Phase 1 Evaluation of Jenfitch as an Antimicrobial Process-Aide for RAC Produce Wash Water

Interim Progress Data Overview

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Procedure: Trial 1

Rough-Surface Fruit (total n=505)

Inoculum preparation

- Inoculate with **Attn Sal** (PTVS 337) and **Path Sal cocktail** (PTVS 26, 42, 45 and 73)
- Let dry at room temp for 6 h
- Hold for 4 days @ 15 °C

Antimicrobial Asssessment

- Surface sanitation by immersion in chilled water (4°C)
- Disinfectants used
  - JC 9450/JC 9465 100 PPM (ideal 600/700 ORP, and pH 9)
  - PAA @ 12.5 and 37.5 PPM
  - HOCl @ 10 and 15 PPM, pH 6.5
- Submerge uninoculated (n=40) and inoculated (n=20) fruit.
- Remove 20 uninoculated and 10 inoculated fruit after 30 and 60 sec respectively.
- Recover post-storage population on 'unwashed control' fruit top verify starting challenge (n=5)

Recovery

- Fruit were placed individually in bags with 20 ml of potassium phosphate buffer amended with 0.05% tween and 15% sodium thiosulfate (antimicrobial neutralizer).
- Fruits were the hand massaged for 1 min then discarded.
- 1 ml aliquot of buffer was plated on CHROMagar Salmonella + rifampicin 80 PPM and incubated at 37 °C for 18 hr

Enrichment

- 20 ml of 2X buffered peptone water + rifampicin 80 PPM was added to the remaining buffer in all bags and incubated at 37 °C for 18 hr.
- Three 33.3 µl droplets (100 µl total) per sample were spot plated onto CHROMagar Salmonella + rifampicin 80 PPM and incubated at 37 °C for 18 hr.

Rough-fruit Surface disinfection and cross-contamination assessment

Disinfectant	Conc.	Residence time	n	log CFU/avocado	log reduction	% enrichment positives	Cross Contamination (n=20 per time point)
HOCl*	**Before**	**0 sec**	**5**	**4.33 ± 0.54**			
	10 PPM	30 sec	10	2.29 ± 0.87	2.04	100%	0%
		60 sec	10	1.63 ± 0.56	2.70	80%	0%
	15 PPM	30 sec	10	2.01 ± 0.60	2.32	90%	0%
		60 sec	10	1.66 ± 0.57	2.67	90%	0%
PAA*	**Before**	**0 sec**	**5**	**4.60 ± 0.47**			
	12.5 PPM	30 sec	10	1.67 ± 0.55	2.93	70%	**13%**
		60 sec	10	2.34 ± 1.04	2.26	80%	0%
	37.5 PPM	30 sec	10	1.88 ± 0.98	2.71	90%	0%
		60 sec	10	1.60 ± 0.79	3.00	80%	0%
Formulation Exp -10	**Before**	**0 sec**	**5**	**4.34 ± 0.37**			
	ATTN*, 100 PPM	30 sec	10	1.51 ± 0.49	2.83	80%	0%
		60 sec	10	1.84 ± 1.01	2.50	70%	0%
	Before	**0 sec**	**5**	**4.60 ± 0.31**			
	PATH¥, 100 PPM	30 sec	10	1.76 ± 0.63	2.84	90%	0%
		60 sec	10	1.90 ± 0.97	2.70	80%	0%
Jenfitch	**Before**	**0 sec**	**5**	**4.46 ± 0.50**			
	ATTN*, 100 PPM	30 sec	10	1.70 ± 0.49	2.76	50%	0%
		60 sec	10	1.29 ± 0.11	3.17	10%	0%
	PATH¥, 100 PPM	30 sec	10	1.38 ± 0.35	3.09	20%	0%
		60 sec	10	1.26 ± 0.00	3.21	**0%**	0%

* Attenuated Salmonella (PTVS 337)

¥ Pathogenic Salmonella Cocktail (PTVS 26, 42, 45, 73)

Water Parameters

Disinfectant	Target conc.		Actual conc. (PPM)		ORP (mV)	pH	Temp (°C)
HOCl	ATTN* LOW	10 PPM	Before	11.6	870	6.38	4.1
			After	11	881	6.68	4.4
	ATTN* HIGH	15 PPM	Before	17.1	950	6.42	3.8
			After	15.3	943	6.73	4.2
PAA	ATTN* LOW	12.5 PPM	Before	14.4	298	5.9	3.9
			After	13.1	287	5.7	4.0
	ATTN* HIGH	37.5 PPM	Before	38.2	301	6.2	4.1
			After	37.8	304	6.4	4.2
Formulation Exp -10	ATTN*, 100 PPM	100 PPM	Before	90	435	6.4	3.8
			After	90	440	6.3	4.1
	PATH¥, 100 PPM	100 PPM	Before	90	433	6.0	4.4
			After	90	457	6.1	4.6
Jenfitch	ATTN*, 100 PPM	100 PPM	Before	100	690	11.2	3.0
			After	100	700	11.6	3.5
	PATH¥, 100 PPM	100 PPM	Before	100	711	11.9	3.2
			After	100	714	11.9	4.0

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Melon Disinfection

Melon disinfection and cross contamination

Disinfectants	log CFU/melon circle			
	n	PATH CX€	ATTN SAL (PTVS 337)	ATTN SAL (isolate D)
Before	**10**	**5.36 ± 0.18**	**5.97 ± 0.18**	**5.57 ± 0.37**
HOCl	**10**	2.36 ± 1.04	5.73 ± 0.23	3.22 ± 0.45
PAA	**10**	2.06 ± 1.05	4.19 ± 0.66	2.66 ± 0.71
Exp-10	**10**	2.54 ± 1.20	5.83 ± 0.32	3.11 ± 0.78
Jenfitch	**10**	2.64 ± 1.13	3.08 ± 0.73	1.67 ± 0.56
Control	**10**	4.14 ± 0.30	5.91 ± 0.21	3.32 ± 0.26

Disinfectants	Cross contamintaion			
	n	PATH CX€	ATTN SAL (PTVS 337)	ATTN SAL (isolate D)
HOCl	**30**	100%	77%	77%
PAA	**30**	100%	100%	100%
Exp- 10	**30**	100%	77%	90%
Jenfitch	**30**	0%	0%	0%
Control	**30**	100%	100%	100%

€ Pathogenic Salmonella Cocktail (PTVS 26, 42, 45, 73)

Percentage decrease after disinfection

Disinfectants	Percentage Decrease		
	PATH CX	ATTN SAL (PTVS 337)	ATTN SAL (isolate D)
HOCl	99.90%	42.46%	99.55%
PAA	99.95%	98.34%	99.88%
Exp- 10	99.85%	27.56%	99.65%
Jenfitch	99.81%	99.87%	99.99%
Control	93.97%	12.90%	99.44%

Water parameter

PATH CX€

Disinfectant	Target conc.	Actual conc. (PPM)		ORP (mV)	pH	Temp (°C)
HOCl	15 PPM	Before	16.8	990	6.1	4.7
		After	14.9	1031	6.5	4.6
PAA	37.5 PPM	Before	38.4	313	5.4	4.2
		After	36.8	309	5.8	4.5
Exp- 10	100 PPM	Before	100	442	6.2	4
		After	100	432	6.6	4.4
Jenfitch	100 PPM	Before	100	694	10.1	4.3
		After	100	686	10.4	4.3
Water		Before		550	8.5	4.4
		After		543	8.7	4.6

ATTN SAL PTVS 337

Disinfectant	Target conc.	Actual conc. (PPM)		ORP (mV)	pH	Temp (°C)
HOCl	15 PPM	Before	16.3	864	6.1	4.0
		After	15.7	780	6.2	4.3
PAA	37.5 PPM	Before	36.6	312	6.2	3.0
		After	34.7	304	6.0	3.4
Exp-10	100 PPM	Before	102	450	5.2	3.2
		After	98	445	5.5	3.3
Jenfitch	100 PPM	Before	100	700	9.5	3.8
		After	100	707	9.8	4.0
Water		Before		540	8.4	4.0
		After		550	8.6	4.2

ATTN SAL - Isolate D

Disinfectant	Target conc.	Actual conc. (PPM)		ORP (mV)	pH	Temp (°C)
HOCl	15 PPM	Before	**16.3**	971	6	4.4
		After	**15.7**	1027	6.1	4.6
PAA	37.5 PPM	Before	**36.6**	290	6.2	4.0
		After	**34.7**	281	6.3	4.5
Exp - 10	100 PPM	Before	**102**	442	5.1	4.1
		After	**98**	447	5.7	4.2
Jenfitch	100 PPM	Before	**102**	680	10.8	5.4
		After	**98**	701	9.8	5.6
Water		Before		511	8.1	4.0
		After		498	8.2	4.4

€ Pathogenic Salmonella Cocktail (PTVS 26, 42, 45, 73)

Trial 2

Rough-Surface Fruit Treatment and Cross-contamination Assessment: Trial 2

Disinfectant	Concentration (PPM)		residence time	log CFU/avocado ± std dev	log reduction	% enrichment positives	Cross Contamination (n=20 per time point)
HOCl*	Before		0 sec	4.26 ± 0.58			
	PATH LOW	12.5	30 sec	1.72 ± 0.39	2.54	100%	0%
			60 sec	1.38 ± 0.27	2.88	60%	5%
	PATH HIGH	15	30 sec	1.67 ± 0.59	2.59	100%	0%
			60 sec	1.83 ± 0.79	2.43	90%	0%
PAA*	Before		0 sec	4.11 ± 0.31			
	PATH LOW	12.5	30 sec	1.45 ± 0.40	2.66	100%	0%
			60 sec	1.53 ± 0.31	2.58	70%	0%
	PATH HIGH	37.5	30 sec	1.46 ± 0.27	2.65	60%	0%
			60 sec	1.45 ± 0.33	2.66	80%	0%
Formulation Exp -10	Before		0 sec	4.30 ± 0.61			
	ATTN¥	450	30 sec	1.41 ± 0.38	2.74	50%	0%
			60 sec	1.42 ± 0.51	2.47	30%	0%
	Before		0 sec	4.20 ± 0.46			
	PATH	450	30 sec	1.34 ± 0.26	1.89	30%	0%
			60 sec	1.26 ± 0.02	2.24	70%	0%
Jenfitch	Before		0 sec	4.06 ± 0.22			
	ATTN¥	100	30 sec	1.32 ± 0.16	2.89	90%	0%
			60 sec	1.59 ± 0.57	2.88	80%	0%
	Before		0 sec	4.12 ± 0.15			
	PATH	100	30 sec	2.23 ± 0.89	2.86	100%	0%
			60 sec	1.88 ± 0.87	2.94	90%	0%

* Attenuated Salmonella (PTVS 337)

¥ Pathogenic Salmonella Cocktail (PTVS 26, 42, 45, 73)

Water Parameters

Antimicrobial	Target Concentration (PPM)		Measured conc.		ORP (mV)	pH	Temp (°C)
HOCl*	PATH LOW	12.5	Before	10	870	6.1	4.3
			After	8	863	6.3	4.2
	PATH HIGH	15	Before	16	910	5.9	4.9
			After	14	913	6.2	5.1
PAA*	PATH LOW	15	Before	16	313	6.4	3.1
			After	16	323	6.4	3.4
	PATH HIGH	37	Before	42.5	350	6.3	3.4
			After	41	355	6.4	3.5
Formulation EXP-10	ATTN¥	450	Before	450	505	4.0	3.3
			After	450	527	4.0	3.3
	PATH*		Before	450	518	4.1	3.0
			After	420	525	3.9	3.2
Jenfitch	ATTN¥	100	Before	100	954	5.8	3.9
			After	100	981	5.7	4.1
	PATH*		Before	100	1006	6.0	3.0
			After	100	1025	6.2	2.9

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¥ Attenuated Salmonella (PTVS 337)

Trial 2 Melon

- Data summaries in progress