a broad oxidant that appears to disrupt the outer membranes of Gram-negative bacteria as well as protein synthesis.[94] In water, inactivation of Gram-negative bacteria is very rapid under conditions simulating drinking water treatment.[94] Chlorine dioxide produces less carcinogenic byproducts than free chlorine.

Treatment of broccoli sprouts with 50 ppm chlorine dioxide for 5 min reduced populations of *E. coli* O157:H7 by 2.39 log.[95] With iceberg lettuce leaves, however, 20–200 ppm of aqueous chlorine dioxide resulted in less than 1 log of inactivation.[96] Cells incorporated in a biofilms or internalized in the tissues were largely unaffected by the surface wash.[96] Chlorine dioxide acts synergistically with drying to inactivate these pathogens on radish seeds. Treatment with 200 μg/mL of ClO_2 followed by drying at 25°C for 24 h resulted in approximately 3 log of inactivation.[97] These authors suggested that chlorine dioxide should be considered as a good alternative treatment for these fruits. In a recent study by the same research group, Bang et al.[98] achieved more than 5 log of inactivation of *E. coli* O157:H7 on radish sprouts by treating them with 500 μg/mL ClO_2 followed by air drying at 25°C for 2 h and heat treatment at 55°C for 36 h. Although the bacteria were not completely eliminated from the sprouts, Bang et al.[98] stated that optimization of this treatment should lead to the eventual elimination of these bacteria on radish sprouts and seeds. In other studies, aqueous chlorine dioxide in combination with 0.5% fumaric acid was more effective than chlorine dioxide alone and has been proposed as a suitable treatment to extend the shelf-life of broccoli sprouts and alfalfa sprouts and to reduce the microbial health risk.[98]

Studies have also looked at the use of chlorine dioxide gas, which, in general, is a very potent disinfectant. For example, treatment with chlorine dioxide gas at a 5.0 mg/L for 14.5 min at 22°C did achieve at least 5 log of inactivation of *E. coli* O157:H7.[99] However, it also negatively affected the quality of the lettuce, leaving the leaves discolored.[99] In a separate study, Mahmoud et al.[100] performed similar experiments with chlorine dioxide gas and reported 4.6 log of inactivation on cantaloupe with 5.0 mg/L for 10 min. On the other hand, chlorine dioxide gas application (1.2 to 2.1 mg/L) for 1 h only resulted in 0.7 log of inactivation on fresh spinach leaves.[101] These researchers had more success with a 2% solution of lactic acid, which yielded 2.7 log of inactivation.

Acidic electrolyzed water is generated using sodium chloride to yield sodium hypochlorite by electrolysis. Acidic electrolyzed water has a pH of about 2.5 and has been reported to be a strong bactericidal agent for use in the food industry.[90,102] In a study with inoculated green onions, that acidic electrolyzed water reduces *E. coli* O157:H7 inocula to below detection in just over 1 h.[102] The researchers hypothesized that the high oxidation potential, the low pH and several forms of chlorine compounds can be responsible for this inactivation potential. Similar results were observed with fresh greens treated with acidic electrolyzed water (pH 2.1, free chlorine of 30–35 ppm).[103] However, the presence of organic matter drastically hindered the bactericidal action of the acidic electrolyzed water, thus requiring the need for a pre-washing step prior to disinfection.[103] This suggests that additional studies are required to optimize the use of acidic electrolyzed water.

The application of lime (10 g/L CaO) to organic wastes from abattoirs completely killed cells of *E. coli* O157:H7.[104] This result may be due to the increased pH disrupting the cell membrane, the sudden temperature increased by the exothermic hydration of CaO, or the increased evaporation and desiccation of the wastes due to hydration of CaO.[104] Lime application should be further studied in the future, especially in relation with the burial and disposal of livestock carcasses.[104] Finally, the application of essential oils, as natural sanitizing agents, has been shown to reduce the growth and survival of pathogens such as *E. coli* O157:H7 in food processing under optimal conditions.[105,106] Essential oils such as eucalyptus (*Eucalyptus globules*), tea tree (*Melaleuca alternifolia*), rosemary (*Rosmarinus officinalis*), mint (*Mentha piperita*), rosa moschata (*Rosa moschata*), clove (*Syzygium aromaticum*), lemon (*Citrus limonum*), oregano (*Origanum vulgare*), pine (*Pinus silvestrys*), sweet basil (*Ocimum basilicum*), conehead thyme (*Coridothymus capitatus*), Chinese cinnamon (*Cinnamomum cassia*), Greek oregano (*Origanum heracleoticum*), winter savory (*Satureja montana*) and true cinnamon (*Cinnamomum verum*) all had excellent activities against *E. coli* O157:H7, suggesting that these natural products may be more commonly used in the future.[105]

Food irradiation. In the United States, food irradiation was first approved in the 1980s for certain products such as potatoes and spices. In 1997, the US Food and Drug Administration approved the use of irradiation in red meats mostly to control bacterial pathogens such as *E. coli* O157:H7, which was the key issue to be addressed.[107,108] Irradiation technologies typically use ionizing radiation such as gamma rays, low-dose electron beam (e-beam) or X-rays. Food irradiation has the potential to destroy DNA beyond repair, thus killing cells and viruses in a variety of products. In a study that emphasized food quality, Park et al.[108] indicated that use of gamma rays irradiation up to 10 kGy on hamburger patties are useful in reducing bacterial populations with no adverse effect on meat quality. E-beam radiation and X-ray irradiation both at 2.0 kGy were also tested with *E. coli* O157:H7 in frozen beef patties and found to reduce this pathogen below detection.[109] Various other products have also been studied. In one study, *E. coli* O157:H7 was stored on iceberg lettuce for 24 h at 4°C.[110] The lettuce was then irradiated with doses up to 0.25 kGy using a low-energy X-ray irradiator, yielding a D-value of 0.04 min. With ten stacked lettuce leaves exposed to a surface dose of 1 kGy, about 1 log of *E. coli* O157:H7 inactivation was observed in the center of the stack.[110] This suggests that this might be a promising technology for leafy greens. Another treatment consisted of 17 h of dry heat at 50°C followed by a dose of 1.0 kGy to completely eliminate *E. coli* O157:H7 from radish seeds without affecting their germination.[111] Broccoli and alfalfa seeds only required a dose of 0.25 kGy to achieve the same level of inactivation without affecting the seeds.[111] Low-dose electron beam (e-beam) radiation has also been successfully tested with *E. coli* O157:H7 using fresh baby spinach without any damage to the products.[112] A dose of 0.7 kGy inactivated about 4 log of this bacterium, whereas a dose of 1.07 kGy removed at least 6 log without any detectable levels after 8 d of storage. Neal et al.[112] indicated that "low-dose e-beam radiation may be a viable tool for reducing microbial populations or eliminating *E. coli* O157:H7 and Salmonella from spinach without product damage."

UV irradiation. UV irradiation is a physical disinfectant that uses low wavelength germicidal light to cause DNA mutations that can be lethal to cells and viruses. UV has been tested (and commercially used in some cases) for a variety of applications including drinking water treatment, beverage, fruits, liquid egg products and even meats.[113,114] In clear apple juice, a D value of 0.49 min was obtained when irradiating cells with a 15 W UV lamp at a distance of 55 cm (the fluence was not reported).[114] Pulsed UV with doses up to 13.1 J/cm^2 was tested with apple juice and apple cider as substrates.[114] Static treatments yielded about 2.5–3.2 log of inactivation, whereas turbulent treatments produced more than 5 log of inactivation, suggesting a very good potential for this technology.[114] UV irradiation of fruits such as strawberries and raspberries has been studied with pulsed UV.[115] As expected, reduction in cell numbers with pulsed UV does not follow a log-linear trend and further research on the effect of the physical structures of fruits (and other foods) are warranted.[115] Bialka and Demirci[116] showed that reductions of 3.9 log of *E. coli* O157:H7 could be achieved on raspberries with pulsed UV at 72 J/cm^2. On strawberries, reductions of 2.8 log were seen with 34.2 J/cm^2. These researchers pointed out that no observable damage to the fruits was visible and that pulsed UV has potentials as a decontamination method for these fruits. Pulsed UV has also been used for decontamination of salmon filets, producing approximately a 1 log reduction with a 1 min treatment.[117] With sprouts, a combination of 0.5% fumaric acid and short-wave UV (UV-C) irradiation at 1 kJ/m^2 for 3.3 min resulted in about 3 log of inactivation, whereas UV-C alone only inactivated about 1 log of *E. coli* O157:H7 on the sprouts.[109]

Pulsed electric field and high pressure. Pulsed electric field (PEF) pasteurization is a non-thermal food treatment method, consisting of short bursts of electrical current (25 to 75 kV).[118] This technology can be used for beverages (juices, milk) and liquid eggs. It is suspected that PEF treatment can increase cell membrane porosity by electroporation.[119] Recently, *E. coli* O157:H7 was found to be relatively resistant to pulsed electric field (PEF) pasteurization.[120,121] However, when used in combination with increased temperatures and low pH values, PEF pasteurization shows a great potential for achieving an effective control of *E. coli* O157:H7.[121] In fresh liquid egg yolk for example, a 5 log reduction was obtained at 40°C and a PEF treatment of 30 kV/cm.[122] Increased temperatures, to 45–55°C, was also used in combination with PEF pasteurization to inactivate a non-pathogenic *E. coli* strain in a variety of different tropical fruit smoothies.[123] PEF treatment was also considered to be an option for increasing the shelf-life of fresh orange juices without significantly impacting the quality of the product.[124] Timmermans et al. used a pre-heating temperature of 38°C prior to PEF treatment at 23 kV/cm. Finally, Garcia et al.[120] also showed that these bacteria were relatively resistant to PEF treatment at 35 kV/cm when evaluated immediately, but they appeared much less capable of surviving for long periods of refrigeration, suggesting that PEF can result in sub lethal injuries.

High pressure processing (e.g., 600 MPa for 1 min) also showed promises as an alternative technology for the elimination of *E. coli* O157:H7 in foods.[125] As pointed out by Malone et al.[126] pressure alone may not necessarily be sufficient to inactivate various strains of *E. coli* O157:H7. Seventeen strains of *E. coli* O157:H7 showed considerable variable levels of resistance (from 0.6 to 3.4 log inactivation) to high pressure at 500 mPa.[121] One of the most pressure-resistant strains (EC-88) was further studied. Using microarray analysis, Malone et al.[126] showed that several genes provide resistance to high pressure. These include genes for the sigma factor (*RpoE*), lipoprotein (*NlpI*), thioredoxin (*TrxA*), thioredoxin reductase (*TrxB*), a trehalose synthesis protein (*OtsA*) and a DNA-binding protein (*Dps*). Malone et al.[126] concluded that the wide variations in pressure resistance must be addressed before ultrahigh pressure technologies are widely used. Neetoo et al. demonstrated that high pressure at 600 MPa followed by heat treatment at 40°C for 2 min was sufficient to eliminate 5 log of *E. coli* O157:H7 cells on alfalfa seeds. This procedure did not have any significant impact on the viability of the seeds. High pressure at 400 MPa also reduced *E. coli* O157:H7 by 3 log in ground beef 20°C, also rendering the surviving more susceptible to additional milder treatment such as heating, freezing, acidity and salts.[128] The application of high pressure (400 MPa) in combination with a solution of tert-butylhydroquinone showed a synergistic effect, leading to significant pathogen reduction.[129] The combination of high pressure and tert-butylhydroquinone is also promising as an antimicrobial agent against more pressure resistant mutants of *E. coli* O157:H7.[130] However, hydrostatic pressure treatment (300 mPa for 5 min at 4°C) did not inactivate this pathogen in frozen hamburger patties.[109]

Reduction by fermentation in dry sausages. Dry fermented sausages have been linked with outbreaks of *E. coli* O157:H7, often because the products are consumed uncooked.[26] Consequently, regulatory agencies in the United States and elsewhere have developed guidelines to ensure that dry sausage manufacturers demonstrate that food processing steps reduce a significant amount of bacterial pathogens.[131,132] In general, studied have shown that the manufacturing process of most dry sausages results in a significant level of *E. coli* O157:H7 reduction. For example, fermentation at a pH of 4.8 and storage at temperatures above 21°C are very effective conditions for reducing pathogens such as Listeria, Salmonella and *E. coli* O157:H7.[131] These researchers also showed that, regardless of pH or temperature, soudjouk-style sausage did not support the growth and survival of *E. coli* O157:H7.

Conclusion

The data and research findings presented in this review demonstrate that the pathogenic *E. coli* O157:H7 has a tremendous potential to survive various stresses in the environment and to remain viable for long periods under certain conditions and in certain environments. This organism has a repertoire of genes that can provide some level of resistance against various stressors. This environmental persistence explains in part its ubiquity and its relevance to public health. At the same time, a tremendous amount of research has been conducted in recent years to either optimize current disinfection procedures and sanitizing technologies or to develop new ones. These procedures have a wide range of applications for the food and water industries. It is apparent from the research that no single technology will likely solve all the problems. Some products may be more suitable for certain technologies, whereas other products will benefit from the synergistic effects of two or three methods. As additional serogroups of *E. coli* become better known and understood, more research will be needed to continue to optimize disinfection processes in order to minimize the risk for human health.

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