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Food-borne pathogens, health and role of dietary phytochemicals

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Infectious diseases transmitted by food have become a major public health concern in recent years. In the USA alone, there are an estimated 6–33 million cases each year. The list of responsible agents continues to grow. In the past 20 years some dozen new pathogens that are primarily food-borne have been identified. Fruits and vegetables, often from the global food market, have been added to the traditional vehicles of food-borne illness; that is, undercooked meat, poultry, seafood, or unpasteurized milk. Such products are minimally processed and have fewer barriers to microbial growth such as salt, sugar or preservatives. The evolution of the epidemiology of food-borne illness requires a rethinking of traditional, though still valid, solutions for their prevention. Among various strategies to prevent food-borne pathogens, use of dietary phytochemicals is promising. The major obstacle in the use of dietary phytochemical is the consistency of phytochemicals in different foods due to their natural genetic variation. We have developed a novel tissue-culture-based selection strategy to isolate elite phenolic phytochemical-producing clonal lines of species belonging to the family Lamiaceae. Among several species we have targeted elite clonal lines of thyme (*Thymus vulgaris*) and oregano (*Origanum vulgare*) against *Escherichia coli* and *Clostridium perfrigens* in fresh and processed meats. We are also evaluating high phenolic profile-containing clonal lines of basil (*Ocimum basilicum*) to inhibit gastric ulcer-causing *Helicobacter pylori*. Other elite lines of the members of the family Lamiaceae, rosemary (*Rosmarinus officinalis*) and salvia (*Salvia officinalis*) also hold promise against a wide range of food pathogens such as *Salmonella* species in poultry products and *Vibrio* species in seafood.

Keywords: diet, food-borne pathogens, phytochemicals, plant biotechnology, antimicrobials, herbs, thyme, Lamiaceae, thymol, carvacrol.

Introduction

In the USA alone there is an estimated 6–80 million cases and 9000 related deaths due to food-borne illness. Most are cases not reported because the episodes are mild and do not require medical attention. Also, many cases are simply referred to as the ‘stomach flu’ rather than an intestinal illness due to contaminated food. In other cases people do not associate their illnesses with food because of the incubation period, which is up to two days for bacteria and a month for certain viruses and protozoa. Finally, most physicians and health professionals treat patients who have diarrhea without ever identifying the responsible pathogen, or else the laboratory does not have the ability to identify the pathogen.

The increased number of cases of food-borne illness is due in part to better reporting but also to changes in the food supply. Modern animal husbandry procedures, such as crowding large numbers of animals together, can promote the spread of food-borne pathogens. This is in addition to the well-known risk factors such as holding perishable foods at room temperature or undercooking foods. The number of individuals in high-risk groups is increasing; for example, children in group settings such as daycare centres, people with weakened immune systems, and the growing elderly population.

Well known foods responsible for food-borne illness, such as raw meat and poultry, have been supplemented by new vehicles such as salami, raw milk and orange juice. As the demand for ‘fresh’ products has increased so too have incidents due to fruits (melon, strawberries) and vegetables (alfalfa sprouts, tomatoes, unpasteurized apple cider and juices). Add to this mixture the more tolerant nature of emerging pathogens (e.g. acid tolerance of *E. coli* 0157:H7; growth at refrigerated temperatures by *Listeria*) and low infectious doses and the result is more serious and unexpected sources of food-borne pathogens. All of this is in addition to costs associated with food-borne illness, which range in the billions of dollars around the world.

In addition to fresh fruits and vegetables consumer demand for convenient, tasty and ready-to-eat foods has led to the introduction of minimally processed entrees which have fewer barriers to microbial growth such as salt, sugar, or preservatives. Minimally processed foods rely on low temperatures for safety. Pathogens in addition to *Listeria* have the ability to grow at refrigerated temperatures. Furthermore, along with emerging pathogens such as *E. coli* and *Listeria* there is an upward trend in the numbers of bacteria like *Salmonella* that are resistant to multiple antibiotics.[1,2] Resistant infections are associated with increased morbidity, prolonged hospital stays, greater costs and greater opportunities for the spread of infection.[3]

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Although proper cooking and pasteurization are effective weapons in the arsenal against microbial food-borne pathogens, their use has not been sufficient or, in the case of food such as fruits and certain vegetables, not appropriate. Other proven technologies such as irradiation have been slow to be approved and adopted. New processing technologies on the horizon include the use of ozone, high-intensity pulsed electric fields, and high hydrostatic pressure. In developing countries these will most likely have minimal impact for some time and other reliable and appropriate technologies must be pursued.

Many naturally occurring compounds found in edible and medicinal plants, herbs and spices have been shown to possess antimicrobial activities against food spoilage and food-borne pathogens. Examples include the essential oils of thyme, cinnamon, bay and cloves.[4–6] These phytochemicals must be exploited for food preservation and for improving food safety.

Plant metabolites and food preservative applications

The food processing industry uses chemical preservatives to inhibit food spoilage caused by chemicals, enzymes and micro-organisms. Among the preservatives used are antioxidants (BHA, BHT, TBHQ and gallates) that retard food spoilage linked to lipid peroxidation and therefore can increase shelf-life of foods.[7,8] An antioxidant may be defined as a substance which, when present at low concentrations compared with that of an oxidizable substrate, significantly delays or prevents the oxidation of the substrate.[9] Also used in food are chemicals that are antimicrobials (benzoic acid, sorbic acid and sulfites) which inhibit the growth of spoilage micro-organisms and therefore reduce associated food deterioration.[10–12] The international market for commercial food preservatives amounted to US $1.5 billion in l992 and is projected to reach US$2.0 billion by 2002.[13] In the USA the current demand for preservatives is about US$600 million or 40% of the world market.[13]

Due to the safety concerns[14] and consumer preference for natural products, the use of natural preservatives to replace synthetic forms is gaining importance and the consumer is willing to pay higher prices for natural products.[13] This trend has led to increased research on natural preservatives.[15–19] Though natural antioxidants like tocopherol and ascorbic acid are widely used, the activity of these compounds are much lower than synthetic forms.[14] Therefore, the search for more active natural and plant-based antioxidants[14,19–26] and antimicrobials is being actively pursued.[13,27–31]

At Rutgers University, the pioneering effort of Dr Stephan Chang on the development of natural antioxidant from rosemary extract[20] is currently marketed by Kalsec Corporation of Michigan.[13] In view of the importance of natural preservatives in food processing and preservation, new biotechnological approaches will have a substantial impact on the industrial food preservative market. The current commercial source of natural preservatives like thyme, rosemary and oregano extract are derived from genetically heterogeneous plants imported from overseas markets.[17,19,20,32] There exists tremendous genetic and source variation in antioxidant content due to natural cross-pollination typical of plants like rosemary, oregano, basil and other species in the family Lamiaceae.[33,34] In order to produce uniform and enhanced levels of important antioxidants and antimicrobials, it is essential and imperative to develop methods for rapid clonal propagation of elite metabolite-producing plants originating from a single heterozygous seed. The current focus, therefore, is to develop rapid clonal propagation systems in thyme (*Thymus vulgaris*) and subsequently screen for high phenolic-producing clones. Using the strategy of clonal propagation coupled to elicitor (*Pseudomonas*)-mediated physiological stimulation, specific targeting of enhanced production of rosmarinic acid (RA) and other specific phenols like thymol and carvacrol in elite oregano and thyme clones could bring substantial benefits to herb growers and preservative manufacturers in the United States.

Rosmarinic acid occurs widely in the family Lamiaceae, including basil and thyme. Rosmarinic acid is a food antioxidant[13,19] and has pharmaceutical applications and anti-inflammatory and complement inhibiting properties.[35–40] Rosmarinic acid also has potential as an antimicrobial.[38–40] For example, as Thyme is a highly valued culinary herb it is an excellent target to develop high phenolic (thymol and carvacrol) and RA-producing clones for diverse value-added applications in food processing, and as medicine. High phenolic and RA-producing clones can be developed as high-value crops for intensive greenhouse production or elite clones can be used in plant-breeding programs to develop elite seeds for field production in rural areas of the world. Extracts of high phenolic and high RA-producing clones of thyme can be targeted for post harvest preservation of food for sustainability, quality and to enhance exports of excess food. The natural thyme extract treated food also could have additional medicinal benefits through enhanced antioxidant activity in meats/seafood and antimicrobial properties against food pathogens like *E. coli*: 0157:H7. Antimicrobial properties can also be targeted against ulcer-causing *Helicobacter pylori*. *Helicobacter pylori* is known to be susceptible to plant phenolic such as capsaisin,[40] and with currently available drug treatments having side-effects,[41,42] phenolics from thyme could have potential as functional antimicrobials in chronic cases.

Applications of plant biotechnology

We have targeted thyme and related species like oregano for use as dietary phytochemical sources against food-borne pathogens. Thyme (*Thymus vulgaris L.*) is an evergreen, small bushy herb indigenous to the Mediterranean regions. It has woody stems covered with epidermal hair. The opposite, sessile leaves are one-fourth to half an inch long, slightly rolled at the edges with a pale, hairy underside. Thyme belongs to the family Labiatae (Lamiaceae), which includes rosemary (*Rosmarinus officinalis*) and oregano (*Origanum vulgare*).

There are over 100 varieties of thyme which have been cultivated since ancient times. The genus 'thymus' is a Greek word for 'courage'. Historically, thyme has had a medicinal reputation for overcoming shyness, gaining strength and courage and preventing nightmares.[43] Thyme was given to knights as they went to battle.

Thyme's leaves are used for flavorings. It is one of the most popular herbs used in European cooking. Wild, garden and lemon thyme are the most commonly used. Thyme works well in almost any dish. It is often used in stews, soups, meats and stuffings. The potential of thyme extracts as sources of food preservatives and specifically as food antioxidants is being realized.[32,44]

The oils extracted from leaves and flowers are used in perfumery and medicine. Thyme is believed to strengthen the immune system and thyme oil has been used in tonics to treat depression, colds and muscular pain.[45] Thyme oils contain more than 40% v/v of phenols.[46] Its major constituents include thymol (41%), carvacrol (3.6%) and RA.[47] Thymol has antiseptic and antifungal functions and is a urinary tract antiseptic.[46] Due to its antibacterial properties, it is often used in mouthwash and toothpaste.[48,49]

Unlike other economic crops, herbs and spices continue to be cultivated in the same way as they were grown thousands of years ago. A large proportion of these plants are still wild or semiwild, their main habitats being forests, uncultivated land and home yards. Due to the cross-pollinating nature of their breeding characteristics, herbs and spices are genetically heterogeneous. Adding to this problem of quality variation is the fact that herbs and spices are imported from all over the world. Furthermore, diseases, climate, geographical and even political factors may also affect the yield of these crops and quality of the final products.

The major objective of plant improvement is the development of these plants as economic crops whose production should be highly efficient and cost-effective.[50–52] Different ways to cultivate plants have been employed to increase yield, reduce cultivation space and most importantly, maintain product uniformity. Biotechnology plays an essential role in plant improvement. Tissue and cell cultures are the principal techniques in generating plants under controlled conditions.[53] This includes aseptic culture of organs (e.g. shoot tips), cells and protoplasts *in vitro*.[52,54,55]

Clonal plant propagation is also known as 'micropropagation' or simply as 'tissue culture'.[52] The earliest propagation by tissue culture was done in l960, when Morel demonstrated virus elimination from dahlia stock through shoot apex culture.[56] This biotechnological approach has many advantages over the traditional practices. Tissue culture offers rapid plant growth and large production in a relatively small space.[55] Cultivation *in vitro* under a controlled environment ensures freedom from changes in climate, temperature and other natural factors. Propagation can be carried out year-round under an artificial environment. Thus, non-seasonal products can serve a more diverse market in different areas of the world. Plants grown in sterile environment are free from many kinds of contamination.[53] Furthermore, tissue culture ensures genetic integrity and phenotypic uniformity even from genetically heterogeneous plant populations.[55] It helps to bring about improvements through genetic engineering, including gene transfer and enzyme modification.[55]

Plant tissue and cell culture provide detailed knowledge of biosynthetic pathways of primary and secondary metabolites. For example, the amino acid sequence of certain key enzymes such as phenylalanine ammonia-lyase have been determined. In relation to the production of secondary metabolites, plant tissue culture offers the possibility of producing novel secondary metabolite compounds by adding specific precursors of a biosynthetic pathway.[51] It can also be done by manipulating or deleting key enzymes to alter the nature of the end product.[53] The pursuit of higher yields of secondary metabolites includes: (i) Selection of high-yielding strains from the heterogeneous population; (ii) selection of mutant cell line; and (iii) application of stress such as UV radiation, high/low temperatures and microbial elicitors.[57]

Multiplication of axillary shoot can be encouraged by the suppression of apical dominance using a growth hormone cytokinin. Individual shoots can serve as the source of further shoot induction or root development. Another form of culture is callus culture, where undifferentiated plant cells (callus) can be induced by appropriate growth hormones (cytokinin and auxins) to generate shoots and roots, respectively.

Use of shoot cultures of thyme for regulation of secondary metabolite synthesis has the following advantages:

l. Shoot clones are genetically more stable than callus cultures.
2. Pseudomonas species-treated elite clones have increased mechanical strength and can be easily adapted to greenhouse or field conditions.
3. Shoot clones will allow the characterization of the light-regulated biochemical pathways associated with RA synthesis.
4. Shoot clones are better systems to study thymol and carvacrol synthesis as they are produced only in differentiated oil glands.

These shoot clones are being used in our laboratory to investigate the regulation of phenolics of thyme by chemical precursors and microbial elicitors.

Thyme and antimicrobial applications in food

Therapeutic interventions for the treatment of *E. coli* 0157:H7 are limited, reinforcing the need to prevent its presence and reproduction in the food manufacturing process. The recent outbreaks of *E. coli* 0157:H7 through meat products has renewed interest in potential inhibitors of this organism.[58,59] It is beyond the scope of this paper to review all chemicals which are currently used for their antimicrobial activity. They include sorbates, benzoates, propionates and sodium chloride. Others are added for colour and flavor (nitrites) or for their antioxidant properties (BHA and BHT) but also possess antimicrobial activity. The food industry relies heavily on the use of synthetic antimicrobial agents to extend shelf life and preserve freshness. However, these synthetic preservatives in the food industry are coming under increasing scrutiny and reappraisal, resulting in the search for natural biochemicals from plants, including herbs and spices.[13] Thus, there has been renewed interest in the antimicrobial properties of herbs and spices. In fact, about 20% of spices sold in the USA are used in the meat industry.

Most essential oils of spices and herbs are considered generally regarded as safe (GRAS) and are considered to contain the antimicrobial activity. The antimicrobial activity of plant extracts, including spices and essential oils, has been reviewed.[60] Carvacrol and thymol are major volatile components of oregano, thyme, and savory. Generally, the thymol:carvacrol ratio in thyme is 10:1 whereas the carvacrol:thymol ratio in oregano is 20:1.[61] Carvacrol and thymol are known to have 1.5X and 20X the antimicrobial activity of phenol, respectively.[62] In l960 Katayama and Nagai reported the antimicrobial activity of thymol and carvacrol against *Salmonella enteritidis*, *Staphylococcus aureus*, and *E. coli* (serotype not given).[63] Similarly Beuchat reported that the growth of *Vibrio parahaemolyticus* was delayed by the presence of 100 p.p.m. of the essential oils of oregano and thyme.[64] More recently, Farag *et al.* reported that of six essential oils examined (sage, rosemary, cumin, caraway, clove, and thyme) thyme oil was the most effective against three gram-negative bacteria, with *E. coli* (serotype not given) being the most sensitive.[65] The same workers showed that thyme oil reduced the total bacterial count in butter stored at room temperature. This is the only report documenting the antibacterial effectiveness of essential oils in a food system.[66]

The specific modes of action of plant extracts remain poorly defined but there is some indication that they may cause a depletion of cellular energy.[67] Since many of the components of essential oils, such as thymol, are similar in structure to phenolic antimicrobials it seems reasonable that their modes of action would be similar.

Finally, environmental conditions may play a role in the effectiveness of natural antimicrobials. In particular, the antifungal properties of thymol and carvacrol were affected by pH.[68] There is no similar information on its antibacterial properties but its effectiveness in meat might be affected by such considerations. Our preliminary results demonstrating the effectiveness of thymol and carvacrol against *E. coli* in laboratory media (Fig. 1) were conducted at pH 6.5, near that of meat. This indicates that, at least with these two essential oils, pH alone would have a minimal adverse effect.

Preliminary results

Tissue culture of Lamiaceae

Novel approaches have been developed to isolate high phenolic and rosmarinic acid-producing clonal lines in the family Lamiaceae including thyme. Species belonging to this family are an excellent source of valuable phenolics which have pharmaceutical and food preservative applications. These species are genetically heterogeneous since the breeding character is influenced by natural cross pollination.[34] This results in tremendous plant to plant variation even among seeds from the same source. However, using tissue culture-based clonal propagation, genetically uniform clonal lines have been isolated. *In vitro* shoot organogenesis has been used to isolate high phenolic-containing clonal lines originating from a single seed in oregano (*Origanum vulgare*),[69] thyme (*Thymus vulgaris*)[70,71] and rosemary.[72] Results indicate that this approach is successful in thyme and is being extensively explored for commercial development. Elite thyme clonal lines can be developed as a new crop for preservation of potatoes and meats for export and concurrently can also be a source of chemopreventive medicinal; for example, against chronic infections of ulcer-causing *Helicobacter pylori* and urinary infection-causing *E. coli*.

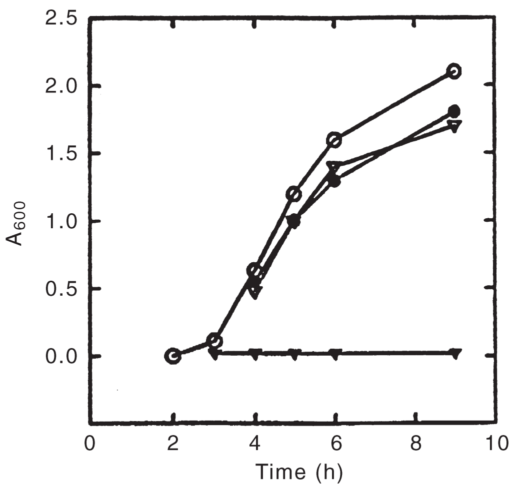

Figure 1. Inhibition of growth of *E. coli* 0157:H7 in trypticase soy broth by the essential oils thymol and carvacrol. (○), control; (●), carvacrol (75 p.p.m.); (▽), thymol (75 p.p.m.); (▼), carvacrol or thymol (150 p.p.m.).

Selection of high phenolics-producing clonal lines using **Pseudomonas** *species*

Clonal lines of oregano were isolated using *in vitro* shoot organogenesis.[73] During the isolation of clonal line 0–4, a seed-borne *Pseudomonas* was also isolated. The contamination was not lethal to oregano. However, it stimulated the phenylpropanoid pathway and enhanced the mechanical strength of all the *in vitro*-produced clonal plants from clonal line 0–4. This enhanced the acclimation to the outside environment of the bacterium-treated clonal lines.[73] It was confirmed that only polysaccharide-producing *Pseudomonas* or purified polysaccharide elicited phenolic synthesis and the subsequent enhanced acclimation response. The phenolic stimulation in response to *Pseudomonas* varied among clonal lines. This Pseudomonas-induced variability was utilized to isolate high phenolics stimulated clonal lines of oregano,[69] thyme[70,71] and rosemary.[72] This approach is being applied in our laboratory to select high phenolics-producing clonal lines of thyme.

Selection of elite clonal lines of thyme

A high phenolics-containing clonal line (T-12) of thyme (*Thymus vulgaris L.*) was isolated from a heterogeneous seed population by plant tissue culture techniques.[70] This clonal line was isolated from among 10 clonal lines, with each originating from different genetically heterozygous, single germinating seedlings. All clonal lines were generated via shoot organogenesis through adventitious bud proliferation from apex explants.

Optimum shoot organogenesis was induced on Murashige and Skoog (MS) medium with benzyladenine (1 mg/L) as the growth hormone. Multiple shoots originating from apex explants of single heterozygous seedlings were further multiplied on the aforementioned benzyladenine-containing MS medium to subsequently generate a larger number of clonally identical plants. Shoots from each individual clonal line were inoculated with a novel *Pseudomonas* species. Following growth on hormone-free MS medium for 25 days, total phenolics were determined spectrophotometrically. Using this approach, high phenolics-stimulated clonal line T-12 and moderate phenolics-stimulated clonal time T-16G were isolated. These clonal lines attained the higher level of phenolics following *Pseudomonas* inoculation and also had uninhibited shoot growth compared with the corresponding uninoculated control.

Several low phenolics clonal lines, which had inhibited shoot growth in response to *Pseudomonas* species, were also isolated. Thymol levels of uninoculated shoots of all clonal lines after 60 days of growth were also measured by gas chromatography–mass spectroscopy. The high-to-medium phenolics-containing clonal lines (T-12 and T-16G) had basal thymol levels in the range of 150 μg/g fresh weight (FW). The thymol levels of low phenolics-containing clonal lines were in the range 10–70 μg/g FW. This *Pseudomonas* species-mediated selection provides a potentially novel biotechnology-based strategy to isolate high phenolics and thymol-containing clonal lines of thyme from a genetically heterogeneous population.

Regulation of phenolics in shoot-based clonal lines of thyme

We investigated the extent of stimulation of total phenolics and RA levels in three *in vitro* shoot culture-based thyme clonal lines in response to the novel *Pseudomonas* species mentioned above.[71] Clonal lines chosen for this study were the high phenolic line T-12 and the medium- and low-phenolic lines T-16G and M-3. Results indicate that different strains of *Pseudomonas* stimulated total phenolic levels and RA accumulation to varying degrees. Mucoid strain M4 elicited the highest levels of RA in clonal line T-12 on day 25. A moderate degree of RA stimulation in response to M4 was observed in clonal line M-3. Mucoid strain F elicited the highest total phenolic levels in T-12 on day 25. Non-mucoid strain NMA elicited stimulation of total phenolic compounds and RA to some degree only: N clonal line T-16G.

A 30 day time course analysis in line T-12 indicated that strains F and NMA elicited the highest phenolic levels. RA synthesis appeared to be stimulated in response to M4 at later stages of growth and reduced stimulation was apparent in response to F and NMA. The mechanical rigidity of T-12 shoots appeared to be highest in response to mucoid strain M4, indicating the possibility of increased lignin formation. This *Pseudomonas*-thyme clonal system provides a foundation to investigate the microbially elicited stimulation of biosynthetic pathways leading to novel antimicrobials and antioxidants.

***Comparision of inhibitory and lethal effects of carvacrol and thymol on food spoilage yeast,* Debaromyces hansenii**

The inhibitory and lethal effects of phenolic compounds found in common herbs and spices were compared on food spoilage yeast, *Debaromyces hansenii*.[30] Seperate treatments of trans-anethole, carvacrol, eugenol and thymol were investigated in potato dextrose broth (PDB) suspension cultures. Inhibitory activity was studied for all compounds at concentrations of 25, 50, 75, and 100 p.p.m. over a 55 h incubation while lethality was investigated for trans-anethole, carvacrol, and thymol at 100 and 125 p.p.m. over a four-day incubation. All compounds exhibited at least minor inhibitory activity at a concentration of 25 p.p.m.

During the 55 h incubation period, the minimum concentration for total inhibition by trans-anethole was 75 p.p.m., while for carvacrol and thymol it was 100 p.p.m. The maximum level of eugenol examined, 100 p.p.m., did not completely inhibit outgrowth. Growth curve data were described by the logistic equation which provided for quantitative comparision of inhibition. Lethality was achieved with trans-anethole, carvacrol, and thymol at 100 and 125 p.p.m. as determined by colony forming units (CFU) on potato dextrose agar over four days incubation. These findings demonstrate an approach for quantitatively describing inhibition and evaluating the lethal effects of plant metabolites on a spoilage yeast. This research should prove useful in studies identifying active compounds, determining their effectiveness, and providing strategies for incorporation into food systems.

Expected benefits to global food and agricultural systems

Solutions to problems of global agriculture and especially rural areas must focus attention on the following: (i) solving food safety problems such as inhibition of pathogens in processed foods and street foods; (ii) developing new uses of local crops for value-added processing through biotechnology; (iii) enhancing transport of excess foods and agricultural products by better preservation using local crops and resources; (iv) developing products that help environmental sustainability; and (v) developing plant phytochemicals that have disease-preventive functions. For example, developing elite, thymol-producing clonal lines of thyme for inhibition of *E. coli* and oxidation-induced deterioration in processed meats and seafood is an excellent example of the kind of new strategies required to meet the new challenges of rural agriculture. Elite thyme clonal lines generated via tissue culture techniques can be developed as a new crop for several value-added applications. In addition to being potentially targeted for inhibition of *E. coli* in processed meat it can also be used as an antioxidant in meat.[32,44]

The use of natural phenolic metabolites in vegetables, meats and seafood will enhance the potential for processing and preserving food and increase the marketability of excess food products into Asia and Europe where natural plant extracts are widely used and readily accepted compared to synthetic chemicals. Since thyme phenolics like thymol, carvacrol and rosmarinic acid have antioxidant properties, it could also be an excellent base for potential skin care products serving as UV protectants and cancer chemopreventive metabolites following clearly defined clinical studies. Selecting elite clonal lines with specific phenolic profiles can also harness the synergistic effect of phenolic metabolites. Further, natural thymol containing extracts can be used in oral hygiene products such as mouthwashes and as antifungals in animal-care products.

The antimicrobial properties of herbs, spices, and their essential oils have been known for some time. As mentioned, many previous studies have used either whole plant materials or essential oils containing mixtures of active ingredients from heterogeneous plant materials. The high degree of variability in the level of active ingredients poses serious problems for their routine use in foods. A unique aspect of our work is that we are envisioning the production of elite clones of *Thymus vulgaris*, which will contain uniform and higher levels of the natural antimicrobial compounds thymol and carvacrol. This plant has been selected because of its high content of these two compounds. The normal ratio of thymol to carvacrol in *T. vulgaris* is 10:1. Tissue culture-based genetic selection of plant species containing specific levels of proven antimicrobial agents has never been attempted. In addition, and importantly, thyme is currently used in meats (as flavoring agents). Meats are a particularly attractive food system because their lipids enhance the solubility of these two non-polar compounds.

Although not directly related to food safety issues, it should be pointed out that safety concerns (real or imagined) have prompted investigations of the use of natural antioxidants in place of synthetic molecules such as BHA and BHT. In addition to their antimicrobial properties, thymol and carvacrol have received attention as possible antioxidants.[16,32,44] Thus, these natural compounds may have a dual function in lipid-containing foods such as meats. In terms of food safety, the results of our work could identify an additional 'barrier' to exposure to food-borne pathogens by global consumers.

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