Smarter arrow now available in the food safety quiver

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COMMENTARY

Smarter arrow now available in the food safety quiver

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Food safety is a critical issue worldwide, and responsibility for ensuring and enhancing safety in the food chain is collectively shared by all involved, from producers to preparation to food service. Just over a century ago, the issues of food safety and production were brought to the forefront of public debate and action following the publication of *The Jungle* by Upton Sinclair. Public pressure and outrage rapidly catalyzed passage in 1906 of the "Pure Food and Drug Act," which ushered in a new age of focusing on and improving food safety. Over the past 100 y, we have dramatically improved food safety, and, consequently, public health around the world has been enhanced (1, 2). Despite the tremendous success in reducing foodborne illnesses over time and the resources that have been devoted to eliminating foodborne pathogens, too many foodborne illnesses still occur each year. In PNAS, Schulz et al. (3) describe a method of controlling the critical foodborne pathogen enterohemorrhagic *Escherichia coli* (EHEC, such as the widely known *E. coli* O157:H7) using an antimicrobial protein (colicins) originally produced by nonpathogenic *E. coli* strains; but in this novel study, the colicins were instead produced by plants. This advance in antimicrobial protein production and delivery finally makes colicins available in quantities sufficient to be used as a weapon specifically targeted at EHEC, but can also be used to reduce other foodborne pathogens in a variety of food production environments.

Human foodborne illnesses can be caused by the bacteria *Salmonella enterica* spp., *Campylobacter*, *Listeria monocytogenes*, and EHEC (e.g., O157:H7), which have all been isolated from a wide variety of foods. Collectively, these key pathogenic bacteria cause more than 2 million illnesses and 750 deaths and cost the US economy more than $8 billion annually in direct and indirect costs (1, 4). *E. coli* O157:H7 and other related Shiga-toxin producing *E. coli* (STEC, including EHEC) are widely known as the "hamburger bug." These pathogens are highly virulent, and as few as 10 cells can initiate an infection with potentially catastrophic results, especially in children. Following the onset of bloody diarrhea, hemolytic uraemic syndrome (HUS), a life-threatening disease that causes severe kidney damage, can develop. Because of the high consequences of infection with this pathogen, the food industry has expended well in excess of $2 billion to specifically combat *E. coli* O157:H7/EHEC in foods.

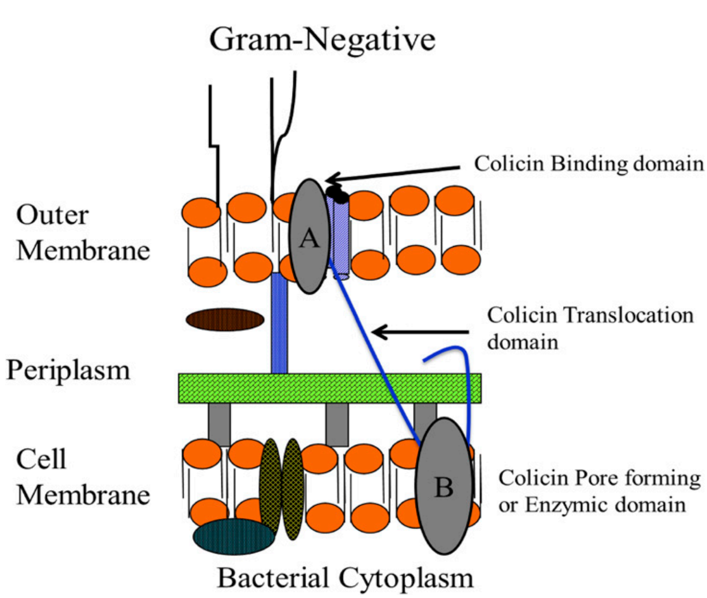

Fig. 1. Stylized mode of action of an antimicrobial protein colicin in Gram-negative bacteria. Domain B represents the active domain, and may form a pore or act enzymically within the bacterial cell. Domain A depicts a stylized binding domain attachment.

Food Safety Improvements

Although the incidence of foodborne illness has decreased with the relatively recent (25 y) implementation of the Hazard Analysis and Critical Control Points (HACCP) process in food production along with best production practices, the consequences of foodborne illness have seemingly increased, at least in public perception. With a rapidly aging populace and a growing population of immunocompromised persons, the deleterious impacts of outbreaks have become more significant from a public health perspective, thus emphasizing the need to develop and implement novel methods to improve food safety throughout the food chain. Naturally, most food safety enhancement efforts have been focused between harvest and the consumer. However, as food safety has improved markedly, we have reached a point of diminishing returns in postharvest interventions strategy implementation; as a result, strategies that can reduce the pathogen load on the farm and during transit to packaging facilities have been developed in recent years and are in increasing demand (5, 6). Because foodborne pathogenic bacteria are unevenly distributed in foods and the food chain, and foods must be rapidly presented to consumers before spoilage becomes an issue, pathogen reduction treatments must be rapidly and broadly applicable on a large scale. Although there is no "magic bullet" that can completely

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prevent all foodborne illnesses, our arsenal of weapons to combat foodborne pathogenic bacteria has grown in both scope and sophistication in recent years.

Colicins, a Smart Arrow

Bacteria in the environment frequently engage in natural chemical warfare against other bacteria occupying the same or similar ecological niches. Antibiotics and other antimicrobials, such as colicins, are secreted into the environment to provide bacteria with an advantage over their nearest competitors. Colicins are small (29–75 kDa in size) antimicrobial proteins produced by some nonpathogenic *E. coli* strains that kill or slow the growth of other competing *E. coli* (or closely related) bacterial strains (7, 8). *E. coli*, as well as *Salmonella*, are Gram-negative bacteria, meaning they are surrounded by two lipid bilayers and a periplasmic space, which provides a measure of physical insulation against many typical antimicrobial proteins that are active against Gram-positive species, which is only surrounded by a single bilayer. Colicins, however, are capable of some rather remarkable gymnastics (Fig. 1) that include binding to the outer membrane, translocating across the outer membrane, and spanning the periplasmic space and inserting into the inner membrane (7, 9, 10). Following insertion into the inner membrane, the pore-forming colicins (e.g., colicin E1, A, and N) create a voltage-dependent pore that allows ions to flow out of the cytoplasm, destroying the electrochemical gradients and the proton-motive force that bacteria depend upon (11–13). Other colicins, (e.g.,colicin E2, E6, E7, and M) act by enzymatically inhibiting DNA, RNA, or cell wall constituent formation in the cytoplasm or in the periplasmic space (13). Because of the mode of action of colicins, the target spectrum for these antimicrobial proteins is relatively narrow; therefore, the potential of colicins was quickly seized upon as a strategy to kill foodborne pathogens (14). Colicins have been shown to inhibit *Salmonella* spp., *Listeria*, and *E. coli* strains, including the critical foodborne pathogenic strain *E. coli* O157:H7, both in and on foods (14–17).

Because colicins are secreted in relatively low concentrations by nonpathogenic *E. coli*, the amount available for use has been limited to the scale for laboratory study only. To get around this limitation, field or animal-level studies typically used colicin-producing *E. coli* as a probiotic or an additive that would persist in the environment (15), but this solution was not always viable in real-world conditions. In recent years, molecular biology has allowed colicins to be produced in greater amounts from different recombinant host systems so that proof-of-concept studies could be performed (17–19). These studies demonstrated that colicins could be used to reduce populations of several species of foodborne pathogenic bacteria on food products, and in live animals (20). The present study by Schulz et al. (3) indicates that plant-made recombinant protein colicins can provide relatively large amounts of a variety of colicin types active against *E. coli* O157:H7 for use as treatments of crops, live animals, or finished foods. This exciting result indicates that for the first time to our knowledge, colicins as specific purified proteins will finally become a viable solution to be used to reduce the foodborne pathogenic bacteria burden entering the food supply. Colicins with a variety of modes of action were successfully produced in this study and, as a result, offer promise not only to preemptively combat the development of colicin resistance but to allow application of colicins as a broad food treatment that is effective against more than one bacterial species simultaneously. This development also opens the door to allow production of colicins as targeted therapeutics for use in human and veterinary applications. Based upon this exciting development, more applications for colicins and related antimicrobial proteins are open for further exploration, which can enhance our understanding of the microbiome around us and how we interact with, and are driven by, the microbial world within and on us.

Plant-made recombinant protein colicins can provide relatively large amounts of a variety of colicin types active against *E. coli* O157:H7 for use as treatments of crops, live animals, or finished foods.

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