Combining biocontrol with chlorine dioxide and other intervention technologies for inactivation of foodborne pathogens on produce

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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

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Source: CDC, Atlanta, GA

Non-thermal Intervention Technologies for food safety

- Physical measures: Irradiation (gamma irradiation, UV), cold plasma.
- Biological control measures (Antagonistic microbes, competitive bacteria, predatory microbes e.g. *Bdellovibrio*, *Bacteriovorax* sp., and phages.
- Chemical intervention measures e.g. chlorine dioxide, electrolyzed water, ozone, hydrogen peroxide and others.

Objectives

- Assess the recovery of *E. coli* O157:H7 from spinach as well as *Salmonella*, and *Pseudomonas* strains from tomatoes.
- Determine the efficacy of *P. fluorescens* and *P. chlororaphis* for biocontrol of *Ec* on spinach and tomatoes.
- Assess the efficacy of chlorine dioxide and *Pseudomonas* strains on the survival of *Salmonella* serovars on tomatoes.

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Materials and methods

Bacterial strains

- *Escherichia coli* O157:H7 – ATCC strains 43894, 43895 & 35150 (USDA-ARS, ERRC).
- *Pseudomonas fluorescens* – strains 2-79, Q2-87, & Q8 R1 (non-pathogenic, non-pectolytic) – (USDA-ARS, Pullman).

Bacteriological media

- *E. coli* O157:H7 chromogenic medium (RFCM), Pseudomonas Agar F (PAF) & CT- SMAC.


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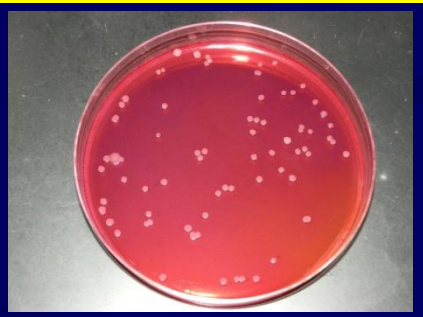
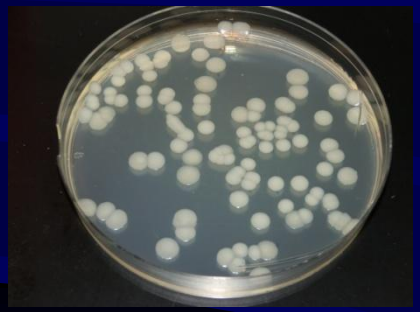
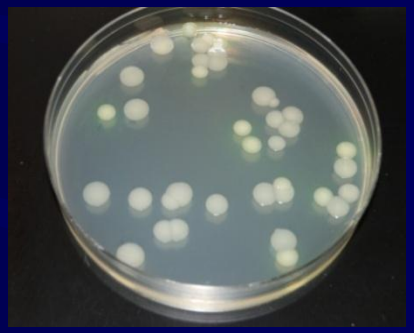
Vortex Genie 2

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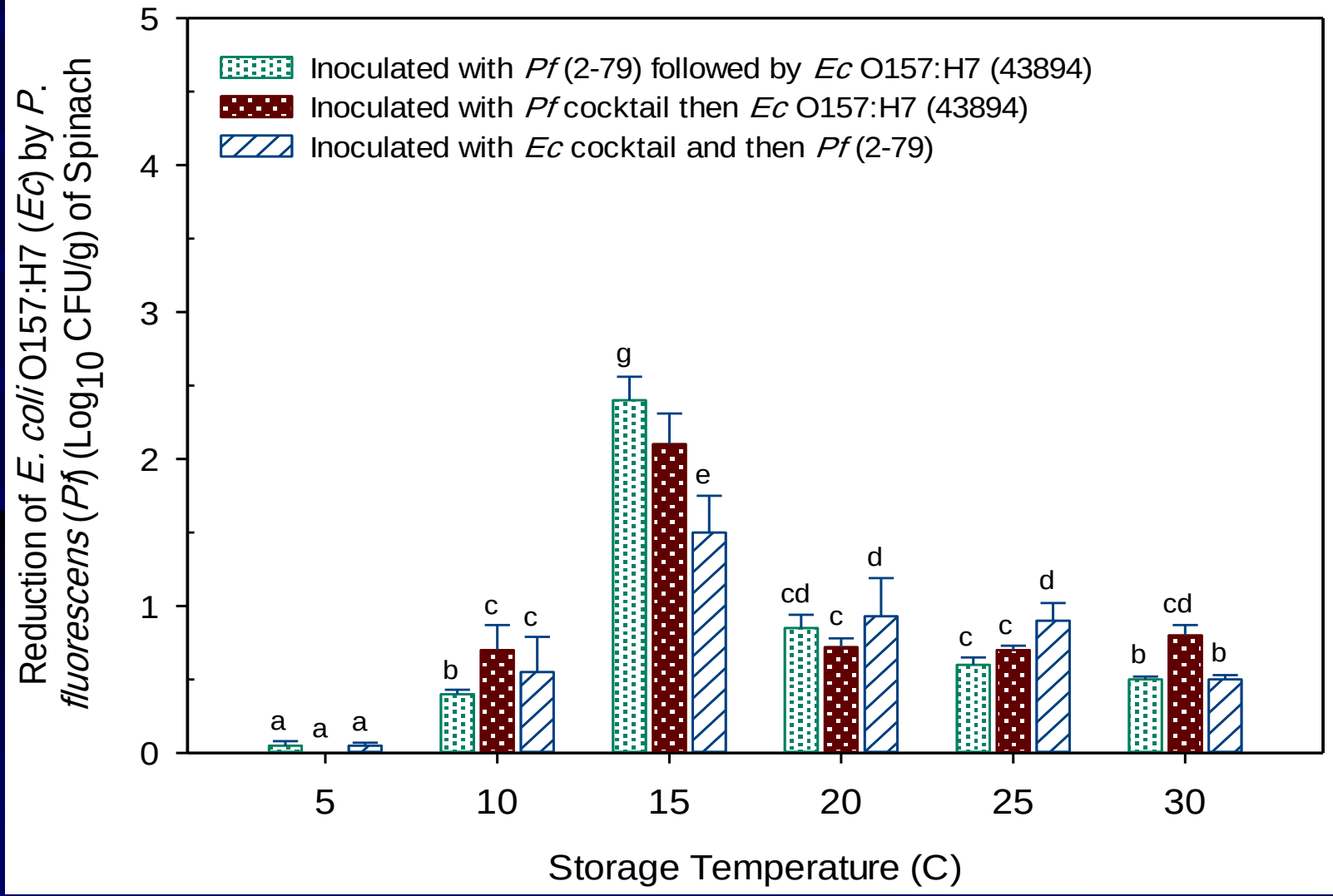
Biocontrol of *E. coli* O157:H7 (*Ec*) by *P. fluorescens* (*Pf*) at various temperatures



Summary

- Reduction of *E. coli* (Ec) 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 had no effect on biocontrol efficacy. Pre-emptive inoculations, simultaneous & post-pathogen inoculations imply pre-emptive and competitive exclusions contributing to biocontrol efficacy.

Efficacy of gaseous chlorine dioxide and *P. chlororaphis* on the survival of *Salmonella* serovars on tomatoes

Materials and methods

Bacterial strains

- *Salmonella* Montevideo – strain G4639 and *S.* Typhimurium – ATCC 2380, *P. chlororaphis*, 30-84.

- *P. chlororaphis* - B-997 (NRRL-Peoria), *P. fluorescens* – strains 2-79, Q2-87,Q8R1

Bacteriological media

- Pseudomonas Agar F (PAF) & XLT-4 Media.

Chlorine dioxide generator / Humidity chamber

- ClorDiSys Minidox-L gas generator (PA), Environmental RH /Temp Chamber (Thermal product solutions, PA).

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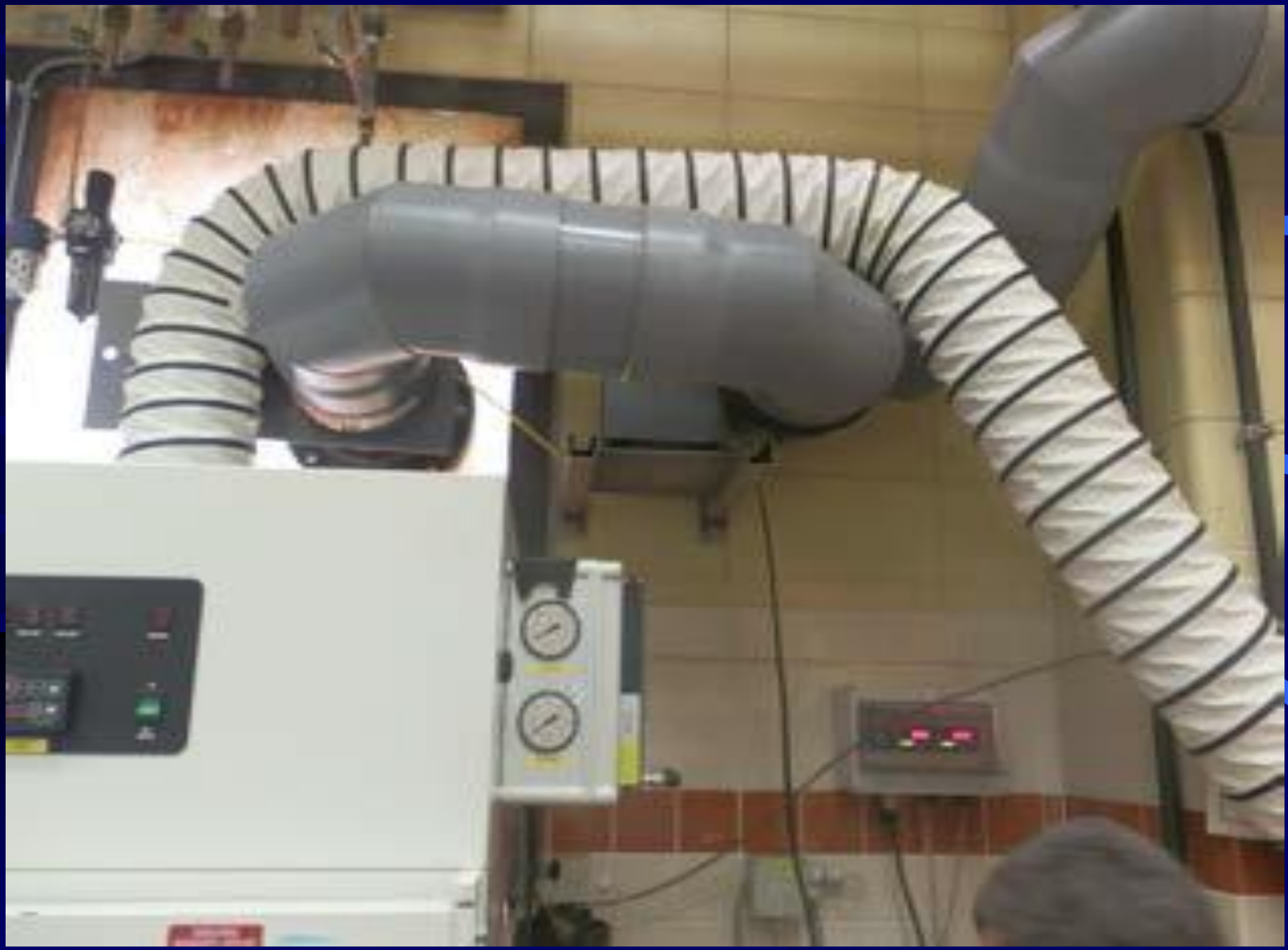


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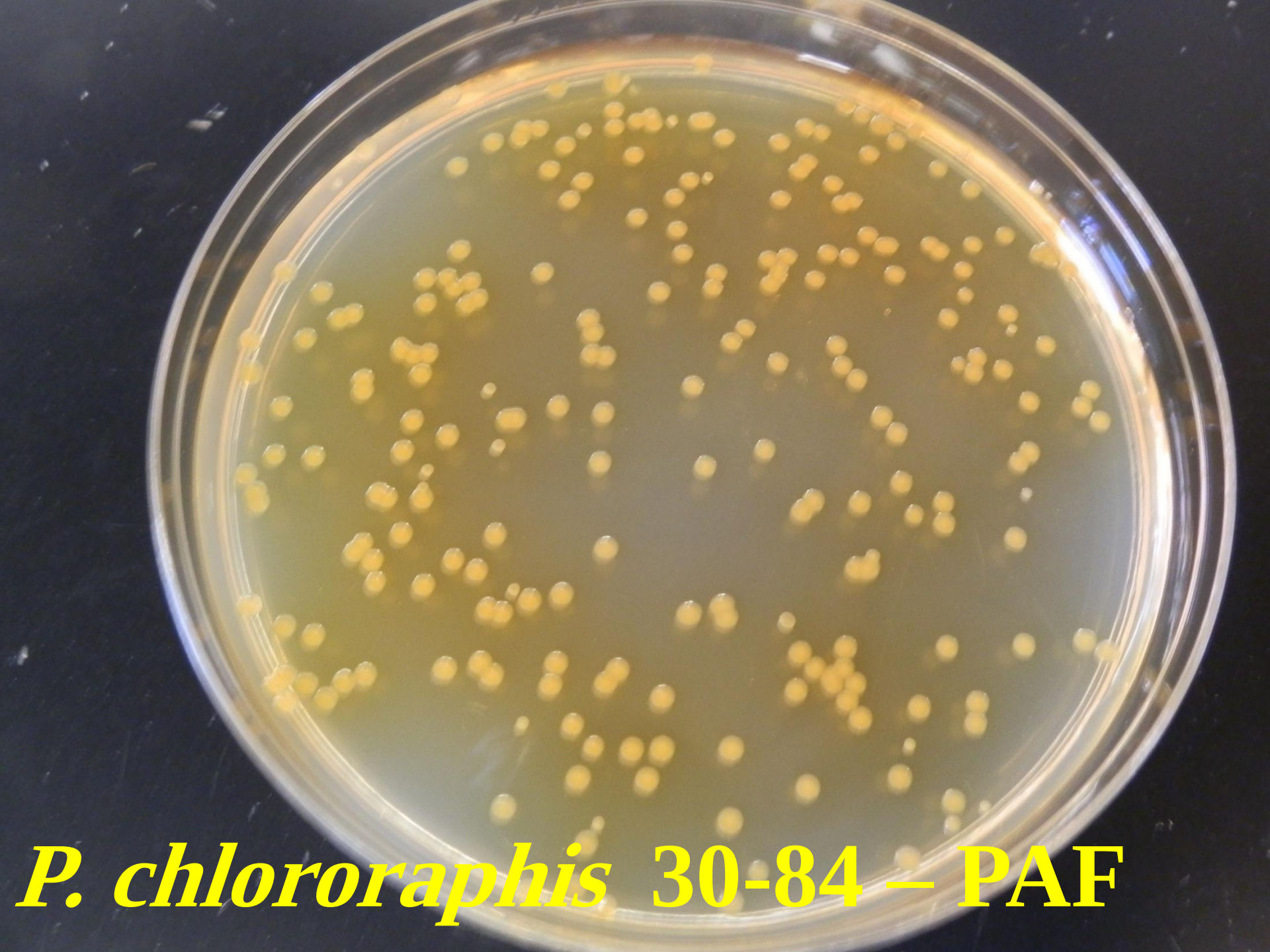
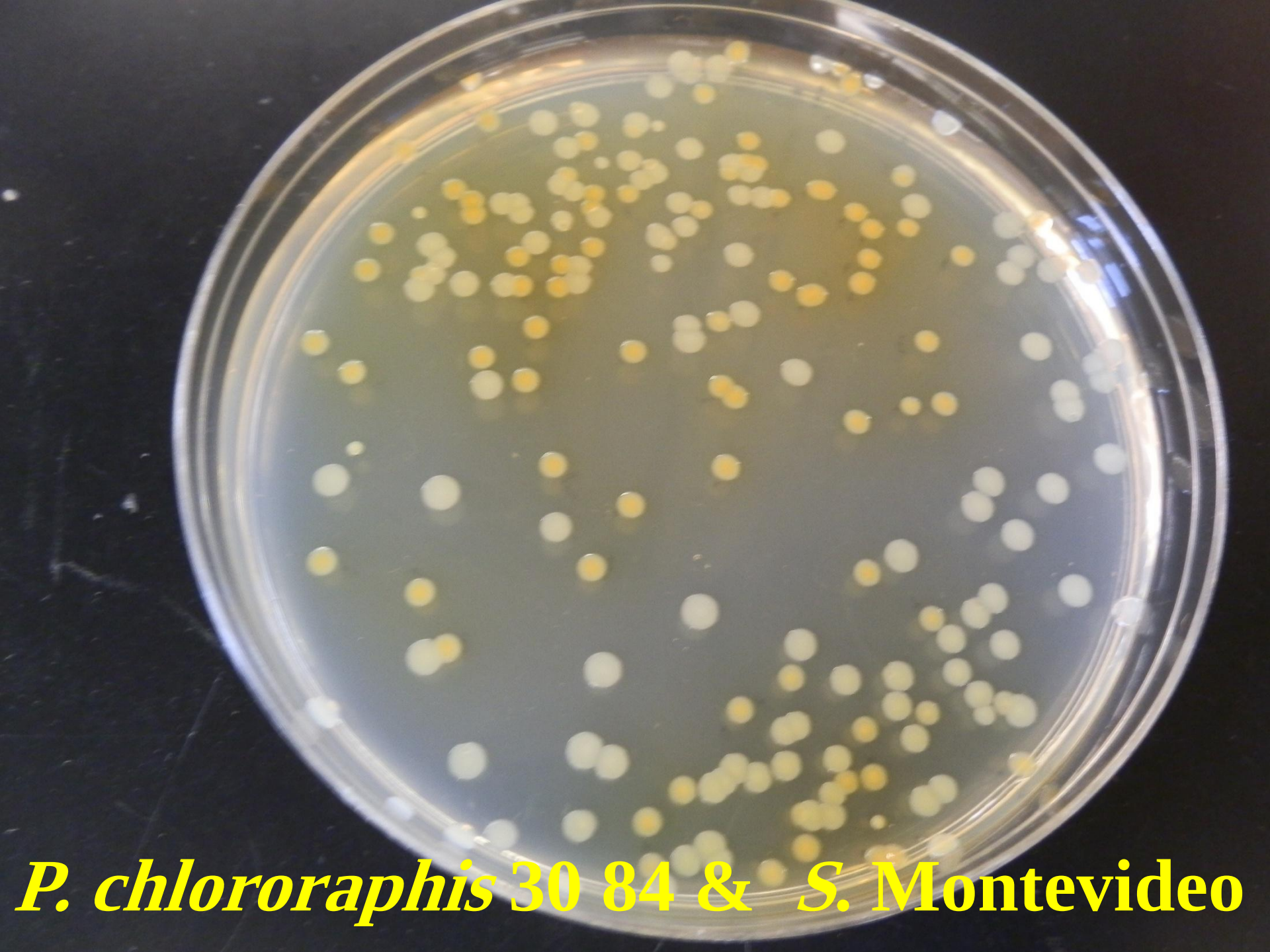
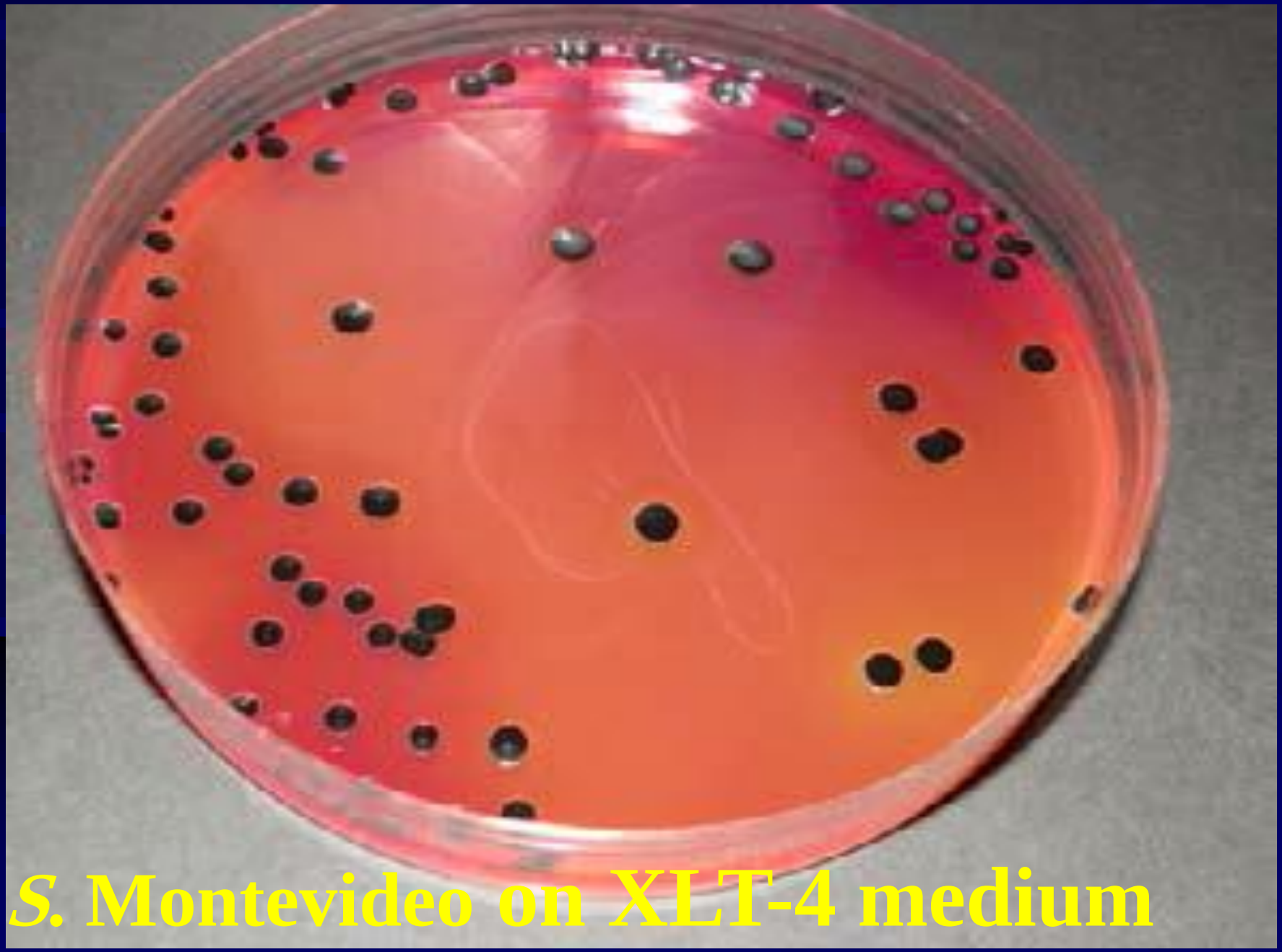
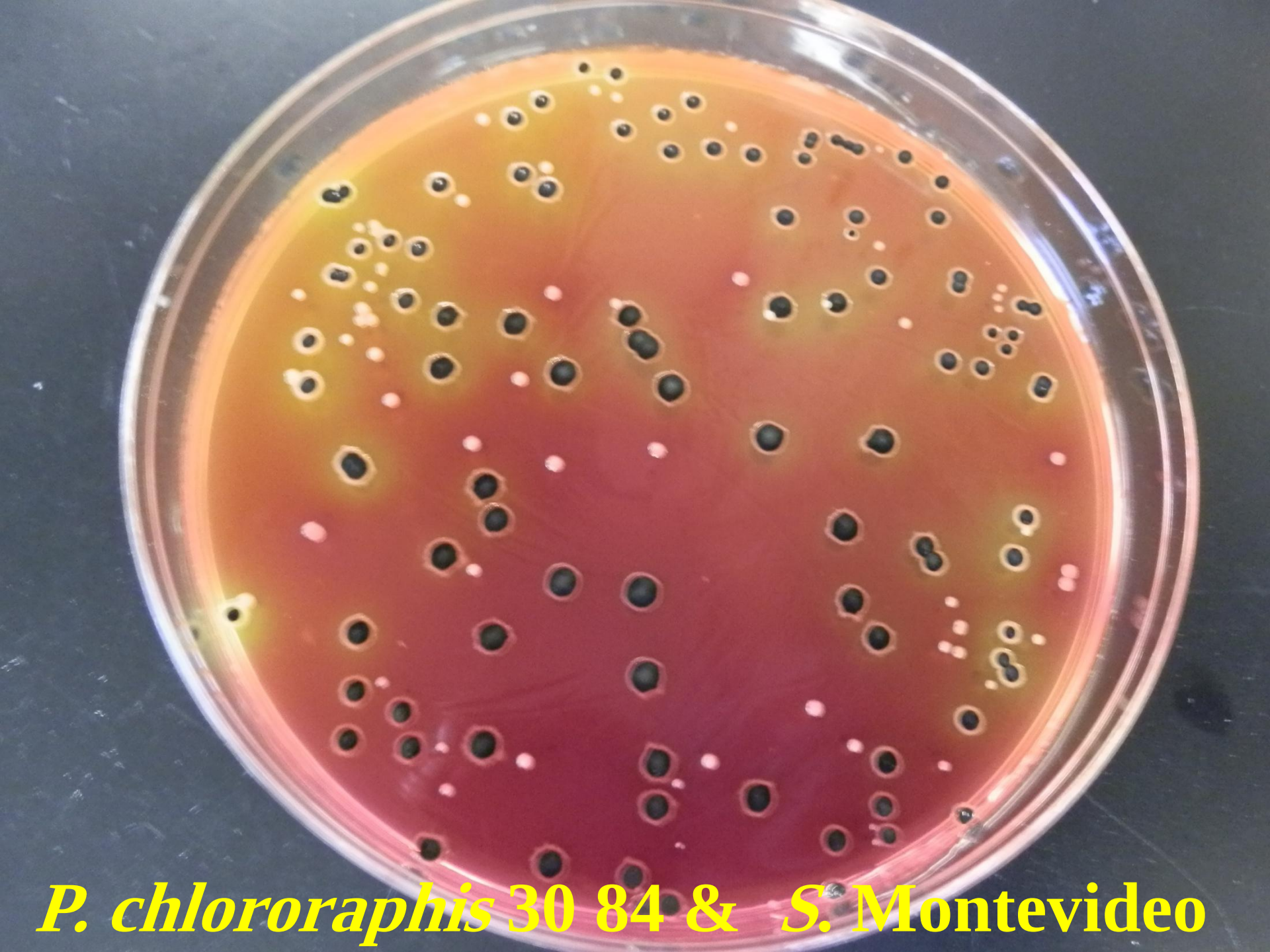
Vortex Genie 2


P. chlororaphis **30-84 – PAF**


P. chlororaphis 30 84 & *S.* Montevideo


S. Montevideo on XLT-4 medium


P. chlororaphis 30 84 & *S.* Montevideo

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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Effects of gaseous chlorine dioxide on the survival of *Salmonella enterica* Montevideo, Typhimurium, and *Pseudomonas chlororaphis* on tomatoes

Time* (Hrs)	Microbes	Untreated (control) (Log CFU/g)	Chlorine dioxide 0.4 mg/L (Log CFU/g)
2	*S.* Montevideo	5.27±0.39b	0.83±0.00a
	S. Typhimurium	5.63±0.2ab	< 0.30a
	P. chlororaphis	6.47±0.35a	3.41±1.10b
4	*S.* Montevideo	5.42±0.00b	<0.30a
	*S.*Typhimurium	5.37±0.15b	<0.30 a
	P. chlororapis	5.84±0.01b	2.59 ±1.46b

*Chlorine dioxide was applied at 0.4 mg/L, 90% R.H., and 13 °C.

Survival of *S. enterica* sequentially inoculated with *P. chlororaphis* on tomatoes and gassing with chlorine dioxide

Time* (Hrs)	Microbes	Untreated (control) (Log CFU/g)	Chlorine dioxide 0.4 mg/L (Log CFU/g)
2	*S.* Montevideo	5.17±0.34a	<0.30a
	S. Typhimurium	5.40±0.16a	<0.30a
4	*S.* Montevideo	3.77±0.01b	<0.30a
	S. Typhimurium	4.13±0.06b	<0.30a

*Chlorine dioxide was applied at 0.4 mg/L, 90% R.H., and 13 °C.

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Summary

- *Pseudomonas* strains (biocontrol agent) and *Salmonella* serovars were selectively recovered and enumerated on PAF and XLT-4 media when co-inoculated on tomatoes.
- Inoculum of *P. chlororaphis*, *S.* Montevideo and *S.* Typhimurium recovered from tomatoes were similar (4.5 log CFU/g)
- On tomatoes, mean reductions of *Salmonella* Montevideo and *S.* Typhimurium ranged from 0.51-0.95 and 0.46-2.00 log CFU/g of produce (low to moderate).
- The reductions of *Salmonella* serovars by *P. chlororaphis* were relatively higher than by *P. fluorescens* (biocontrol microbe).

Summary

- Gaseous chlorine dioxide (0.4 mg/L) reduced bacterial populations compared to the untreated control.

- The reductions of populations of *Salmonella* Montevideo and Typhimurium on tomato by chlorine dioxide (4 hrs) were in excess of 5 log CFU/g.

- *P. chlororaphis* was also significantly ($P<0.05$) reduced by chlorine dioxide application and suviving populations were 3.41 and 2.59 log CFU/g at 2 and 4 hrs of gaseous treatment.

Acknowledgements

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- For further questions: Dr. Modesto Olanya at e-mail: modesto.Olanya@ars.usda.gov