

## Pretreatment with Allura Red or Tartrazine Increase Resistance of *E. coli* and *Staphylococcus aureus* to some Preservatives

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### Abstract

Coloring agents such as allura red and tartrazine are frequently used in food and pharmaceutical industries. Also, many types of preservatives are added to food and pharmaceutical products to prevent their spoilage. This study examined the effect of pretreatment with nutrient broth containing allura red (AR) or tartrazine (TA) on survival of *E. coli* and *Staphylococcus aureus* in media containing preservatives (eg ascorbic acid, citric acid, or sodium chloride). Pretreatment of *E. coli* for 4 days with AR or TA before exposure for 3 days to nutrient broth containing NaCl (20%) significantly increased its resistance to sodium chloride (20%). Pretreatment of *E. coli* for 4 days with AR, TA, or nutrient broth alone before exposure for 3 days to nutrient broth containing ascorbic acid (1%) or to nutrient broth containing citric acid (0.5%) killed all cells. For *S. aureus*, pretreatment with AR or TA before exposure for 3 days to nutrient broth containing citric acid (0.3%) significantly increased its resistance to citric acid (0.3%). Pretreatment of *S. aureus* for 4 days with AR, TA, or nutrient broth alone before exposure for 3 days to nutrient broth containing citric acid (0.3%) reduced number of cells by 4.37, 2.54, and 6.97 logs, respectively. Pretreatment of *S. aureus* for 4 days with AR, TA, or nutrient broth alone before exposure for 3 days to nutrient broth containing ascorbic acid (0.5%) reduced number of viable cells by 3, 3.6, and 3.07 logs, respectively. Pretreatment of *S. aureus* for 4 days with AR, TA, or nutrient broth alone before exposure for 3 days to nutrient broth containing NaCl (20%) increased number of cells by 0.74, 0.7 and 1.1logs, respectively. These results have implications in food and pharmaceutical industries.

**Keywords:** Allura red, tartrazine, *S. aureus*, *E. coli*, preservatives

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## INTRODUCTION

Allura red and tartrazine are synthetic azo dyes that are used widely in food industry. They are used to give red or yellow color to food or pharmaceutical products.<sup>1</sup> They are added to many types of food such as jellies, pudding, sweets, soft drinks, fruit juices, chips, dairy products, frozen meat, fish and seafood.<sup>2,3</sup>

*Staphylococcus aureus* is one of the common causes of foodborne diseases in developed and developing countries.<sup>4</sup> Symptoms of food poisoning result from consumption of preformed enterotoxin(s) that are produced in the food contaminated with *S. aureus*.<sup>5</sup> Many strains of *E. coli* are pathogenic causing mild or severe diarrhea. Food poisoning outbreaks usually result from ingestion of fresh vegetables or fruits or undercooked food contaminated with these microorganisms.<sup>6</sup>

Chemical preservatives are frequently added to food to prevent their spoilage. To my knowledge, there are no studies on the effect of pretreatment with coloring agents (such as allura red, tartrazine) on subsequent inhibition of *S. aureus* and *E. coli* by preservatives. Therefore, this study was undertaken to investigate the effect of pretreatment with allura red or tartrazine on the growth of *S. aureus* and *E. coli* in media containing preservatives (eg ascorbic acid, citric acid, or sodium chloride).

## MATERIALS AND METHODS

### Strains of bacteria

Strains of *E. coli* and *S. aureus* were obtained from a central laboratory. For preparation of the inoculum, nutrient broth (20 mL) was inoculated with appropriate cultures and incubated at 31°C for 20h.

### Experimental design

#### Growth in nutrient broth

A 0.5mL of the overnight grown bacterial culture was transferred to 10 mL nutrient broth containing 0.1% allura red or 0.1% tartrazine (these concentrations are representatives of the concentrations of AR or TA that may be added to food or pharmaceutical products), and incubated for 4 days at 31°C. Then 0.5 mL of each culture was added to 10 mL nutrient broth containing NaCl (20%), or nutrient broth containing ascorbic acid (0.5% or 1%), or nutrient broth containing citric

acid (0.3% or 0.5%). At time 0 and after 1 day and 3 days, samples were withdrawn and appropriate dilutions were made. To enumerate bacterial cells, dilutions were plated on nutrient agar, and plate counts were recorded after incubation at 31°C for 24h. The lower limit of detection for this method was 10 CFU/ mL.

For control experiments, 0.5 mL of the overnight grown bacterial culture was transferred to 10 mL nutrient broth alone and incubated for 4 days at 31°C. Then, 0.5 mL of the culture was added to 10 mL nutrient broth containing the preservatives as described above. At time 0 and after 1 day and 3 days, samples were withdrawn and treated as previously described to enumerate bacterial cells.

#### Growth in fish homogenate

Fish homogenate was prepared and sterilized as previously described.<sup>7</sup> Then, 10 mL portion of the cooled homogenate was dispensed into flasks, and allura red (0.1%) or tartrazine (0.1%) was added to flasks. A 0.5 mL of the overnight grown bacterial culture was transferred to the flasks, and incubated for 4 days at 31°C. Then, 0.5 mL of each culture was added to 10 mL fish homogenate containing the preservatives as described above. At time 0 and after 3 days, samples were withdrawn and treated as previously described to enumerate bacterial cells. For control experiment, 0.5 mL of the overnight grown bacterial culture was transferred to 10 mL fish homogenate alone and incubated for 4 days at 31°C. Then, 0.5 mL of the culture was added to 10 mL fish homogenate containing the preservatives as described above. At time 0 and after 3 days, samples were withdrawn and treated as previously described to enumerate bacterial cells.

#### Statistical analysis

Experiments were done four times with consistent results, the values presented are the mean values. To determine the significant differences ( $p < 0.05$ ), student's t-test was used.

## RESULTS AND DISCUSSION

### Effect of pretreatment with allura red or tartrazine on growth of *E. coli* in the tested preservatives

Pretreatment with allura red (AR), tartrazine (TA), or nutrient broth alone (control experiment) before exposure for 1 day to nutrient broth containing ascorbic acid (1%) greatly reduced

**Table 1.** Effect of pre incubation for 4d in nutrient broth containing allura red (A) or nutrient broth containing tartrazine (B) on growth of *E. coli* in nutrient broth containing preservatives

Exposure time to the preservative(d)	A		
	NB + 20% NaCl	Log CFU/ml NB + 1% ascorbic acid	NB + 0.5% citric acid
0	5.79	5.4	5.8
1	3.4 (- 2.39)	1.6 (- 3.8)	3.6 (- 2.2)
3	1.3 (- 4.49)	0	0

  

Exposure time to the preservative (d)	B		
	NB + 20% NaCl	Log CFU/ml NB + 1% ascorbic acid	NB + 0.5% citric acid
0	6.41	6.1	6.2
1	4.11 (- 2.3)	2.1 (- 4)	3.32 (- 2.88)
3	1.41 (- 5)	0	0

Numbers in parenthesis represent: log CFU/ ml at time 0 – log CFU/ ml at time 1d or 3d.

- : indicates decrease in growth; d: days

**Table 2.** Effect of pre incubation for 4d in nutrient broth alone on growth of *E. coli* in nutrient broth containing preservatives

Exposure time to the preservative (d)	Log CFU/ml		
	NB + 20% NaCl	NB + 1% ascorbic acid	NB + 0.5% citric acid
0	6.56	6.2	6.3
1	4.32 (- 2.24)	2.65 (- 3.55)	0
3	0	0	0

Numbers in parenthesis represent: log CFU/ ml at time 0 – log CFU/ ml at time 1d or 3d.

- : indicates decrease in growth.

( $p < 0.05$ ) number of viable cells, number of cells decreased by 3.8, 4, and 3.55 logs, respectively (Table 1 and 2). Pretreatment with AR, TA, or nutrient broth alone before exposure for 3 days to nutrient broth containing ascorbic acid (1%) killed all cells (Table 1 and 2). The pH after exposure for 3 days to nutrient broth containing ascorbic acid (1%) was 3.1, 3.21, and 3.17, respectively.

Incubation in AR, TA, or nutrient broth alone before exposure for 1 day to nutrient broth containing citric acid (0.5%) significantly decreased ( $p < 0.05$ ) number of *E. coli* cells; but cells incubated in AR or TA decreased to a lesser extent than cells incubated in nutrient broth alone (number of cells was decreased by 2.2, 2.88, and 6.3 logs, respectively) (Table 1 and 2). Citric acid can chelate metal ions and may remove Mg<sup>++</sup>

ions from the outer membrane of Gram- negative bacteria (such as *E. coli*) which disrupts the cell wall causing death of bacterial cells. *E. coli* can grow in media containing allura red or tartrazine (unpublished data) and may degrade these dyes; and some of the resultant products may protect *E. coli* from complete inhibition by citric acid (0.5%). However, incubation in AR, TA, or nutrient broth alone before exposure for 3 days to nutrient broth containing citric acid (0.5%) killed all cells (Table 1 and 2).

For growth in nutrient broth containing NaCl (20%), pretreatment with AR, TA, or nutrient broth alone before exposure for 3 days to nutrient broth containing NaCl (20%) decreased ( $p < 0.05$ ) number of cells by 4.49, 5 logs and killed all cells, respectively (Table 1 and 2).

**Table 3.** Effect of pre incubation for 4d in nutrient broth containing allura red (A) or nutrient broth containing tartrazine (B) on growth of *S. aureus* in nutrient broth containing preservatives

Exposure time to the preservative(d)	A		
	NB + 20% NaCl	Log CFU/ml	NB + 0.3% citric acid
0	6.04	5.3	6.32
1	6.32 (+ 0.28)	3.81 (- 1.49)	2.64 (- 3.68)
3	6.78 (+ 0.74)	2.3 (- 3)	1.95 (- 4.37)

  

Exposure time to the preservative (d)	B		
	NB + 20% NaCl	Log CFU/ml	NB + 0.3% citric acid
0	6.1	5.9	6.2
1	6.5 (+ 0.4)	3.23 (- 2.67)	3.99 (- 2.21)
3	6.8 (+ 0.7)	2.3 (- 3.6)	3.66 (- 2.54)

Numbers in parenthesis represent: log CFU/ ml at time 0 – log CFU/ ml at time 1d or 3d.

+ : indicates increase in growth.

- : indicates decrease in growth.

**Table 4.** Effect of pre incubation for 4d in nutrient broth alone on growth of *S. aureus* in nutrient broth containing preservatives

Exposure time to the preservative (d)	Log CFU/ml		
	NB + 20% NaCl	NB + 0.5% ascorbic acid	NB + 0.3% citric acid
0	6.69	6.57	6.97
1	7.60 (+ 0.91)	5.38 (- 1.19)	0
3	7.79 (+ 1.1)	3.5 (- 3.07)	0

Numbers in parenthesis represent: log CFU/ ml at time 0 – log CFU/ ml at time 1d or 3d.

+ : indicates increase in growth;

- : indicates decrease in growth.

Similar results were obtained for pretreatment of *E. coli* in fish homogenate containing AR, fish homogenate containing TA, or fish homogenate alone before exposure to ascorbic acid (1%), citric acid (0.5%), or NaCl (20%) (data not shown).

#### Effect of pretreatment with allura red or tartrazine on growth of *S. aureus* in the tested preservatives

The logs of number of cells of *S. aureus* pretreated with allura red (AR) or tartrazine (TA), then subjected to growth in nutrient broth containing preservatives are shown in Table 3. Pretreatment with AR or TA before exposure for 3 days to nutrient broth containing ascorbic acid (0.5%) greatly reduced ( $p < 0.05$ ) number of viable cells, number of cells decreased by 3 and 3.6 logs, respectively (Table 3). Logs of number

of cells of *S. aureus* incubated in nutrient broth alone before exposure to preservatives is shown in Table 4. The decrease in log number of viable cells after exposure for 3 days to nutrient broth containing ascorbic acid (0.5%) was nearly similar to that observed when cells were pretreated with AR or TA before exposure to the preservatives.

For exposure for 3 days to nutrient broth containing citric acid (0.3%) after pretreatment with AR or TA, number of cells of *S. aureus* decreased by 4.37 and 2.54 logs, respectively (Table 3). In control experiment (incubation in nutrient broth alone before exposure to preservatives), all cells of *S. aureus* were killed after exposure for 3d to nutrient broth containing citric acid (0.3%) (Table 4). Citric acid decreases pH and chelates metal ions 8 which are needed

for synthesis of enzymes and other components of Gram positive bacteria (such as *S. aureus*). Because *S. aureus* can grow in media containing allura red or tartrazine (unpublished data), some of the resultant products may protect bacterial cells grown in AR or TA from complete inhibition by citric acid (0.3%). However, application of higher concentration of citric acid may prevent growth of *S. aureus*.

For exposure for 3 days to nutrient broth containing NaCl (20%) after pretreatment with AR, TA, or nutrient broth alone, number of cells increased by 0.74, 0.7 and 1.1 logs, respectively (Table 3 and 4). *S. aureus* is one of the osmotolerant bacteria and can survive in high concentrations of sodium chloride.<sup>9</sup> However, pretreatment with AR or TA did not increase its tolerance to sodium chloride.

Similar results were obtained for pretreatment of *S. aureus* in fish homogenate containing AR, fish homogenate containing TA, or fish homogenate alone before exposure to ascorbic acid (0.5%), citric acid (0.3%), or NaCl (20%) (data not shown).

## CONCLUSION

Pretreatment of *E. coli* with AR or TA before exposure for 3 days to nutrient broth containing sodium chloride (20%) increased its resistance to sodium chloride (20%). Pretreatment of *S. aureus* with AR or TA before exposure for 3 days to nutrient broth containing citric acid (0.3%) increased its resistance to citric acid (0.3%). This has implications in food and pharmaceutical industries, where AR or TA is used as coloring agent and citric acid or sodium chloride as a preservative.

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None.

## FUNDING

None.

## DATA AVAILABILITY

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

## ETHICS STATEMENT

Not applicable.

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