

Influence of Medicinal Spices on the Acid Tolerance of a Health Beneficial Bacterium *Streptococcus thermophilus* ST-M5

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ABSTRACT

There is a great deal of public interest in the use of herbal remedies. Garlic is said to antiviral and antifungal and along with ginger; they are antibacterial and preventatives for cardiovascular diseases. Ginger is very effective against nausea and has analgesic properties. Onion reduces the risk of developing diabetes and like garlic, has anticancer properties. *Streptococcus thermophilus* is a lactic acid bacterium that produces lactase, which facilitates the digestion of lactose in milk, decreases the symptoms of malabsorption, and reduces the risk of antibiotic associated diarrhea. Acid tolerance is an important probiotic characteristic and it is an indication of the ability of the microorganism to withstand the acidic pH of the stomach. Freshly thawed culture was inoculated in acidified MRS broth at pH 2 and 1% v/v of freshly extracted spice juice was added. Control was without spice juice. Growth was determined hourly during 2 hours of incubation at 37°C. Bacterial culture treated with all three spices individually showed a significant increase in counts at 0 and 1 hours of incubation when compared to control. After 2 hours of incubation, culture exposed to ginger showed no significant difference compared to control, while there were slight yet significantly lower counts for culture exposed to garlic and onion individually. Among the spices, ginger had the best overall effect. These three spices can be used with *Streptococcus thermophilus* enabling health benefits from both sources.

Keywords: Spices; Garlic; Ginger; Onion; Acid Tolerance; *Streptococcus thermophilus*

1. Introduction

Some medicinal plants are used as spices. Garlic (*Allium sativum*) is said to be antibacterial, antiviral and antifungal [1] and also prevent cardiovascular diseases [2] and some types of cancer [3]. Ginger (*Zingiber officinale*) is effective against nausea [4] and cardiovascular diseases [5] and is also an analgesic and has antibacterial properties [6]. Onion (*Allium cepa*) is effective against the common cold [7] and reduces the risk of developing diabetes [8]; it also has anti-inflammatory [9], anti-cholesterol [10], anticancer [11] and antioxidant [9] properties.

Functional foods are foods that have a health benefit over and above their regular counterparts [12]. In 2009, the US was the second largest market for functional foods, with a worth of \$7.123 billion dollars. It is estimated to be worth \$8.618 billion dollars in 2015 [13]. The importance of a balanced diet and the presence of probiotic bacteria in the gut are well recognized in maintaining general gut health [14].

Streptococcus thermophilus is a lactic acid bacterium commonly used in yogurt manufacture. This bacterium replicates within the human gastrointestinal tract and produces lactase, which facilitates the digestion of lactose in milk [15]. It also can decrease the symptoms of malabsorption, which often go along with acute infections diarrhea and it reduces the risk of antibiotic associated diarrhea [16].

Probiotics are microorganisms introduced orally in the gastrointestinal tract that are able to contribute positively to the activity of intestinal microflora and, therefore, to the host health [17]. The term probiotic means “for life” and it is currently used to name bacteria associated with beneficial effects for humans and animals [18]. Probiotic microorganisms should be capable of surviving passage through the digestive tract, be resistant to gastric juices, be capable to proliferate in the gut and be able to grow in the presence of bile under intestinal conditions [17]. When a probiotic reaches the stomach, it is exposed to an acidic solution that contains hydrochloric acid, potassium chloride and sodium chloride, which brings the stomach

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pH to a range between 1 and 2 [19,20]. In view of the fact that the stomach acid is one of the first stress barriers encountered by the probiotics upon ingestion, it is very important that the probiotic be acid tolerant. Whether or not spices with medicinal properties have the capacity of enhancing the acid tolerance of *Streptococcus thermophilus* is not known. The objective of this study was to elucidate the influence of spices with medicinal properties namely, garlic, ginger and onion on the acid tolerance of *Streptococcus thermophilus* a health beneficial bacterium.

2. Materials and Methods

2.1. Experimental Design

Freshly thawed *Streptococcus thermophilus* ST-M5 (F-DVS, Chr. Hansen's Laboratory, Milwaukee, WI) culture was suspended in sterilized 0.1% peptone water and 1% of freshly extracted spice juice (garlic, ginger or onion) was added. The three spice juice treatments were performed in a random manner. The control was the sample in which no spice juice was added. Acid tolerance was determined by plating the control and the treated samples every hour for 2 hours of incubation. The experimental design was a completely randomized design (CRD). Three replications were conducted for each experimental condition.

2.2. Sample Preparation

Fresh spices were peeled, washed and dried. Using a juice extractor, (JM480S, Juiceman), the juice of each spice was obtained just before it was needed and it was added immediately to the inoculated peptone water. This step was done in this manner to avoid oxidation reactions. Control and spice treated samples for acid tolerance analysis were prepared by inoculating 11 mL of freshly thawed culture of *Streptococcus thermophilus* ST-M5 (Chr. Hansen's Laboratory, Milwaukee, WI) into 99 mL of sterile acidified MRS broth at pH 2 containing 1 mL juice of each spice (garlic or ginger or onion).

2.3. Preparation of Media

2.3.1. pH Modified MRS Broth

The MRS broth was prepared according to the manufacturer's instructions (Difco™, Dickinson and Company, Sparks, MD). The pH of the broth was adjusted to 2 using 10M HCl.

2.3.2. *Streptococcus thermophiles* Agar

The appropriate amount of distilled water was added to a 1L graduated cylinder. The ingredients used to make *S. thermophilus* agar were weighed (10 g of Bacto Tryptone (Becton, Dickinson and Co, Sparks, MD), 10 g of Sucrose

(Amresco, Solon, OH), 5 g of Bacto yeast extract (Becton, Dickinson and Co, Sparks, MD), 2 g of K₂HPO₄ (Fisher Scientific, Fair Lawn, NJ) per L using individual plastic weighing boats and transferred to a 1 L Erlenmeyer flask. The mix was stirred to dissolve the ingredients. The pH was adjusted to 6.8 ± 0.1 with HCl 10M. Next, 6 mL of 0.5% bromocresol purple solution and 12 g of agar were added to the medium. The medium was autoclaved at 121°C for 15 minutes.

2.4. Acid Tolerance

The acid tolerance was determined according to the method proposed by Pereira and Gibson [21] with slight modifications. The bacterial culture *Streptococcus thermophilus* ST-M5 was inoculated in acidified MRS broth (Difco, Becton, Dickinson and Co, Sparks, MD) previously adjusted to pH 2 using 10 M HCl. The inoculated acidified MRS broth was incubated aerobically at 37°C. At 0, 1 and 2 hours of inoculation, 11 mL of the inoculated broth were serially diluted in 99 mL of 0.1% peptone water and 1mL of each dilution was pour plated using *Streptococcus thermophilus* agar [22]. The Petri dishes were incubated aerobically at 37°C for 48 hours after which the colonies were counted.

2.5. Statistical Analysis

Differences of least square means were used to determine significant differences at $P < 0.05$ for main effects (spice and time), and two way interaction effects (spice * time). Data is presented as mean ± standard error of the means. Significant differences were determined at $\alpha = 0.05$. Significant differences ($P < 0.05$) among the main effects were analyzed using Tukey's adjustment. Data was analyzed using Proc Mixed model of Statistical Analysis System (SAS®).

3. Results and Discussion

The acid tolerance of *Streptococcus thermophilus* ST-M5 as influenced by various health beneficial spice juices was tested and is shown in **Figure 1**.

All three spices showed a significant ($P < 0.05$) increase in counts at 0 hours of incubation when compared to control. After 1 hour of incubation, all three spices had significantly ($P < 0.05$) higher counts than the control. After 2 hours of incubation, ginger showed no significant ($P > 0.05$) difference compared to control (5.5176 Log cfu/mL), while there were slight yet significantly lower counts for garlic (4.2579 Log cfu/mL) and onion (5.0709 Log cfu/mL) (**Table 1**).

Mean log reduction of the viable counts of *S. thermophilus* subjected to different spice juices were obtained by subtracting counts at 2 hours from 0 hours and are shown in **Table 2**.

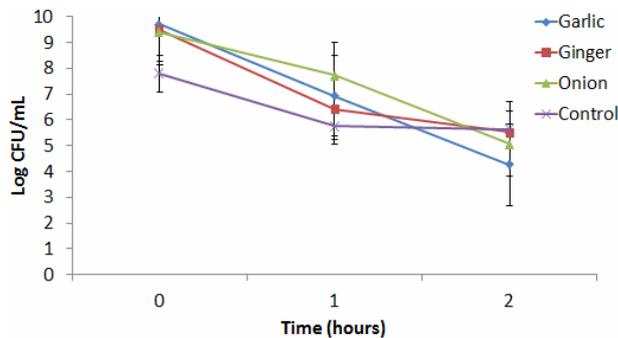


Figure 1. Acid tolerance of *Streptococcus thermophilus* ST-M5 under the influence of garlic, ginger and onion juice.

Table 1. Pr > F of acid tolerance of *Streptococcus thermophilus* ST-M5 exposed to different spice juices to control.

Treatment (spice)	0 hr	1 hr	2 hr
Garlic	<0.001	<0.001	<0.001
Ginger	<0.001	<0.001	0.9571
Onion	<0.001	<0.001	<0.001

Table 2. Mean log reduction of the viable counts of *Streptococcus thermophilus* ST-M5 when exposed to spice juice for 2 hours.

Treatment (spice)	Log CFU/mL
Garlic	5.4522
Ginger	3.9684
Onion	4.3373
Control	2.1431

In **Table 2**, a high number indicates high bacterial death and a lower number indicates low bacterial death. The log reduction of ginger showed the lowest bacterial death when compared with the rest of the spices.

An important characteristic of a probiotic is its survival at low pH [23]. A protective coating of exopolysaccharides (EPS) may allow the bacterium to better withstand stomach acid and bile salts [24]. According to a study by Boke *et al.* [25], it was demonstrated that EPS protected the bacteria in gastrointestinal conditions and they suggest that the bacterial EPSs are thought to play a role in the protection of microbial cells against low pH, like that of the stomach.

Mater and others [26] reported that *S. thermophilus* by itself resisted a pH as low as 2 and 2.5 and maintained its viability. Furthermore, Lick and others [27] found that *Streptococcus thermophilus* is able to survive gastrointestinal passage *in vivo* and detected viable counts in human duodenal samples after fresh yogurt ingestion.

4. Conclusion

Among the spices, ginger had the best overall effect, showing the lowest bacterial death, while garlic showed

the highest, but still with viable counts. These three spices can be used with *Streptococcus thermophilus* enabling health benefits from both sources.

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