The Synergistic and Individual Anti-microbial Impacts of Green Tea and Ginger on Common Gastro-Intestinal Bacteria

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Abstract

Ginger and green tea, common substances found increasingly in the diets of the global population, have known anti-microbial effects and are commonly used together in teas. In the following experiment, the synergistic impact of green tea extract and ginger on two bacteria, *Escherichia coli* and *Enterococcus faecalis*, is investigated along with each supplements individual properties as an anti-microbial agent. In examining the effect that each test substance has on bacteria lawns in various combinations, we found no synergistic impact due to experiment design flaws. Both ginger and green tea had antimicrobial effects on *E. faecalis* but not on *E. coli.* The potential impacts of these findings are discussed.

Introduction

In 1928, Andrew Flemming discovered Penicillin. That moment marked a turning point in the world of western medicine. Antibacterial medication has become as commonplace as hand washing soap. Everything from acne to life-threatening infection is fought with antibiotics, often extremely successfully. Antibiotics and medicine reach beyond the laboratory. Many foods present antibiotic functions that often, unbeknownst to the eater, have reduced or limited the growth of bacteria in their body. Among those antibacterial foods that are becoming more common in our western diet are green tea and ginger. Both of these foods have been valued for anti-microbial properties for thousands of years in Asian cultures (Weil 1995). As this tradition moves west, research is being done to back the anecdotal evidence of healing stories. In research done on the effects of ginger on angiogenesis, a process that is closely connected to tumor growth and metastasis, gingerol (a main ingredient in ginger) is cited as having anti-bacterial tendencies (Kim et al., 2005). Similarly research on epigallocatechin-gallate, the main catechin in green tea extracts, was found to not only have anti-bacterial properties of its own but also to act in synergy to promote the antibacterial activity of tetracycline, a well known antibiotic (Roccaro et al., 2004). Given the current research and many years of successful medicinal use as an anti-microbial, this study set out to test whether green tea acts synergistically with ginger to limit the growth of two bacteria found in the human gastrointestinal system, *Escherichia coli* and *Enterococcus faecalis*.

Escherichia coli is a gram-negative rod that includes a large number of strains ranging from common and benign to extremely pathogenic and linked to many diseases. The common enteric strain that was used for this experiment was chosen based on its important role in digestion of food for many mammals (Schaechter, 1992). *Enterococcus faecalis*, originally in the genus of Streptococcus, is a gram positive round (coccoid) bacteria that commonly occurs in the intestines of humans and other mammals. This bacteria is also know to behave pathogenically when in larger quantities, and are implicated in urinary tract infections and wound infections among other pathogenic activities (Holt, 1986). *E. faecalis* was chosen based on its common presence in the GI tract and its pathogenic nature when overgrown.

The experiment tested both the anti-microbial effects of green tea and ginger separately and in proximity to each other on bacterial lawns of *E. coli* and *E. faecalis* over a 48-hour incubation. We hypothesized, if green tea and ginger act synergistically to limit the growth of both *E. coli* and *E. faecalis* separately, then we will see less bacterial growth, fewer rogue colonies, and greater zones of inhibition on plates that ginger and green tea appear together than those that have each test substance separately. The results of this testing shed light into the anti-microbial abilities of both test substances, potentially providing ground for natural alternatives to pharmaceutical antibiotic medication.

Methods

The methods used in this experiment followed the basic procedure outlined by Morgan and Chase in their *Investigating Biology Laboratory Manual, Fifth Edition*. A total of eighteen tryptic soy agar (TSA) plates were prepared with bacterial lawns created with sterile swabs, nine with lawns of *E. coli* and nine with lawns of E. faecalis. One plate from each set of nine was inoculated with the control, sterile de-ionized water. Two plates from each bacterial set were inoculated with organic green tea extract acquired from a natural food store (brand name, Gaia Herbs). The extract from organic green tea leaves was mixed with spring water and 35-45% organic grain alcohol. Two plates from each bacterial set were inoculated with ginger purchased from a local grocery store. The ginger was placed in the approximate center of the TSA plate. Two plates from each bacterial set were inoculated with both green tea extract and ginger, on separate sides of the TSA plate, as if the TSA plate were divided into two equal halves. Two more plates were prepared from each bacteria set by dividing them into thirds, placing ginger in one third, green tea extract in the second third and the control in the final third of the plate. Two final plates were prepared without bacterial lawns, one inoculated with ginger and the other with green tea. All inoculations done with green tea or water were done with sterile paper discs soaked in the respective testing medium. The ginger used for inoculation was peeled by hand, shredded using the small grated of a standard metallic kitchen shredder, shredding the ginger directly into a sterile holding dish. The ginger was placed directly on the bacterial lawns in portions slightly smaller than the diameter of the sterile paper discs, and as flat as possible. The plates were incubated for 48 hours at 37 degrees Celsius. After 48 hours the plates were refrigerated until data could be taken (within one week of inoculation). Measurements were taken using a standard clear ruler and a dissecting microscope, measuring for the zone of inhibition or impact. Rogue colonies (bacterial growth in the middle of a zone of inhibition) were counted and the presence of bacterial growth on the ginger, green tea, and control was investigated as well using a simple flashlight pen.

Results

The antimicrobial abilities of green tea extract and ginger against *Escherichia coli* and *Enterococcus faecalis* were determined by examining the TSA plates after 48 hours of incubation. Both green tea and ginger had some antibiotic effect against *E. faecalis* however neither showed signs of effectiveness against *E. coli* as can be seen be seen in Table 1. It is important to note that growth over the top of both testing substances was demonstrated for plates containing *E. coli* bacteria. No noticeable effect was had by green tea extract or ginger when examined under a dissecting microscope. Bacterial growth and fungal growth was found on the ginger in the plate with out bacterial lawn, however neither was identified. This same fungal and bacterial growth was not present in the complementary plate for green tea extract. There was no zone of inhibition for either substance on the *E. coli* plates, thus there were no rogue colonies found.

Green tea extract had an effect on the bacteria *E. faecalis*. It displayed zones of inhibition in every test plate it was present in. The zones ranged from 11 mm to 14 mm in diameter. No rogue colonies were found under dissecting microscope with the possible exception of one found in the same plate containing ginger. No growth was found on the sterile disc soaked in green tea extract in the TSA plates with *E. faecalis* bacteria.

Ginger displayed two effects on the *E. faecalis* bacteria. In all test plates containing ginger, a small irregularly shaped zone of inhibition could be found. These zones

measured no more than 1 mm, with areas around the fresh ginger not displaying zones at all. One plate was an exception to this; it displayed an irregular zone of inhibition of approximately 1-2 mm in diameter. In addition to these zones of inhibition, 5 of the 6 plates testing ginger against *E. faecalis* displayed a zone of impact. This zone was characterized by a distinct lighter sheen in a circle with proportional circumference around the ginger. These circles were 16mm to 20 mm in diameter. Inside the zone of impact, there is a clear growth of *E. faecalis*. There were a small number of rogue colonies within the zones of inhibition displayed in the plated testing ginger against *E. faecalis*. The zones of inhibition were generally too small to denote rogues however.
The synergistic effect of green tea and ginger was tested in two ways against each of the two bacteria. Both the plates divided into thirds (containing ginger, green tea extract, and the control equally spaced) and the plates divided into half (containing ginger and green tea extract equally separated), displayed no overlap in the zones of inhibition against *E. coli* and *E. faecalis*.

Discussion

The evidence sought in the experiment to accept the hypothesis that green tea and ginger have a synergistic impact to limit the growth of *E. coli* and *E. faecalis* was not present due to experiment design error. We expected to see greater zones of inhibition, less growth of bacteria, and less growth of rogue colonies in plates where ginger and green tea were both present in comparison to plates were only one test substance was present. This would indicate a synergistic anti-microbial response between the green tea and the ginger. While the results show clearly a difference between the impacts of ginger and green tea on *E. coli* and *E. faecalis*, the synergistic impact of both is inconclusive. Further testing is being done that corrects clear experiment design issues: the impact zones of ginger and green tea were too far apart to provide any results to inform if the two substances have any impact on each others effectiveness as antimicrobial agents that work together.

This does not render the results with out value. Particularly considering the commonality of the two bacteria and the availability of both ginger and green tea to the general public. Green tea and ginger had no visible impact under dissecting microscope on the *E. coli* bacterial lawns. This result can be interpreted as disappointing as we search for ways to control pathogenic bacteria (*E. coli* has strains other than the one used for this experiment that fit in this category) or positive considering the fact that *E. coli* bacteria is commonly found in the GI tract of humans and serves important functions in our digestion. If both ginger and green tea, being common in Asia and becoming increasingly common in the United States, had strong antibiotic effects on *E. coli*, the digestive health of many people could be put into upheaval.

This result could prove of even greater value considering the impact of ginger and green tea on the second test bacteria, E. faecilis. The antimicrobial impact of green tea seen in the consistent creation of a zone of inhibition measuring between 10 and 14 mm shows green tea is more effective at controlling the growth of *E. faecalis*. This result reinforces research that finds green tea extract has an antimicrobial impact (Roccaro et al., 2004). Ginger was less effective, but results under dissecting microscope did show a zone of impact, different than the zone of inhibition as ginger did not prevent the growth of the bacteria but did seem to effect it in some way to change its pattern of growth. This impact should be researched in future experiments. It is possible that the impact that ginger has on *E. faecalis* could be manipulating the bacteria in some way. The zones of inhibition that ginger imposed on *E. faecalis* were usually insignificant and irregular with areas within that zone showing rogue colonies or growth next to the ginger itself. Though the amount of inhibition should not be neglected in consideration of possible future research. The results indicating a positive antimicrobial effect on *E. faecalis* by green tea, and to a lesser extent ginger, signifies the possible use of each substance as a target antibiotic that could allow the positive flora of the gut to remain while preventing the further growth of *E. faecalis* if it were to occur in pathogenic levels. While *E. faecalis* is found naturally in the human GI tract, it is often implicated in many infections. In these cases, it would benefit the person infected to control *E. faecalis* without destroying other flora of the GI tract that have positive impact on an individual's digestive health.

Future research into ginger should take into account the results found regarding the presence of fungus on the ginger itself. Steps should be taken in preparing the ginger sample used for testing that could prevent the presence of fungus in the ginger or isolated the source of the fungus found. Sterile equipment should be used including gloves, scalpel and surface. The ginger used for this experiment was prepared using a household shredder; no gloves were used to shred ginger directly into a sterile holding dish. While it is possible that the fungus present in the ginger on the agar plate that contained no bacteria was contaminated for the brief time it was open or by the ginger itself (as it is a root, the presence of bacteria should be considered, possible future testing could be done on this topic), it seems equally likely that it was contaminated in the process of peeling or shredding by contaminated equipment or hands. In either event, it would be beneficial to isolate the possible sources of this contamination.

Additional information should be sought by comparing the impact of green tea on *E. faecalis* to other antibiotics that are known to control the growth of this bacteria.

Statistical evidence may be able to be provided to determine there is statistically significant difference between the two effects.

In conclusion, the synergic effect of green tea and ginger on two common enteric bacteria should be studied in further experiments taking into account the zone of inhibition that both display for the bacteria that they will be tested against. As green tea and ginger are commonly found in diets around the world today, the results implicating that green tea, and to a lesser extent ginger, do act as antimicrobials for *E. faecalis* but not *E. coli* should provide encouragement that forms of infection associated with *E. faecalis* can be minimized with out destroying the rest of an individual's microbial gastrointestinal flora.

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	E. faecalis #1	*E. faecalis #2*	*E. coli #1*	*E. coli #2*
Ginger Plates				
Growth	Yes	Yes	Yes	Yes
Zone (Inhibition, mm)	< 1	< 1	N/A	N/A
Zone (Impact, mm)	16	None	N/A	N/A
Rogues	None	None	N/A	N/A
Green Tea Plates				
Growth	None	None	Yes	Yes
Zone (Inhibition, mm)	14	11	N/A	N/A
Zone (Impact, mm)	N/A	N/A	N/A	N/A
Rogues	None	None	N/A	N/A
Control Plates				
Growth	Yes		Yes	
Zone (Inhibition, mm)	N/A		N/A	
Zone (Impact, mm)	N/A		N/A	
Rogues	N/A		N/A	
Ginger/Green Tea Plates				
Ginger				
Growth	Yes	Yes	Yes	Yes
Zone (Inhibition, mm)	< 1	< 1	N/A	N/A
Zone (Impact, mm)	17	19	N/A	N/A
Rogues	None	None	N/A	N/A
Green Tea				
Growth	None	None	Yes	Yes
Zone (Inhibition, mm)	12	11	N/A	N/A
Zone (Impact, mm)	N/A	N/A	N/A	N/A
Rogues	1	None	N/A	N/A
Ginger/Green Tea/ Control Plates				
Ginger				
Growth	Yes	Yes	Yes	Yes
Zone (Inhibition, mm)	< 2	< 1	N/A	N/A
Zone (Impact, mm)	20	20	N/A	N/A
Rogues	3	None	N/A	N/A
Green Tea				
Growth	None	None	Yes	Yes
Zone (Inhibition, mm)	11	13	N/A	N/A
Zone (Impact, mm)	N/A	N/A	N/A	N/A
Rogues	None	None	N/A	N/A
Control				
Growth	Yes	Yes	Yes	Yes
Zone (Inhibition, mm)	N/A	N/A	N/A	N/A
Zone (Impact, mm)	N/A	N/A	N/A	N/A
Rogues	N/A	N/A	N/A	N/A
No Bacteria Plate:	Ginger: Fungus and bacteria formation Green Tea: No growth			

Table 1
Ginger and Green Tea Effects