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Antimicrobial Properties of Chili Peppers

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Abstract

Chili peppers are used worldwide in foods for their pungent flavor, aroma, and to prolong food spoilage. With capsaicin contents ranging from zero to millions of Scoville heat units, the different varieties offer a wide range of options for people all over the world. In addition to their use in cuisines, chili peppers have been explored for their antimicrobial and antifungal properties. Consequently, research is underway to determine the potential for the application of chili pepper extracts in the food industry in place of artificial preservatives. As new antibiotic-resistant food borne pathogens emerge, the discovery of natural antimicrobials in chili peppers will be invaluable to food scientists. This review goes over some relevant research that has already been done in this area. In addition it lays the ground for the new research that is emerging testing new varieties of chili peppers for nutrient content, flavor profiles, and for antimicrobial activities against numerous human pathogens.

Keywords: Chili peppers; Chile peppers; Antimicrobial; Foodborne pathogen

Introduction

Human use of chili peppers dates back to prehistoric times. Preserved peppers have provided evidence that South Americans ate and grew *aji*, (chili in English), in 2500 B.C. The peppers became increasingly common and integrated into the diet of particular cultures. However, chili peppers and similar spices remained isolated in these cultures until the 13th century, when they became available to civilizations throughout the world [1]. The pungency of chili peppers is due to the accumulation of capsaicinoids (also known as capsinoids, a group of naturally produced compounds that are unique to the *Capsicum* genus [2,3]. The chili pepper is a member of the *Solanaceae* family. It is a diploid, facultative, self-pollinating crop, and closely related to potato, tomato, eggplant, tobacco and petunia. It is one of the oldest domesticated crops in the Western hemisphere, the most widely grown spice in the world, and is a major ingredient in most global cuisines. *Capsicum* species are commonly grown in warm humid regions such as the tropics and subtropics and their fruits are mainly used in local cuisine.

Chili peppers are widely used as spices in traditional Mexican foods. The flavor and pungent power of these peppers varies widely and so do their contents of capsaicin and its capsaicinoid analogs [2]. When eaten, many chili peppers evoke a sensation of heat and/or pain to the neurological systems in mammals, and these adverse effects can be overcome through the consumption of foods containing casein such as milk, cheese, or yogurt. Studies of the botanical pharmacopoeia of the indigenous Mayan inhabitants of Mesoamerica have shown that chili peppers (*Capsicum* species) are incorporated into a number of medicinal preparations. These preparations were applied for a variety of ailments including respiratory problems, bowel complaints, earaches, and sores. Early European observers noted the omnipresent nature of chili peppers in the Mayan diet, reporting that nothing was eaten without them. While typically regarded as a spice, the substantial role that chili peppers occupy in this culture's diet may have important nutritional consequences for these people [4,5].

Chili peppers have a wide range of uses, including pharmaceutical, natural coloring agents and cosmetics, as an ornamental plant, and as the active ingredient in most defense repellants (i.e. pepper sprays) .Capsaicin, a well-studied chemical component of the *Capsicum* species and one of the pungent capsaicinoids found in chili peppers, has already demonstrated a high degree of biological activity affecting the nervous, cardiovascular, and digestive systems [5]. Chemical analysis has demonstrated that *Capsicum* fruits contain relatively high concentrations of several essential nutrients, including vitamin C (up to 6 times the concentration of an orange) [5].

Strong consumer demand for safe and high-quality foods can be attributed in part to the wide spread availability and accessibility of quality health data and information. There are also new concerns about food safety due to increasing occurrences of new food-borne disease outbreaks caused by pathogenic microorganisms. This raises considerable challenges, particularly since there is increasing unease regarding the use of chemical preservatives and artificial antimicrobials to inactivate or inhibit growth of spoilage and pathogenic microorganisms [6]. In addition, currently available treatment options for food-borne pathogen infections have drug-related side effects, bacterial resistance to antimicrobials, and in some cases no medical treatment exists for organisms such as *Escherichia coli* O157:H7. Therefore, newer treatments which are safe, cost effective, and simple to administer are urgently needed. In light of this, the use of nutritional agents is an attractive alternative to conventional therapeutics and warrants further investigation [3]. Consequently, natural antimicrobials, such as chili peppers, are receiving a good deal of attention for a number of microorganism-control issues [6]. Recent reports state that the *Capsicum* genus, among other plant genera, is a good source of antimicrobial and antifungal compounds [7].

Top 14 Food-borne Pathogens

According to the U.S Food and Drug and Administration (FDA), there are several food-borne pathogens that are of concern and harmful to the general public, and are particularly harmful to pregnant women (Table 1) [8].

Aside from these 14, there are other well-known pathogens some of which are foodborne, including *Bacillus cereus, Bacillus subtilis, Enterobacter aerogenes* [5], *Pseudomonas aeruginosa* [5,9]

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Pathogen	Basics	Sources	Symptoms	Incubation	Duration
Campylobacter jejuni	A bacterium that's the most common bacterial cause of diarrhea in the U.S. Must-Know: Children under age 1 have the highest rate of *Campylobacter* infections. Unborn babies and infants are more susceptible on first exposure to this bacterium. In addition, there's a low threshold for seeking medical care for infants.	Raw milk, untreated water, raw and undercooked meat, poultry, or shellfish	Diarrhea (sometimes bloody), stomach cramps, fever, muscle pain, headache, and nausea.	Generally 2 to 5 days after eating contaminated food	7 to 10 days
Clostridium botulinum	A bacterium that can be found in moist, low-acid food. It produces a toxin that causes botulism, a disease that causes muscle paralysis. Must-Know: Don't feed a baby honey - at least for the first year. Honey can contain *Clostridium botulinum* spores. Infant botulism is caused by consuming these spores, which then grow in the intestines and release toxin.	Home-canned and prepared foods, vacuum-packed and tightly wrapped food, meat products, seafood, and herbal cooking oils	Dry mouth, double vision followed by nausea, vomiting, and diarrhea. Later, constipation, weakness, muscle paralysis, and breathing problems may develop. Botulism can be fatal. It's important to seek immediate medical help.	4 to 36 hours after eating contaminated food	Recovery can take between 1 week to a full year.
Clostridium perfringens	A bacterium that produces heat-stable spores, which can grow in foods that are undercooked or left out at room temperature.	Meat and meat products	Abdominal pain, diarrhea, and sometimes nausea and vomiting.	8 to 12 hours after eating contaminated food	Usually 1 day or less
Pathogenic *Escherichia coli (E. coli)*	A group of bacteria that can produce a variety of deadly toxins.	Meat (undercooked or raw hamburger), uncooked produce, raw milk, unpasteurized juice, and contaminated water	Severe stomach cramps, bloody diarrhea, and nausea. It can also manifest as non-bloody diarrhea or be symptomless. Must-Know: It can cause permanent kidney damage which can lead to death in young children.	Usually 3 to 4 days after ingestion, but may occur from 1 to 10 days after eating contaminated food.	5 to 8 days
Listeria monocytogenes	A bacterium that can grow slowly at refrigerator temperatures. Must-Know: Listeria can cause serious illness or death in pregnant women, fetuses, and newborns.	Refrigerated, ready-to-eat foods (meat, poultry, seafood, and dairy - unpasteurized milk and milk products or foods made with unpasteurized milk)	Fever, headache, fatigue, Muscle aches, nausea, vomiting, diarrhea, meningitis, and miscarriages.	48 to 72 hours after ingestion, but may occur from 7 to 30 days after eating contaminated food.	1 to 4 days.
Norovirus (Norwalk-like Virus)	A virus that's becoming a health threat. It may account for a large percent of non-bacterial foodborne illnesses.	Raw oysters, shellfish, coleslaw, salads, baked goods, frosting, contaminated water, and ice. It can also spread via person-to-person.	Diarrhea, nausea, vomiting, stomach cramps, headache, and fever.	24 to 48 hours after ingestion, but can appear as early as 12 hours after exposure.	1 to 2 days
Salmonella enteritidis	A bacterium that can infect the ovaries of healthy-appearing hens and internally infect eggs before the eggs are laid.	Raw and undercooked eggs, raw meat, poultry, seafood, raw milk, dairy products, and produce	Diarrhea, fever, vomiting, headache, nausea, and stomach cramps Must-Know: Symptoms can be more severe in people in at-risk groups, such as pregnant women.	12 to 72 hours after eating contaminated food	4 to 7 days
Salmonella typhimurium	Some strains of this bacterium, such as DT104, are resistant to several antibiotics.	Raw meat, poultry, seafood, raw milk, dairy products, and produce	Diarrhea, fever, vomiting, headache, nausea, and stomach cramps Must-Know: Symptoms can be more severe in people in the at-risk groups, such as pregnant women.	12 to 72 hours after eating contaminated food	4 to 7 days
Shigella	A bacterium that's easily passed from person-to-person via food, as a result of poor hygiene, especially poor hand washing. Only humans carry this bacterium.	Salads, milk and dairy products, raw oysters, ground beef, poultry, and unclean water	Diarrhea, fever, stomach cramps, vomiting, and bloody stools	1 to 7 days after eating contaminated food	5 to 7 days
Staphylococcus aureus	This bacterium is carried on the skin and in the nasal passages of humans. It's transferred to food by a person, as a result of poor hygiene, especially poor hand washing. When it grows in food, it makes a toxin that causes illness.	Dairy products, salads, cream-filled pastries and other desserts, high-protein foods (cooked ham, raw meat and poultry), and humans (skin, infected cuts, pimples, noses, and throats)	Nausea, stomach cramps, vomiting, and diarrhea	Usually rapid - within 30 minutes to 8 hours after eating contaminated food	24 to 48 hours

Vibrio cholerae	A bacterium that occurs naturally in estuarine environments (where fresh water from rivers mix with salt water from oceans). It causes cholera, a disease that can cause death if untreated.	Raw and undercooked seafood or other contaminated food and water.	Often absent or mild. Some people develop severe diarrhea, vomiting, and leg cramps. Loss of body fluids can lead to dehydration and shock. Without treatment, death can occur within hours.	6 hours to 5 days after eating contaminated food	7 days
Vibrio parahaemolyticus	A bacterium that lives in saltwater and causes gastrointestinal illness in people.	Raw or undercooked fish and shellfish	Diarrhea, stomach cramps, nausea, vomiting, headache, fever, and chills	4 to 96 hours after eating contaminated food	2.5 days
Vibrio vulnificus	A bacterium that lives in warm seawater. It can cause infection in people who eat contaminated seafood or have an open wound exposed to seawater.	Raw fish and shellfish, especially raw oysters	Diarrhea, stomach pain, nausea, vomiting, fever, and sudden chills. Some victims develop sores on their legs that resemble blisters.	Usually within 16 hours after eating contaminated food or exposure to organism	2 to 3 days
Yersinia enterocolitica	A bacterium that causes yersiniosis, a disease characterized by diarrhea and/or vomiting.	Raw meat and seafood, dairy products, produce, and untreated water	Fever, diarrhea, vomiting, and stomach pain Must-Know: Symptoms may be severe for children.	1 to 2 days after eating contaminated food	1 to 2 days

Adopted from the FDA website [8]

Table 1: Top 14 food-borne pathogens.

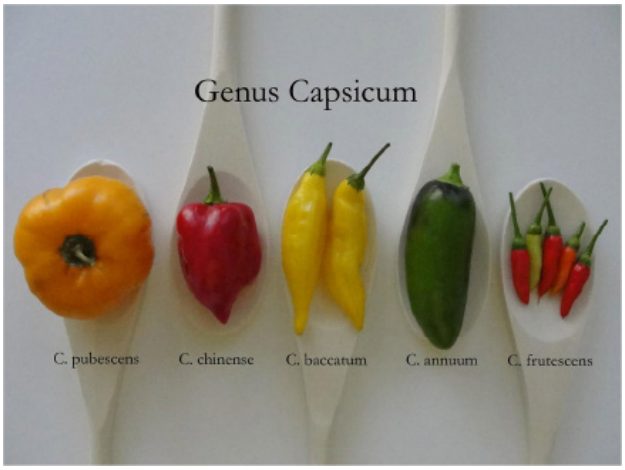

Figure 1: Courtesy of Dr. D. J. Baumler.

and *Helicobacter pylori* [10] which seem to be of interest to research scientists.

Species of the Genus *Capsicum* Presently Known

Capsicum species are small perennial herbs native to tropical South America. The majority of researchers believe that this genus is comprised of more than 20 species. The 5 most common ones believed to be a result of domestication are *C. annuum, C. baccatum, C. frutescens, C. chinense* and *C. pubescens* [5], (Figure 1).

The other species are exotic and not as widely distributed as these five. Below is a list of the other presently known species [11].

- *Capsicum buforum*
- *Capsicum campylopodium*
- *Capsicum cardenasii*
- *Capsicum ceratocalyx*
- *Capsicum chacoense*
- *Capsicum coccineum*
- *Capsicum cornutum*
- *Capsicum dimorphum*
- *Capsicum dusenii*
- *Capsicum eximium*
- *Capsicum flexuosum*
- *Capsicum friburgense*
- *Capsicum galapagoense*
- *Capsicum geminifolium*
- *Capsicum havanense*
- *Capsicum hookerianum*
- *Capsicum hunzikerianum*
- *Capsicum lanceolatum*
- *Capsicum leptopodum*
- *Capsicum lycianthoides*
- *Capsicum minutiflorum*
- *Capsicum mirabile*
- *Capsicum mositicum*
- *Capsicum parvifolium*
- *Capsicum pereirae*
- *Capsicum ramosissimum*
- *Capsicum recurvatum*
- *Capsicum rhomboideum*
- *Capsicum schottianum*
- *Capsicum scolnikianum*
- *Capsicum spina-alba*
- *Capsicum stramoniifolium*
- *Capsicum tovarii*
- *Capsicum villosum*

		MIC (mg/ml of ciprofloxacin for respective strain with/without test molecule (fold reduction)		
Capsaicin (mg/L)	MIC of capsaicin	SA-1199	SA-1199B	SA-1758
Capsaicin (50)	>100	0.12/0.25 (2)	2/8 (4)	0.125/0.125 (0)
Capsaicin (25)	>100	0.12/0.25 (2)	2/8 (4)	0.125/0.125 (0)
Capsaicin (12.5)	>100	0.25/0.25 (0)	4/8 (2)	0.125/0.125 (0)
Reserpine (25)	>100	0.12/0.25 (2)	2/8 (4)	0.125/0.125 (0)

Table adopted from Kalia et al. [13].

Table 2: *In vitro* ciprofloxacin/capsaicin studies.

	Mean PAE (h) ± S.D		
Regimen	**0.25×MIC (2 mg/L)**	**0.5×MIC (4 mg/L)**	**MIC (8 mg/L)**
Ciprofloxacin	0.3 ± 0.1	1.0 ± 0.1	1.3 ± 0.17
Ciprofloxacin + Capsaicin (25mg/L)	1.0 ± 0.2	1.5 ± 0.17	2.4 ± 0.2

PAE = Post Antibiotic Effect
Table adopted from Kalia et al. [13]

Table 3: PAE of ciprofloxacin alone and in combination with capsaicin against *S. aureus* SA-1199B after exposure of 2 h.

Capsaicin (mg/L)	2×MIC (0.5 mg/L)	4×MIC (1 mg/L)	8×MIC (2 mg/L)	16×MIC (4 mg/L)
0	1.47×10^{-9}	7.7×10^{-9}	4.3×10^{-9}	$<10^{-9}$
12.5	13.5×10^{-9}	3.9×10^{-9}	$<10^{-9}$	$<10^{-9}$
25	2.5×10^{-9}	$< 10^{-9}$	$<10^{-9}$	$<10^{-9}$

MIC = Minimum Inhibitory Concentration
Table adopted from Kalia *et al*. [13]

Table 4: Mutation frequency of *S. aureus* ATCC 29213.

Studies on Antimicrobial Effects of Chili Pepper-extracts on Some Foodborne and/or Human Pathogens

Bacillus subtilis (not typically associated with foodborne illness)

According to Molina-Torres et al. [12], capsaicin (pure, purchased from Sigma Aldrich), had a strong inhibitory effect towards *B. subtilis* starting from 25 µg/ml (minimum concentration assayed).

Escherichia coli

Molina-Torres et al. [12] determined that capsaicin (pure, purchased from Sigma Aldrich), at concentrations up to 200 or 300 µg/ml only retarded the growth of *E. coli*.

Salmonella typhimurium

Careaga et al. [9] investigated the antimicrobial effect of *Capsicum* extract on *S. typhimurium* inoculated in minced beef. The minimum lethal concentration of the pepper extract was 1.5 ml/100 g of meat. The combination of sodium chloride and *C. annum* extract tested was not successful to eliminate *Salmonella*. This could be explained by the fact that S*almonella* is tolerant to salt. The researchers proposed using a combination that had less salt and more pepper extract, because any more salt would be too much to eat.

Pseudomonas aeruginosa

In the same study, Careaga et al. [9] investigated the antimicrobial effect of *Capsicum* extract on *P. aeruginosa* inoculated in minced beef. A reduction of *P. aeruginosa* growth was observed between 0.06-0.1 ml/100 g meat, with a bacteriostatic effect between 0.5-1.5 ml/100 g meat. As the extract concentration increased, a drastic bactericidal effect was observed, particularly between 4-5 ml/100 g meat. The combination of sodium chloride and *C. annum* extract tested eliminated *P. aeruginosa* after 3 days of storage.

Staphylococcus aureus

Nitin et al. [12] evaluated the possibility of capsaicin acting as an inhibitor of the NorA efflux pump of *S. aureus*. The minimum inhibitory concentration (MIC) of ciprofloxacin was reduced 2 to 4 fold in the presence of capsaicin. This reduction was more prominent for *S. aureus* SA-1199B (NorA overproducing) as compared with *S. aureus* SA-1199 (wild-type) up to 25 mg/L capsaicin. Beyond that, no concentration dependent effect was observed. *S. aureus* SA-K1758 (*norA* knockout) showed no reduction in the MIC of ciprofloxacin. Table 2 shows *in vitro* ciprofloxacin/ capsaicin combination studies. Table 3 shows post-antibiotic effect (PAE) of ciprofloxacin alone and in combination with capsaicin against *S. aureus* SA-1199B after exposure of 2 h. Ciprofloxacin at 4 mg/L, at which no mutant was selected, was defined as the mutant prevention concentration (MPC). When tested in combination with capsaicin at 12.5 and 25 mg/L, the MPC of ciprofloxacin was reduced to 2 and 1 mg/L, respectively. The MPC of the combination was found to be lower than the C_{max} of the ciprofloxacin (3-4 mg/L), indicating the clinical relevance of these combinations in restricting the selection of resistant mutants. Ethidium bromide fluoresces only when it is bound to nucleic acids inside cells. Only the control cells without capsaicin extruded ethidium bromide, resulting in a significant decrease in florescence over the assay period. In the presence of capsaicin, the loss of florescence was significantly reduced, reflecting a strong interference with ethidium bromide efflux by capsaicin [2]. Table 4 shows the mutation frequency of *S. aureus* ATCC 29213 [13].

Vibrio cholerae

This study examines common spices to determine their inhibitory capacity against virulence expression of *V. cholera* (Table 5). Among them methanol extracts of red chili, sweet fennel and white pepper could substantially inhibit cholera toxin (CT) production (Table 6). As these species act against virulence expression rather than viability of *V. cholerae*, there is a lesser chance of developing resistance [13].

In a different study, Chatterjee et al. [15] determined that the methanol extract of red chili, and purified capsaicin could inhibit cholera toxin (CT) production in recently emerged *V. cholerae* O1 El Tor variant strains without affecting their viability. All 23 strains of *V. cholerae* used in the study (Table 7), were grown in the lab. Crude methanol extract of the red chili pepper was used (individual ingredients

Plant	Scientific name	Specific compound	Target	Mechanism
Wasabi	*Wasabi japonica*	Allyl isothiocynate	*V. parahemolyticus*	Inhibit growth
Green tea	*Camellia sinensis*	Catechins	*V. cholera*	Inhibit growth and CT activity
Guazuma	*Guazuma ulimifolia*	Procyanidins	*V. cholera*	CT activity
Daio (Kampo formulation)	*Rhei rhizome*	Gallate analogues	*V. cholera*	CT activity
Apple	*Malus spp.*	Aplephenon	*V. cholera*	CT activity
Hop	*Humulus lupulus*	Procyanides	*V. cholera*	CT activity
Neem	*Azadirachta indica*	Unknown	*V. cholera*	Inhibit growth
Elephant garlic	*Allium ampleloprasum*	Oil (diallyl sulfides)	*V. cholera*	Inhibit growth
Red bayberry	*Myrica rubra*	Unknown	*V. cholera*	Inhibit CT production
Red chili	*Capsicum annum*	Capsaicin	*V. cholera*	Inhibit CT production

CT = Cytotoxin
Table adopted from Yamasaki et al. [14]

Table 5: Natural compounds identified to act against diarrhoeagenic *Vibrio* spp.

Stain ID	Isolation year	Red chili	Sweet fennel	White pepper	Red pepper	Cassia bark	Star anise
CO_{533}	1994	97	95	86	68	45	50
CRC_{27}	2000	97	92	99	80	79	66
CRC_{41}	2000	90	96	94	53	86	6.0
CRC_{87}	2000	94	85	87	56	78	29

Table adopted from Yamasaki et al. [14]

Table 6: % Inhibition of CT production in *V. cholerae* O1 E1 Tor variant strains (isolated from cholera patients in India) with methanol extracts of 6 different commonly used spices (100 µg/ml).

Serial no.	Strain	Serogroup/biotype	ctxB genotype	Country	Isolation Year
1	NICED-1	O1 El Tor	El Tor	India	1970
2	NICED-10			India	1970
3	NICED-3			India	1980
4	P130			Peru	1991
5	VC190			India	1993
6	VC301	O1 El Tor	Classical	India	1992
7	Al-091	variant		Bangladesh	1993
8	CO533			India	1994
9	CRC27			India	2000
10	CRC41			India	2000
11	CRC87			India	2000
12	B33			Mozambique	2004
13	1'/05			India	2005
14	2'/05			India	2005
15	5'/05			India	2005
16	2680713			Bangladesh	2006
17	2684269			Bangladesh	2006
18	SG24	O139	El Tor	India	1992
19	CRC142		Classical	India	2000
20	VC82	Non-O1/	El Tor	India	1989
21	VC259	Non-O139		India	1991
22	569B	O1 classical	Classical	India	1948
23	O395			India	1964

Table adopted from Chatterjee et al. [14]

Table 7: Vibrio cholerae strains used in the study.

not isolated). Capsaicin was purchased from LKT laboratories Inc., MN. RNA isolation and real-time transcription-PCR (qRT-PCR) assay revealed that capsaicin effectively repressed the transcription of *ctxA*, *tcpA*, and *toxA* genes, but not the *toxR* and *toxS* genes. It enhanced the transcription of the gene *hns* (Table 8).

Based on the experimental results, the researchers proposed a mechanism by which capsaicin and the red chili methanol extract represses the virulence genes of *V. cholerae*. Briefly, the activation of *toxR*, *toxS*, *tcpP*, and *tcpH* is caused by environmental factors such as pH, temperature, and osmolarity. This activation subsequently activates *ctxAB* and *tcpA* transcriptions via activation of transcriptional activator *toxT*. HN-S is a basal repressor of *toxT*, *ctxAB* and *tcpA* genes under nonpermissive conditions. In the presence of capsaicin, while *ctxAB*,

Primer/probe	Primer and probe sequence (5' – 3')	Amplicon size (bp)
ctxA F	GGA GGG AAG AGC CGT GGA T	
ctxA P	CAT CAT GCA CCG CCG GGT TG	**66**
ctxA R	CAT CGA TGA TCT TGG AGC ATT C	
tcpA F	GGG ATA TGT TTC CAT TTA TCA ACG T	
tcpA P	TGC TTT CGC TGC TGT CGC TGA TCT T	**82**
tcpA R	GCG ACA CTC GTT TCG AAA TCA	
toxT F	TGA TGA TCT TGA TGC TAT GGA GAA A	
toxT P	TAC GCG TAA TTG GCG TTG GGC AG	**107**
toxT R	TCA TCC GAT TCG TTC TTA ATT CAC	
toxR F	GCT TTC GCG AGC CAT CTC T	
toxR P	CTT CAA CCG TTT CCA CTC GGG CG	**65**
toxR R	CGA AAC GCG GTT ACC AAT TG	
toxS F	TGC CAT TAG GCA GAT ATT TCA CA	
toxS P	TGA CGT CTA CCC GAC TGA GTG GCC C	**72**
toxS R	GCA ACC GCC CGG CTA T	
tcpP F	TGG TAC ACC AAG CAT AAT ACA GAC TAA G	
tcpP P	TAC TCT GTG AAT ATC ATC CTG CCC CCT GTC	**100**
tcpP R	AGG CCA AAG TGC TTT AAT TAT TTG A	
tcpH F	GCC GTG ATT ACA ATG TGT TGA GTA T	
tcpH P	TCA ACT CGG CAA AGG TTG TTT TCT CGC	**82**
tcpH R	TCA GCC GTT AGC AGC TTG TAA G	
hns F hns	TCG ACC TCG AAG CGC TTA TT	
hns P	CTG CGC TAT CAG GCG AAA CTA AAA CGA AA	**70**
hns R	GGT GCA CGT TTG CCT TTT G	
recA F	CAA TTT GGT AAA GGC TCC ATC AT	
recA P	CTT AGG CGA CAA CCG CGC	**71**
recA R	CCG GTC GAA ATG GTT TCT ACA	

Table adopted from Chatterjee et al. [15]

Table 8: Primers and probes used for qRT-PCR.

Compound	Retention Time (min)*	% Acetonitrile at which the separation was achieved
L-phenylalanine	6.55 ± 0.66	27.91
Caffeic Acid	7.00 ± 0.76	28.83
p-coumaric acid + ferulic acid	8.56 ± 0.52	32.03
m-coumaric acid	9.32 ± 0.52	33.59
o-coumaric acid	11.21 ± 0.70	37.46
Trans-cinnamic acid	18.99 ± 094	53.41
Capsaicin	25.72 ± 0.90	67.20
Dihydrocapsaicin	27.33 ± 0.74	70.50

**Data represent an average of ten replicates* (± S.D.).
Table adopted from Dorantes et al. [2]

Table 9: HPLC profile of standard phenylpropanoid compounds, capsaicin, and dihydrocapsaicin from chili extracts.

tcpA, and *toxT* transcriptions were repressed, the transcription of *hns* was enhanced. Capsaicin may probably repress the virulence genes transcriptions in a direct manner or via modulation of the global regulator *hns* gene. The higher inhibitory impact of red chili methanol extract than capsaicin (43- and 23- fold respectively) indicates the possibility of other unidentified compound(s) in red chilis that can directly inhibit or synergistically act with capsaicin [15].

Helicobacter pylori

In their experiment, Jones et al. [3] determined that capsaicin inhibited growth of *H. pylori* strain LC-11 in a dose-dependent manner at concentrations above 10 μg/ml (ANOVA, $P<0.05$). This bactericidal effect was evident within 4 h of incubation. After 24 h, growth of the bacteria was completely inhibited. The effect of capsaicin was maximal at a concentration of 50 μg/ml. This bactericidal effect was not limited to *H. pylori* LC-11. Growth of LC-32 and LC-28 were inhibited to a similar extent at 500 μg/ml [3].

To examine the possible influence of pH on the bactericidal activity of capsaicin, the growth of *H. pylori* strain LC-11 was compared in broth culture at pH 4.5, 5.4, and 6.4 in the presence and absence of capsaicin. At each of these pH values, the growth of *H. pylori* was inhibited compared to bacterial growth in standard broth culture at pH

Capsinoid	Habanero	Serrano	Morron
o-coumaric acid	0.089 ± 0.01	0.90 ± 0.01	0.18 ± 0.01
m-coumaric acid	-	0.31 ± 0.01	0.21 ± 0.01
Trans-cinnamic acid	-	0.47 ± 0.01	0.21 ± 0.01
Capsaicin	5.88 ± 0.03	0.63 ± 0.01	-
Dihydrocapsaicin	0.86 ± 0.01	0.059 ± 0.01	-

**Data represent an average of three replicates* (± S.D.).
Table adapted from Lidia et al. [2]

Table 10: Content of some capsinoids in the habanero, serrano, and pimiento moron extracts (mg/ml).

Figure 2: Courtesy of Dr. D. J. Baumler.

Bacteria	*o*-coumaric	*m*-coumaric	Cinnamic acid	Capsaicin	Dihydro-capsaicin
B. cereus	Neg	10.0 ± 0.0	8.0 ± 0.8	Neg	Neg
S. aureus	Neg	10.0 ± 0.8	6.0 ± 0.8	Neg	Neg
L. monocytogenes	Neg	6.0 ± 0.6	5.0 ± 0.8	Neg	Neg
S. typhimurim	Neg	2.0 ± 0.8	2.0 ± 0.0	Neg	Neg

**Data represents an average of three replicates* (± S.D.)
Table adopted from Lidia et al. [2]

Table 11: Zone of growth produced by some phenylpropanoids identified in serrano chili peppers (mm)*.

Figure 3: Courtesy of Dr. D. J. Baumler.

7.38. Capsaicin exerted a growth inhibitory effect of 92 ± 3.7% at pH 5.4 and 72 ± 11% at pH 6.4. At pH 4.5, bacterial growth did not differing the presence (93.5 ± 2.4%) and absence (88.4 ± 7.8%) of capsaicin [3].

Listeria monocytogenes

Reverse-phase HPLC analysis was performed to determine the capsinoid-content of the pepper extracts of habanero, serrano, and pimiento chili peppers. Table 9 shows the HPLC profile of standard phenylpropanoid compounds, capsaicin, and dihydrocapsaicin from chili extracts, while Table 10 shows the content of some capsinoids in the habanero, serrano, and pimiento moron extracts (mg/ml) [2]. Lidia et al. do not specify what serotypes of the peppers they used. The following pictures show the most readily available varieties in the market (Figure 2).

The capsinoid compositions of the three pepper extracts are different, and this may influence their antimicrobial effect. The concentration of capsaicin and capsaicinoids used in this study did not show an inhibitory effect on *L. monocytogenes*. Habanero which has the highest content of capsaicin was the least effective as a bacterial inhibitor. The pimiento morron extract, which contains *m*-coumeric acid and cinnamic acid but no capsaicin, showed a good inhibitory effect on the bacteria [2] (Table 11).

Conclusions

As more food scientists, consumers, and members of the medical field gain interest in chili peppers, it is certain that through ethnobotanical observations, *Capsicum* species harbor many economically significant benefits awaiting 'discovery' [6]. There are a variety of methods for testing the antimicrobial activities of chili peppers. These methods strongly affect the observed levels of inhibition. Various reasons may contribute in the differences between results, including inconsistency between analyzed plant materials [7].

In these experiments, crude extracts of chili peppers were used; no separation of pepper components was done, except by Dorantes et al. [2]. Based on the data, it seems that capsaicin had a lesser antimicrobial effect compared to other components of chili pepper extracts. Therefore, future studies should try to determine what compounds in the chili pepper gives the spice its antimicrobial properties, and to do so purification of the extracts is necessary. Capsaicin gives chili peppers the 'hot' sensation, which some people might not like. It would, therefore, be beneficial if there is another substance in the pepper that could be used in the food industry as a preservative without the pungent taste and hotness.

The studies examined herein were done *in vitro*. However, more tests need to be conducted to determine the antimicrobial effects of chili peppers *in vivo*, especially because such a large number of people eat peppers. This could be a potential means through which to minimize the effect of foodborne pathogens when there is an outbreak. Graham et al. [10] were unable to confirm the hypothesis that capsaicin has an inhibitory effect on *H. pylori in vivo*. They believe that natural substances and folk remedies should undergo testing *in vivo* before publication of the *in vitro* results to reduce the possibility of misinforming the public regarding the potential usefulness of these agents.

Varied as these studies may be, they open the doors to greater research on chili peppers. The data already collected and methods of testing offer new directions for future experiments. To obtain more conclusive data, the number of pepper varieties used should be increased since hundreds of thousands of different types of chili pepper plants exist worldwide. The following picture shows some of the most common varieties, including many exotic types sourced from all over the world (Figure 3).

For example the six hottest chili peppers in the world, Bhut Jolokia, Trinidad 7-pot, Trinidad Scorpion ButchT, Trinidad Doughlah, Trinidad Moruga Scorpion (shown in the next photo), and Carolina Reaper (not shown), have not been tested and may possessun discovered antimicrobial compounds and activity [15].

Our lab will be working with over 700 varieties of chili peppers to determine the antimicrobial effects the extracts of leaves and fruits

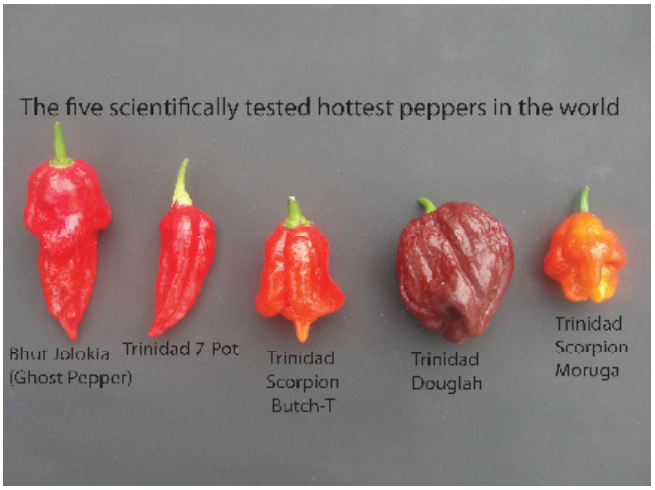

Figure 4: Courtesy of Dr. D. J. Baumler.

have on selected foodborne pathogens (Figure 4). These varieties will include peppers with and without capsaicin from all over the world. Also, purification of the extracts will be done to determine the most effective component of the extract for antibacterial usage. Finally, as mentioned earlier, peppers have vitamins and other nutrients. Our lab will carry out assays to determine the contents of vitamins A, C, E, and folic acid in many of these exotic types of chili peppers. This data will be useful when using peppers as an additive to value added foods.

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