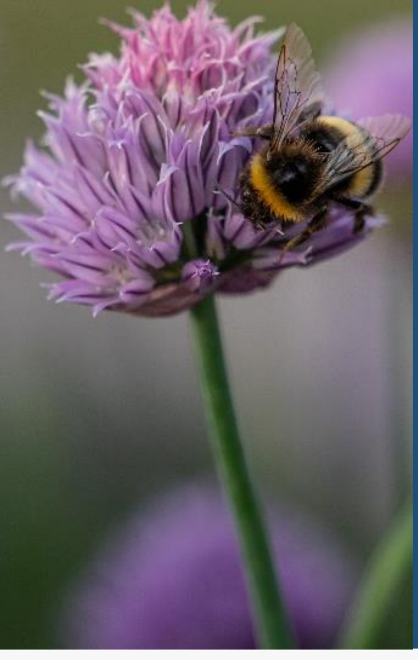

Bioactive Botanical Bacteria: Recovering Antimicrobial Producing Bacteria from Flowering Blossoms

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Introduction

Antimicrobial Resistance continues to threaten our ability to treat bacterial infections and new antibiotics are urgently needed to successfully combat disease. Natural product screening programmes account for most current antibiotics, but diminishing returns mean novel sample types need to be examined. Flower scents comprise volatile organic compounds (VOCs) that can deter insect herbivores and attract pollinators, with studies indicating that microorganisms may add to or alter floral scent profile (Peñuelas et al, 2014; Helletsgruber et al 2017). This study investigated flower-associated bacteria for antimicrobial activity against Gram-positive/negative species.

Materials & Methods

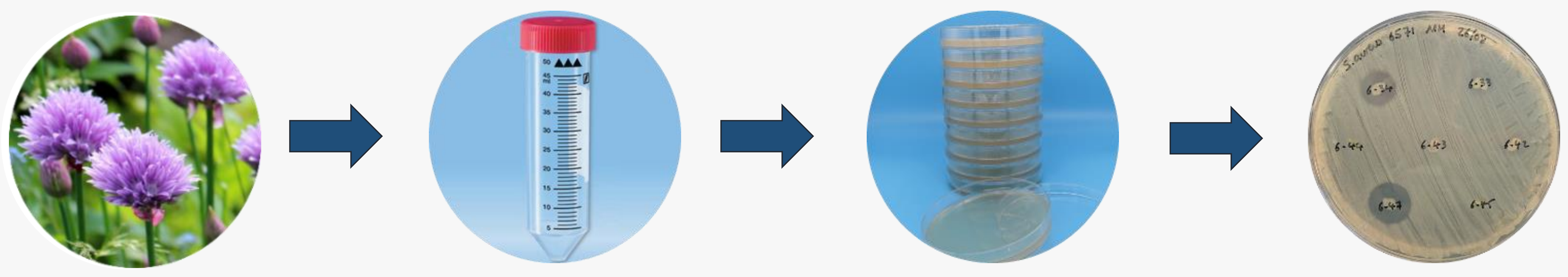
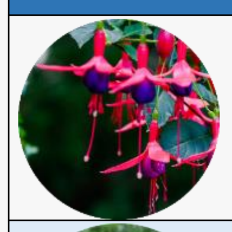
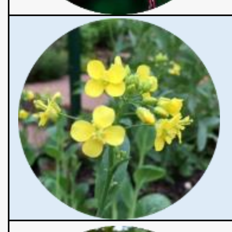
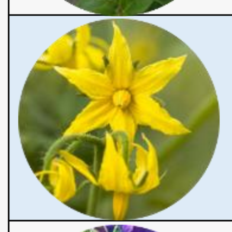
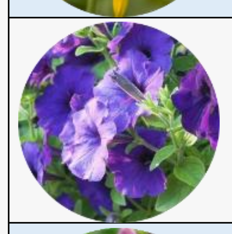
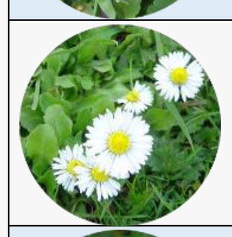
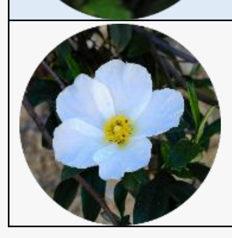


Figure 1. Recovery, culture and antibiotic testing of bacteria from blossoms.

Results

- 385 bacterial isolates were recovered from 11 flowering blossoms (table 1)
- Initial screening found 88 isolates with activity; 88 (100%) against *M. luteus*, 24 (27.3%) against *S. aureus* and 18 (20.4%) against *E. coli* (table 1)
- 5 of the 11 flowers sampled had >30% active isolates (table 1)
- Zones of inhibition ranged from 1-15mm (figure 2)

Table 1. Types of flower sampled, and number of isolates recovered/tested

	Sample #, Flower (Latin name)	# of Isolates	Active Isolates			% active
		Recovered	*M. luteus*	*S. aureus*	*E. coli*	isolates
	#1 Fuchsia (*Fuchsia magellanica*)	35	1	1	-	2.9%
	#2 Pak Choi (*Brassica rapa*)	2	1	1	1	50.0%
	#3 Honeysuckle (*Lonicera periclymenum*)	12	-	-	-	0%
	#4 Tomato (*Solanum lycopersicum*)	20	-	-	-	0%
	#5 Petunia (*Petunia hybrida summer ray*)	3	2	-	-	66.7%
	#6 Chives (*Allium schoenoprasum*)	49 *(2)	16	12	9	32.7%
	#7 Angel's Trumpet (*Brugmansia sp.*)	23 *(9)	3	1	1	13.0%
	#8 Pansy (*Viola tricolor*)	61	7	1	-	11.5%
	#9 Daisy (*Bellis perennis*)	72	28	2	1	38.9%
	#10 Buttercup (*Ranunculus acris*)	84	20	1	-	23.8%
	#11 Cistus (*Cistus ladanifer*)	24 *(2)	10	5	6	41.7%

*(#) number of additional fungal isolates recovered but discarded prior to testing

Results Continued

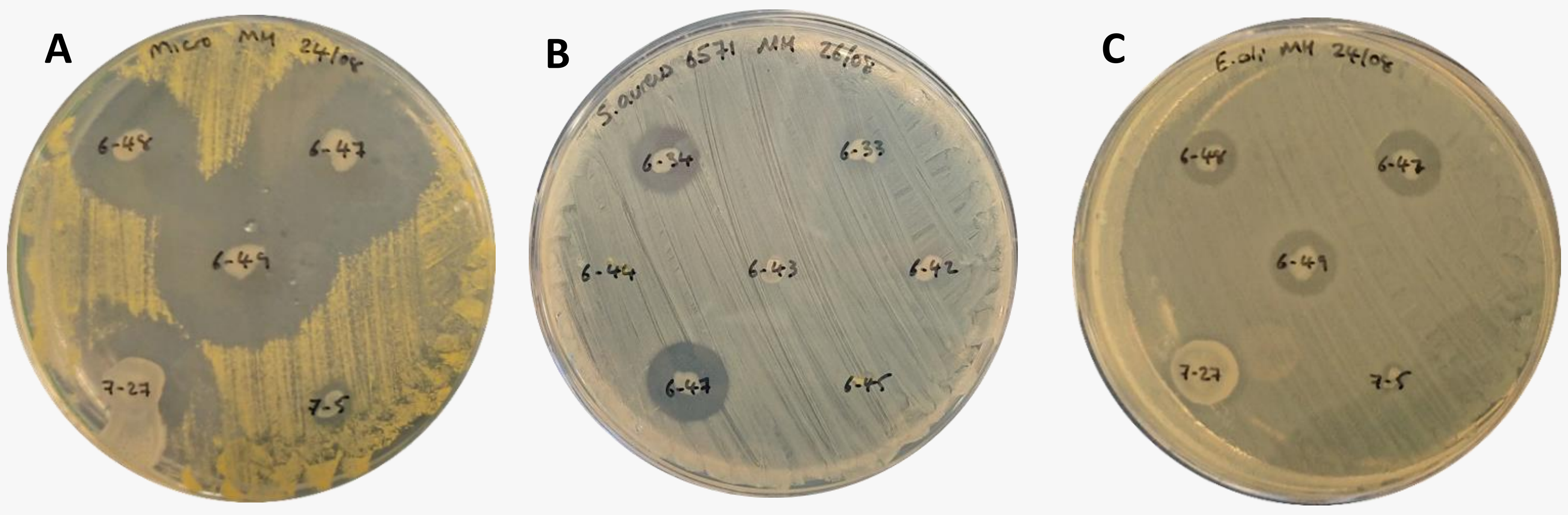


Figure 2. Selected isolates showing activity against *M. luteus* (A), *S. aureus* (B) and/or *E. coli* (C)

- Of the 34 isolates identified to genus level, 27 were G-ve (*Pseudomonas* x 23; *Erwinia* x 2; *Acinetobacter* x 1; *Pantoea* x 1) and 7 were G+ve (*Bacillus* x 6; *Staphylococcus* x 1)
- Isolates with activity against several other human pathogens where predominately recovered from the Chives and Cistus flowers (table 2)

Table 2. Select isolates with activity against the initial *M. luteus, S. aureus* and *E. coli* K12 isolates, plus several human pathogens (zones of inhibition in mm)

	G+ve			G-ve							
Isolate number	*M. luteus* DSMZ 20030	*S. aureus* NCTC 6571	*E. faecalis* NCIMB 775	*E. coli* K12	*E. coli* NCTC 11560	*S. flexneri* DSMZ 4782	*K. pneumonia* DSMZ 681	*A. baumannii* DSMZ 3007	*P. aeruginosa* NCIMB 10817	*C. albicans* NCPF 3179	16S rRNA Gene sequence (% identity)
6-9	10-11	3-5	-	2-3	-	5-6	4-5	-	-	-	*Pseudomonas* (100%)
6-11	12	2-4	-	-	-	3-4	3-4	-	-	-	*Pseudomonas* (100%)
6-13	11	2-3	-	-	-	2-8	2-4	-	-	-	*Pseudomonas* (100%)
6-14	11-13	5-6	<1	2-3	1-2	9-10	6-10	<1	-	<1	*Pseudomonas* (100%)
6-15	9-10	5-7	<1	4	2-3	8-9	6-9	<1	-	<1	*Pseudomonas* (100%)
10-73	5-8	5-8	<1	4-6	4-5	6-7	1	-	-	4	
11-13	8-10	3-6	-	1-2	4	6	1	-	-	1	*Bacillus* (100%)
11-18	7	1-5	-	5	1-4	2-6	-	-	-	1	*Bacillus* (100%)
11-19	4-5	1-4	-	5	2-4	2-6	-	-	-	1	
11-20	5	1-4	-	4	1-3	3-6	-	-	-	1	*Bacillus* (100%)
11-21	5	1-4	-	4	2-5	2-6	-	-	-	1	

Zone of inhibition is radius size in mm, measured at two different points, based on n =3

Discussion & Conclusions

- 22.9% of all isolates recovered were active against *M. luteus*, ~4-5 times greater than those active against *S. aureus* (6.2%) and *E. coli* K12 (4.7%) - suggesting *M. luteus* may not be a suitable indicator organism for screening
- *Pseudomonas* and *Bacillus* isolates from Chive and Cistus flowers showed activity against the greatest range of isolates, however 16S rRNA gene sequencing was not sufficient to discriminate down to species level
  - E.g., 100% identity to *Pseudomonas poae*, *trivialis*, *simiae* and *fluorescens*
- Activity shown by *Acinetobacter guillouiae* was a novel finding
- This is the first study to report on bacteria associated with flowers that show antimicrobial activity against several human pathogens, suggesting this is a potential novel sample type for further examination

References

- Helletsgruber C, Dötterl S, Ruprecht U, Junker RR. 2017. Epiphytic Bacteria Alter Floral Scent Emissions. J Chem Ecol. 43:1073–1077
- Peñuelas J, Farré-Armengol G, Llusia J, Gargallo-Garriga A, Rico L, Sardans J, Terradas J, Filella I. 2014. Removal of floral microbiota reduces floral terpene emissions. Sci Rep. 22;4:6727