Progress on the biocontrol of food-borne pathogens on leafy greens with non-pathogenic microbes

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Outbreaks of food-borne illnesses

Produce	Outbreaks	Year	Pathogens
Frozen product	19 States (35 cases)	2013	*E. coli* O121
Cucumber	18 States (81 cases)	2013	*Salmonella* Saintpaul
Spinach & spring mix	5 States (33 cases)	2012	*E. coli* O157:H7
Raw clover sprouts	11 States (29 cases)	2012	*E. coli* O26
Cantaloupe	24 States (261 cases)	2012	*Salmonella* Typhimurium
Unidentified	9 States (18 cases)	2012	*E. Coli* O145 (STEC)
Romaine lettuce	10 States (60 cases)	2011	*E. coli* O157:H7
Cantaloupes	28 States (147 cases)	2011	*L. monocytogenes*
Cantaloupes	9 States (20 cases)	2011	*Salmonella* Panama
Alfalfa / spicy sprout	5 States (25 cases)	2011	*Salmonella* Entiritidis
Fresh Papaya (pawpaw)	25 States (106 cases)	2011	*Salmonella* Agona

Source: CDC, Atlanta, GA

Inhibition of the growth & survival of human pathogens by *Pseudomonas fluorescens*

Biocontrol	Targeted pathogen	Produce	References
P. fluorescens	*Salmonella enterica*	Alfalfa sprout	Fett 2006
P. fluorescens	*S. enterica* *Listeria monocytogenes* *Escherichia coli* O157:H7	Bell pepper	Liao & Fett, unpublished
P. fluorescens *P. chlororaphis*	*L. monocytogenes*	Endive	Carlin *et al.* 1996
P. fluorescens	*S. enteritidis* *E. coli* O157:H7	In *vitro* assays	Cheng *et al*. 1995
Microbial mix	*S. enterica*	Alfalfa sprout	Matos & Garland 2005

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Objectives

- To determine the efficacy of *P. fluorescens* for biocontrol of *E. coli* O157:H7 on spinach as a post-harvest intervention strategy.
- Evaluate the effects of storage temperatures and time on biocontrol efficacy on leafy green.
- Assess the inactivation of *Salmonella* serovars by *P. fluorescens* and *P. chlororaphis*.


WARING PRO
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COMMERCIAL
LABORATORY BLENDER


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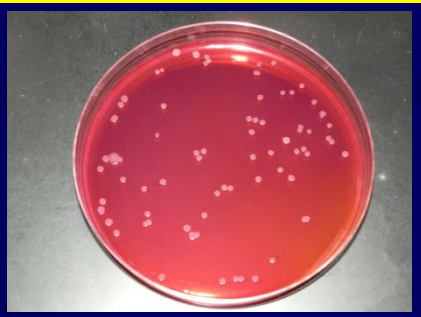
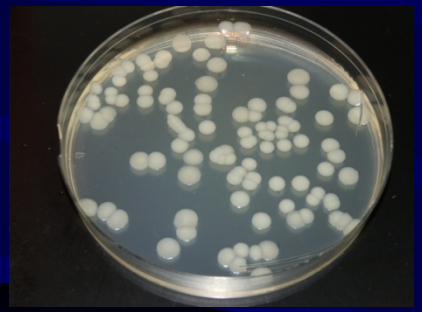
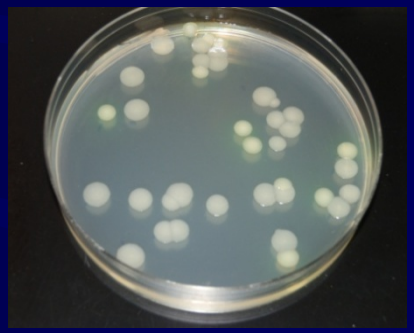
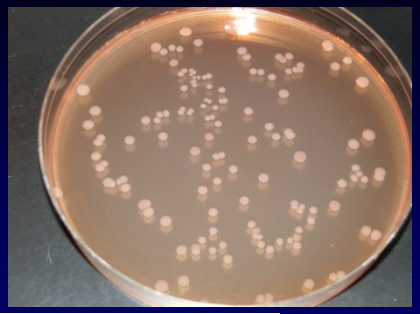
Vortex Genie 2

RESULTS

Progress in biocontrol of *E. coli* O157:H7 and *Salmonella* serovars on spinach and tomato

Recovery of *E. coli* O157:H7 (*Ec*) and *P. fluorescens* (*Pf*) and both bacteria (*Ec* & *Pf*) from broths and inoculated spinach

Ec	*Ec* and *Pf*	*Pf*	
			RFCM
			PAF
			CT-SMAC

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Efficacy of *P. fluorescens* on the reduction of *E. coli* O157:H7 (*Ec*) on spinach (20 ºC)

Treatment	24 Hrs	48 hrs
P. fluorescens (Pf) and *Ec*	Reduction of *Ec* (Log CFU/g)	Reduction of *EC* (Log CFU/g)
***Ec* 43894 + *Pf* 2-79**	0.95±0.45b	0.57±0.12a
***Ec* 43894 + *Pf* Q287**	2.10±0.00a	0.48±0.21a
***Ec* 43894 + *Pf* 2-79**	1.60±0.00ab	0.70±0.13a
***Ec* 43895 + *Pf* 2-79**	1.05±0.65b	0.70±0.02a
***Ec* 43895 + *Pf* Q287**	1.50±0.20ab	0.48±0.24a
***Ec* 43895 + *Pf* 2-79**	0.80±0.16b	0.53±0.11a

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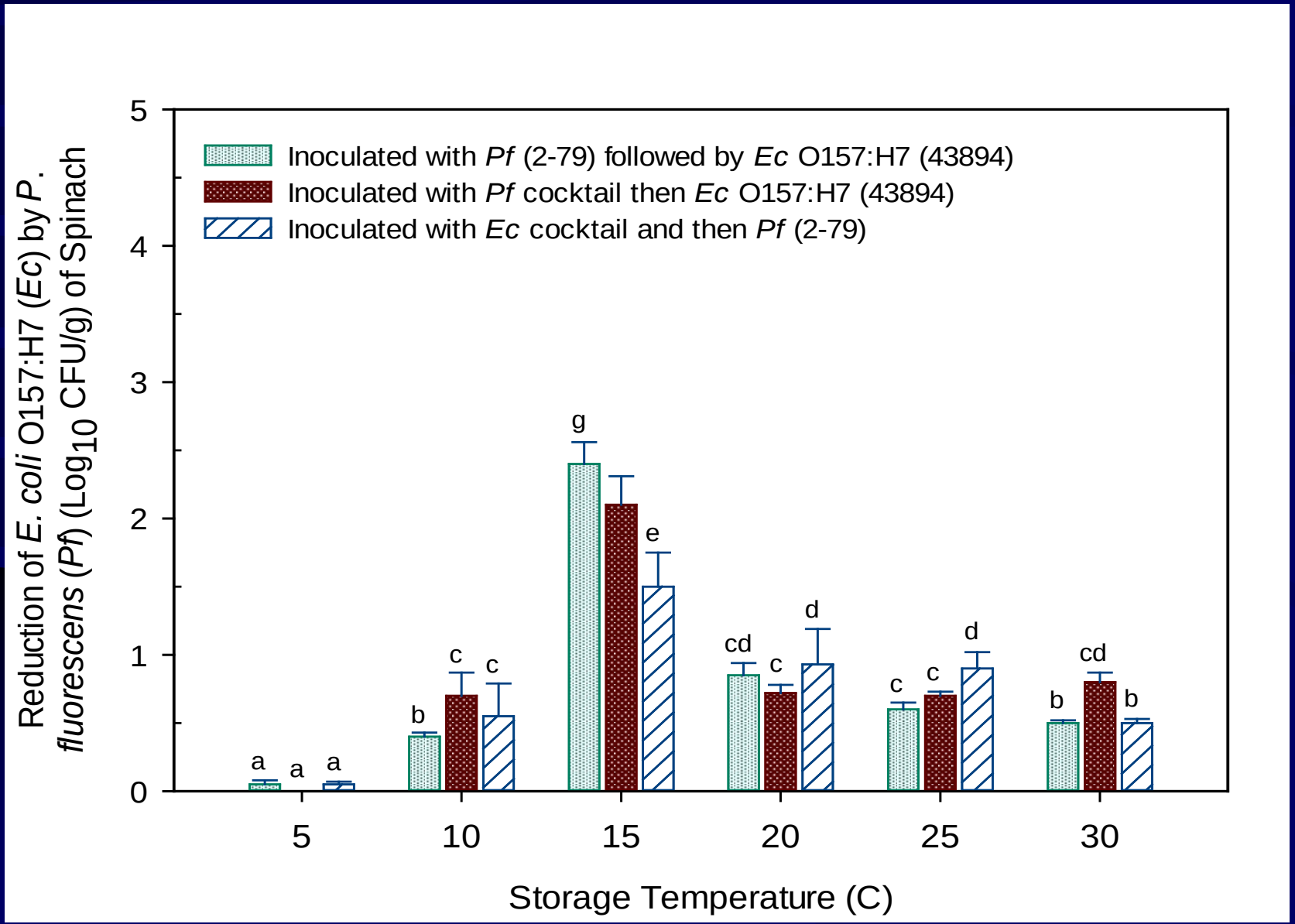
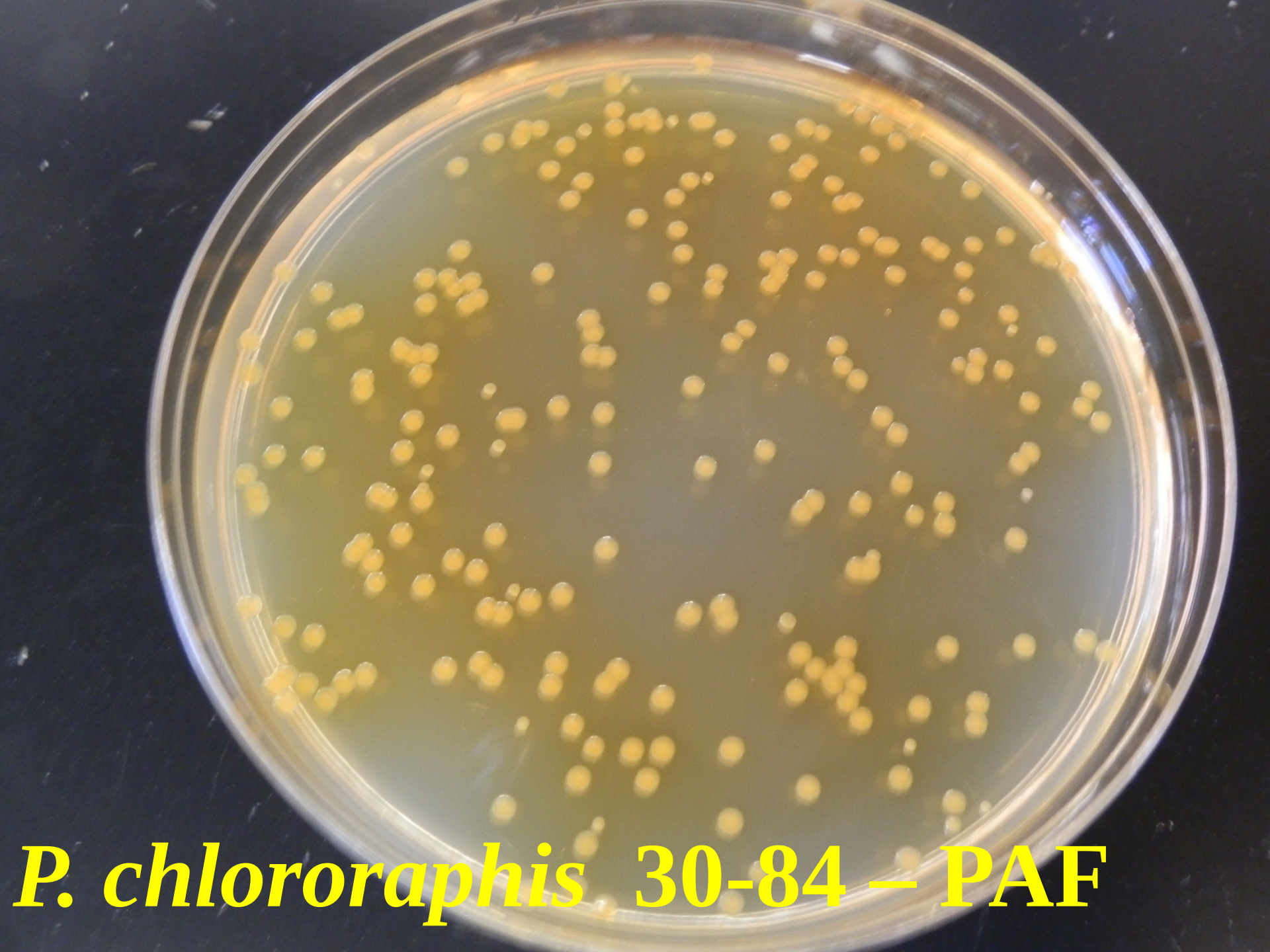
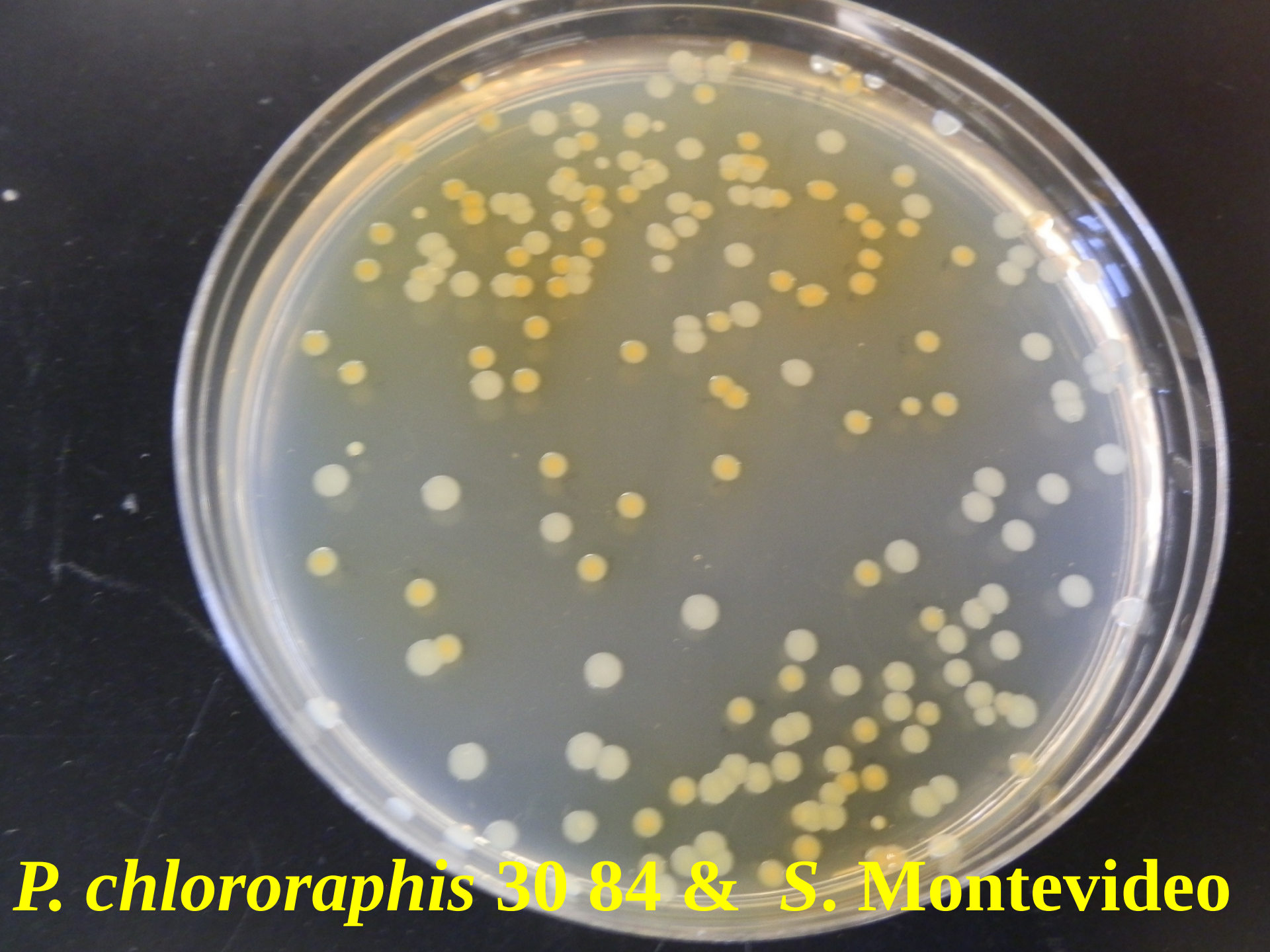
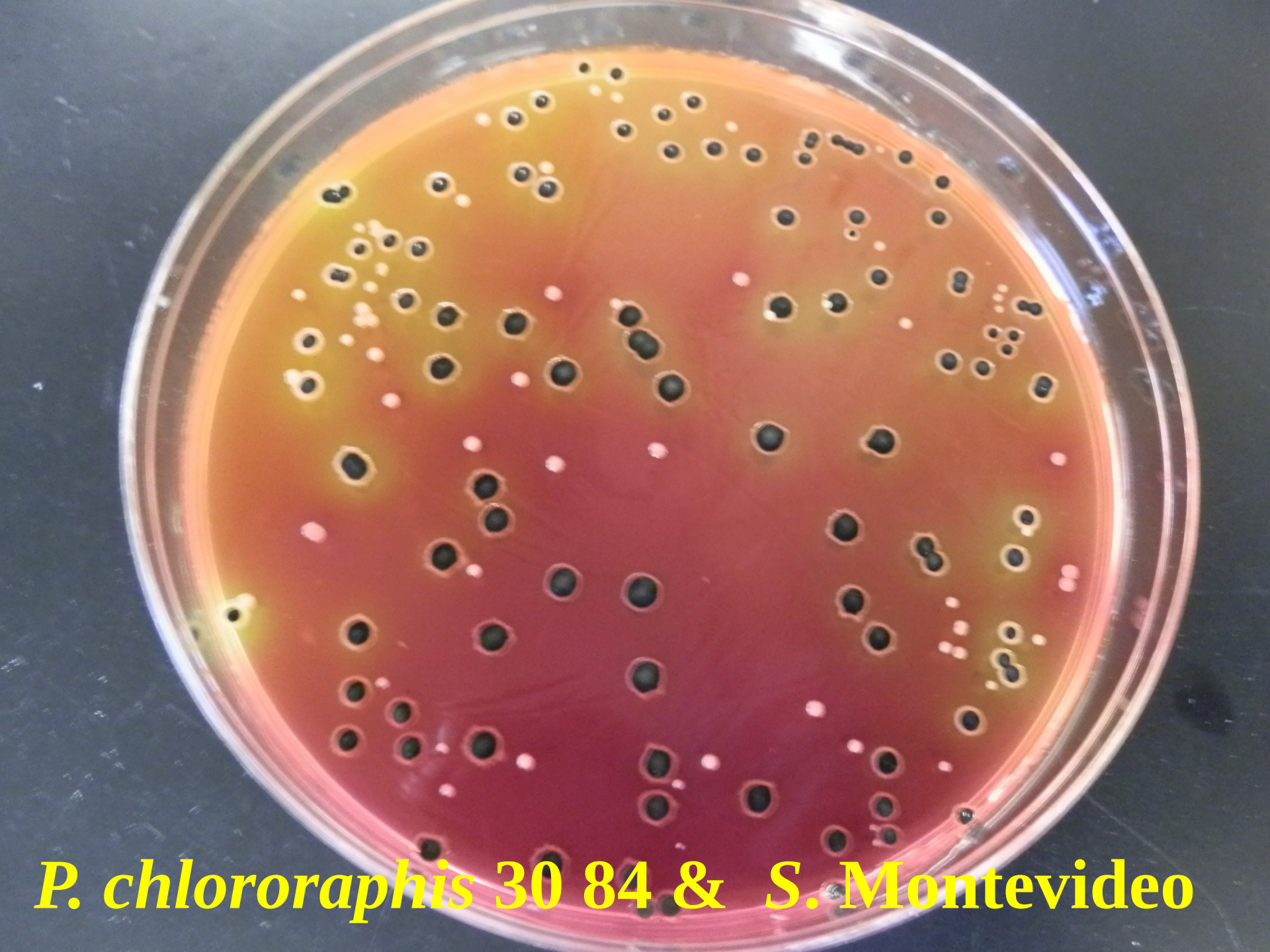
Biocontrol of *E. coli* O157:H7 (*Ec*) by *P. fluorescens* (*Pf*) at various temperatures




P. chlororaphis 30-84 – PAF


P. chlororaphis 30 84 & *S.* Montevideo


P. chlororaphis 30 84 & *S.* Montevideo

Reduction of *Salmonella* populations (Log Cfu/ml) by *P. fluorescens* (*Pf*) and *P. chlororaphis* in co-cultures

Salmonella serovars	*P. fluorescens* 2-79	*P. chlororaphis* 30-84	*P. chlororaphis* B-977
Salmonella **Typhimurium**	0.82a	0.60a	0.70a
Salmonella **Montevideo**	1.36a	0.69a	0.18a
Salmonella **Poona**	0.06b	0.47a	1.47b
Means	0.74	0.58	0.78
LSD **(0.05)**	0.55	0.47	0.62

Efficacy of *Pseudomonas* strains for biocontrol of *Salmonella* serovars on spot-inoculated tomatoes

Biocontrol applications	*Salmonella* Montevideo (Log CFU/g)	*Salmonella* Typhimurium (Log CFU/g)
P. chlororaphis 30-84	0.95±0.18a	1.35±0.24ab
P. chlororaphis B-997	0.74±0.12a	2.00±0.08a
P. fluorescens 2-79	0.90±0.11a	1.07±0.09ab
P. fluorescens Q2 87	0.53±0.13a	0.49±0.11b
P. *fluorescens* Q8 R1	0.51±0.10a	0.46±0.07b

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Summary

- Reduction of *E. coli* by *P. fluorescens* ranged from 0.5-2.1 log Cfu/g of spinach. Low to moderate reductions of *Ec* populations by *Pf* may be attributed to equal ratios of biocontrol to the pathogen .

- Efficacy of biocontrol was significantly ($P<0.05$) affected by storage temperatures. Suppressive effects were greater at 15 ºC (1.5-2.4 log Cfu/g) than at other temperatures (<0.93 log Cfu/g).

- Inoculation sequences affected the efficacy of biocontrol. Pre-emptive inoculations, simultaneous & post-pathogen inoculations imply competitive exclusion and others contributing to biocontrol efficacy.

Summary

- Pseudomonas strains (biocontrol agent) and *Salmonella* serovars were readily recovered and enumerated on PAF and XLT-4 media.
- Biocontrol of *Salmonella* serovars by *P. fluorescens* and *P. chlororaphis* varied in co-cultures.
- On tomatoes, mean reductions ranged from 0.25-2.00 log Cfu/g of produce (low to moderate).
- As a post-harvest intervention measure, the addition of competing bacteria such as *P. fluorescens* or *P. chlororaphis* may supplement physical and chemical measures on produce.

Acknowledgements

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