RESEARCH IN PROCESS

Significance of the Dormant State in the Persistence, Interaction with Growing Plants and Virulence of Shiga Toxin–Producing *Escherichia coli*

SUMMARY

In the natural environment, pathogens primarily exist in the non-growing state that is collectively termed dormancy. Persister is one such state of dormancy, which can be defined as when a cell down-regulates metabolism despite being under conditions to support growth. From an evolution perspective, persister cells exhibit high stress tolerance and can generate false-negative results during culturing due to the delay in growth. The current project has identified the persister state in Shiga toxin–producing *Escherichia coli* (STEC). From the *E. coli* tested, O103 contained a higher proportion of persisters compared to other isolates tested. However, even with O103 the proportion of persisters was <1% of the total population. Indole was found to induce the perister state in STEC, while EDTA had a negative effect. It is anticipated that future work will identify agents that can break the persister state thereby making STEC more stress susceptible along with increasing recovery during culturing.

OBJECTIVES

1. Determine the proportion of dormant cells within populations of the top 7 Shiga toxin–producing *Escherichia coli* (STEC), including isolates implicated in produce outbreaks.
2. Assess the role of different soil types and lettuce root exudates in the induction or breaking of the dormant state.
3. Establish the resistance of dormant STEC to free chlorine.
4. Evaluate the virulence of dormant STEC with respect to Shiga toxin production and attachment.

METHODS

An extended range of O157 and non-O157 STEC will be applied in the study. Persisters will be identified through treating a culture with antibiotics that would inactivate growing cells. The residual non-growing cells (persisters) will be enumerated following up to 5 days incubation. Complementary studies will be performed using microfluidic chips in combination with time-lapse epifluorescent microscopy to directly visualize persister cells (Figure 1).

A range of growth media (animal or plant origin, in addition to defined minimal) will be evaluated in terms of inducing or breaking the persister state. The constituents with soil, manure and root extracts on the persister state within STEC will also be assessed.

Additional studies will recover persisters to determine relative resistance to chlorine and also survival in soil. The virulence of STEC emerging from the perister state will also be assessed to determine pathogenicity potential.

RESULTS TO DATE

STEC have a persister state

The persister state was found to exist in O157:H7 and the Big 6 STEC although it constituted <1% of the populations. From the range of STEC assessed to date a higher proportion of persisters were recovered from *E. coli* O103 and O121 compared to the other serotypes tested (Figure 2).

Persister state can be influenced by media composition depending on the strain

In the majority of cases the proportion of persisters within a STEC population was independent of media used to cultivate or recover populations. Exceptions were the generation of a higher proportion of persisters within O45 when grown in BHI and within O103 in minimal media. The inclusion of indole within the cultivation medium increased the number of persisters in all STEC cultivated on minimal media (Figure 3). EDTA reduced the number of persisters in O103 but had no effect on the other strains tested.

BENEFITS TO THE INDUSTRY

Unlike under laboratory conditions, pathogens such as Shiga toxin–producing *Escherichia coli* spend most of the time in the non-growing (dormant state). There are different types of dormancy encountered, with the persister being considered as most significant. Although persisters only make up a small proportion of the population, the cells are characterized as having high resistance, persistence and delayed growth. This is manifested in real life by residual subpopulations in soil, processing environments or wash tanks, in addition to false-negative diagnostic tests. The project will, for the first time, identify the persister state within STEC and identify which factors promote dormancy. Of greater interest will be to identify agents that break the dormancy to make STEC more susceptible to treatments such as sanitizers and environmental stresses. Moreover, breaking dormancy will be a powerful tool when reducing the number of false-negatives in culture-based diagnostics.

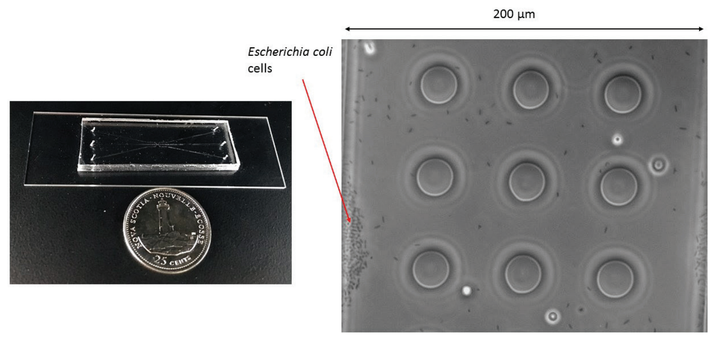

Figure 1. Microfluidic chamber for visualizing STEC. The *E. coli* is introduced into the chamber via a feeder channel. Effector compounds can be introduced via a different channel and change in cell viability captured in real-time.

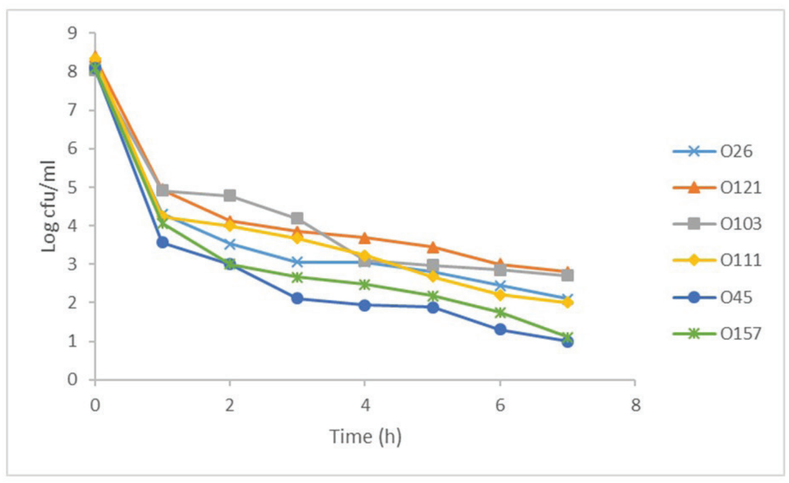

Figure 2. Persisters within different Shiga toxin *Escherichia coli* serotype populations cultivated in LB broth and treated with 200 µg/ml ampicillin. The test *E. coli* strain was cultivated in LB broth to mid-exponential phase before ampicillin was added to the culture (t=0). Samples were then removed periodically and survivors enumerated on LB agar cultivated at 37°C for up to 72 days.

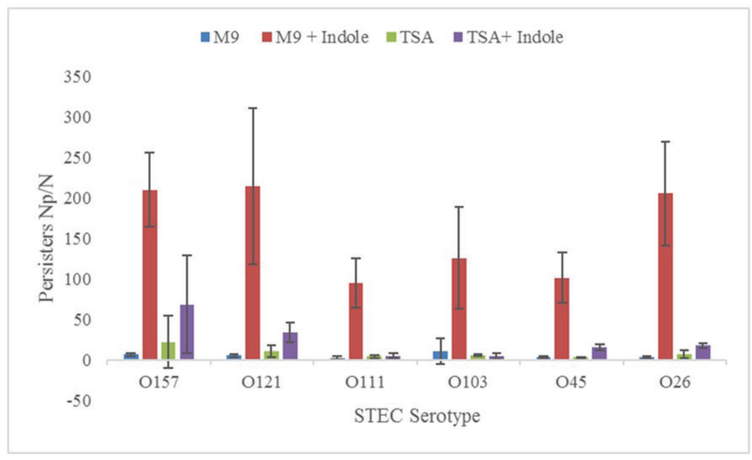

Figure 3. The effect of indole on inducing the proportion of persisters within STEC populations. The test STEC strain was cultivated in M9 minimal media, then inoculated into M9 or tryptic soy broth (TSB) with or without indole (500 µM). The cultures were grown to mid-exponential phase before adding ampicillin and incubating for a further 8h. The persister cells were recovered by plating onto TSA incubated at 37°C for up to 72h. Np – cfu/ml following 8h incubation with antibiotic
N – cfu/ml original culture

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