Research Report

USING PROBIOTIC BACTERIA TO CREATE A NOVEL ORGANIC FLORAL PRESERVATIVE FOR ROSE

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INTRODUCTION

One of the main issues in cut flower postharvest is controlling microorganism growth. The importance of clean cutting instruments, buckets, and solutions are stressed in many general postharvest resources (Sacalis and Seals, 1993; Dole and Wilkins, 2005; Hunter, 2000; Armitage et al., 2004). Research has shown that bacterial growth in vase solutions can lead to stem vasculature blockage causing petal and leaf wilt, bent neck, or similar water stress related symptoms that reduce vase life (Put, 1990; Put, 1986; van Doorn et al., 1991; Zagory and Reid, 1986; de Witte and van Doorn, 1988). The effects of bacteria concentrations in vase solutions differ with cut flower species. Put and Jansen (1989) found cut *Rosa* 'Sonia' vase life was reduced by bacteria concentrations as low as 105 cfu·ml-1, while Jones and Hill (1993) found *Dianthus caryophyllus* 'Medea', *Iris*, *Alstroemeria*, and *Tulipa* to be tolerant to bacterial counts up to 108 cfu·ml-1. Therefore, the number of bacteria in the vase solution may not be the primary cause of wilting or shortened vase life in all cut flower species.

Controlling the species of bacteria present in the vase solution may be more important than just the concentration. Research on microbial growth in vase solutions has identified bacteria that decrease or have little effect on vase life (Jacob and Kim, 2010; van Doorn et al., 1991; Zagory and Reid, 1986, de Witte and van Doorn, 1988; Putt, 1986). Some bacterial species produce exopolysaccharides that can clog the vasculature (Put, 1990), enzymes that degrade plant tissue (Membre and Burlot, 1994), and hormones, such as ethylene, that accelerate senescence (van Doorn, et al., 1991). Several genera of bacteria have been identified from cut flowers, including: *Alcaligenes*, *Pseudomonas* (de Witte and van Doorn, 1988; Put, 1990), *Acetinobacter*, *Achromobacter*, *Bacillus*, *Chromobacterium*, *Citrobacter*, *Enterobacter*, and *Erwinia* (Put, 1990), but none of these studies have ever considered bacteria for improving vase life.

As the number of organic cut flower farms increases, the demand for an organic floral preservative that effectively controls microbial growth also increases. Commercial floral preservatives have three general components: an acidifier, a biocide, and a carbohydrate (sugar). Certified organic sources of the acidifier and carbohydrate are relatively easy, but a biocide is difficult. In our previous studies using *E. coli* K 12 in the vase solution of cut *Zinnia* stems we've shown vase life results similar to a commercial floral preservative. It is important to test this bacterium on different cut flower species to see if the results are species dependent. Rose was selected because it is the most commercially significant cut flower crop in the world.

OBJECTIVE

To evaluate the potential of two bacteria species, *Escherichia coli* K12 and *Pseudomonas fulva*, to be an organic vase solution probiotic for controlling microbial growth in postharvest solutions in order to meet the demand for an organic floral preservative. (Note: The original proposal was amended to include another bacteria species with potential.)

MATERIALS AND METHODS

Stems of three rose cultivars, 'Freedom', 'Mondial', and 'Orange Unique', were received from a commercial supplier and immediately unpacked, cut to 45 cm, labelled, and placed into the treatments outlined below. The experiment was an 8 X 2 factorial design of 16 treatments (8 treatments x 2 water types) with 3-1L vases per treatment and 4 stems per cultivar per vase resulting in 12 total stems per vase. Tap water was sterilized by autoclaving. Vases were randomized in the postharvest environment at 68 °F, 40-60% relative humidity under fluorescent lights for 12 h·d-1 at 20 µmol·m2·s-1 for postharvest evaluation.

Data collected included vase life of each stem, reasons for termination (bent neck, petal blueing, petal wilt, botrytis), initial and final vase solution pH and EC, initial and final fresh weights for one stem per cultivar per jar, solution uptake by weight for each vase when the first stem in the entire study was terminated, a visual vase solution turbidity (cloudiness) rating on a scale from 0-4 (0=clear, 4=very turbid), and solution bacteria quantification by serial dilution. Turbidity rating, bacteria quantification, and photos were taken every two days after the first stem in the entire study was terminated. Data were analyzed using analysis of variance (ANOVA) using general linear models (proc GLM) and means separated by Tukey's Studentized Range using SAS 9.3.

RESULTS AND DISCUSSION

The Floralife treatments had the greatest vase lives (12.8 and 10.8 d), but were not greatly different from the control in plain water (9.3 d), *E. coli* at 107 cfu·mL-1 in plain water (9.2 d), and *P. fulva* at 103 cfu·mL-1 in plain water (8.3 d) (Table 1). These differences in vase life among treatments were not found to be statistically significant, but they may be important to this study. There are some minor variations among cultivars, but trends are similar for each cultivar for the interactions presented above. In general, 'Mondial' and 'Freedom' had similar vase lives, which tended to last longer than 'Orange Unique'. The stem caliper of the 'Mondial' roses was quite large, which may have contributed to its long vase life.

For the treatments using sterilized tap water, vase solution turbidity increased over time. Once vases reached 4.0 on the 0-4 scale (0=clear, 4=very turbid) stems were soon terminated, which likely is correlated to bacteria concentrations which can be seen in Fig. 1. Differences among the vases could have been due to different bacteria species and numbers on the stems or debris falling into the water. Debris was removed as soon as it was noticed. Treatments 1 (103 cfu·mL-1 *E. coli* K12 in sterilized water) and 11 (107 cfu·mL-1 *P. fulva* in sterilized water) reached 4.0 very quickly and were some of the treatments with the lowest vase lives. Treatment 13 (Floralife vase solution in sterilized water) remained clear the entire length of the study due to the biocide in the commercial solution.

Turbidity ratings tended to be lower for treatments that used plain tap water and the flowers lasted longer. None of these treatments averaged higher than 3.5 on the 4.0 scale. Turbidities below 2.0 would be unlikely to be detectable by the general consumer as cloudy and undesirable. Treatment 2 (103 cfu·mL-1 *E. coli* K12 in plain water) reached a high turbidity quickly (by day 9) and was completely terminated before day 11. Bacteria concentrations can be seen for these treatments in Fig. 2.

There were no significant differences among treatments for termination criteria, change in fresh weight, change in vase solution pH and EC, and water uptake.

CONCLUSION

While none of the treatments performed equal to the Floralife vase solution, which contains several other components that contribute to its success, two of the bacteria treatments were similar enough to the tap water controls to warrant further experimentation. The Floralife vase solution has an acidic pH and carbohydrate source proven to improve the vase life of cut roses. In previous studies we have found that *E. coli* K12 can withstand a pH of 3.0 and have re-isolated *E. coli* from the solution and stems. In further studies we will alter the solution pH using organic methods (i.e. citric acid) and the addition of organic sugars. *E. coli* at the 107 cfu·mL-1 concentration and *P. fulva* at 103 cfu·mL-1 both in plain water have the most potential.

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