Biosynthesis and Structure-Activity Relationship of Leudiazen, an Extracellular Chemical Signal

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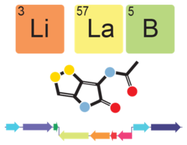

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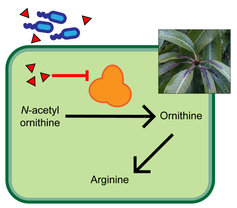
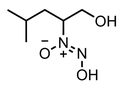
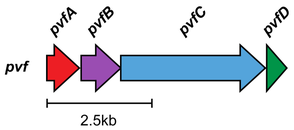
Leudiazen regulates virulence of plant pathogen



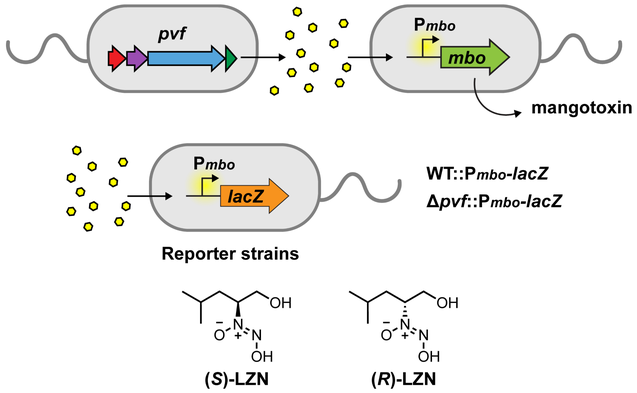
- *Pseudomonas syringae* strains infect many food crops worldwide[1,2]
- Mangotoxin produced by *P. syringae* pv. *syringae* (*Pss.*) contributes to necrosis of mango leaves[3]
- Leudiazen regulates mangotoxin production in *Pss.* UMAF0158 and is biosynthesized by *mangotoxin generating operon* (*mgo*),[1] also known as *Pseudomonas virulence factor* (*pvf*)

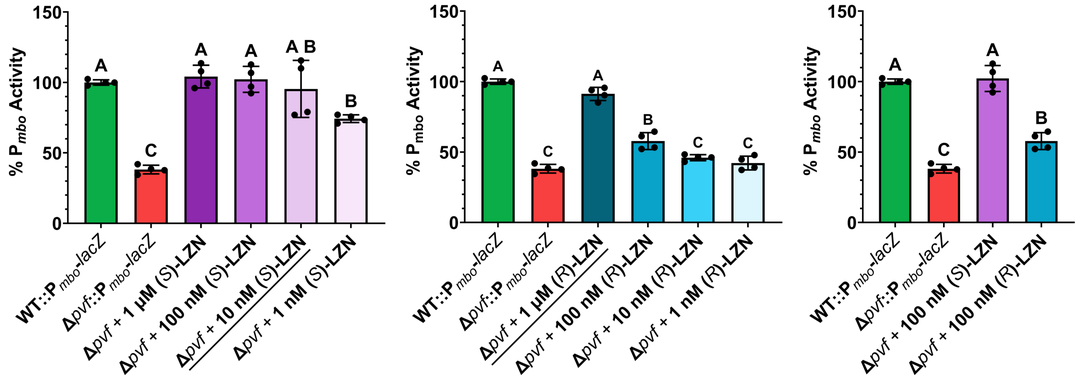


Leudiazen (LZN)



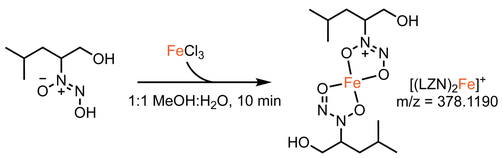
(*S*)-Leudiazen 100X more potent than (*R*)-leudiazen as virulence-regulating signal

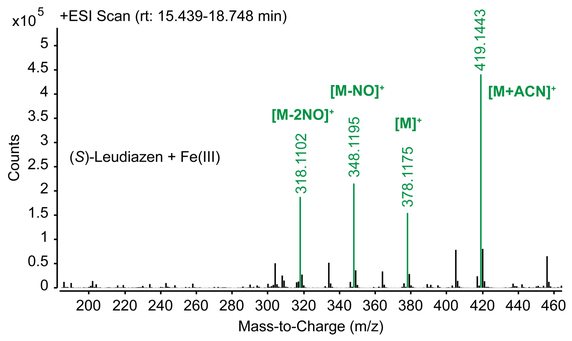


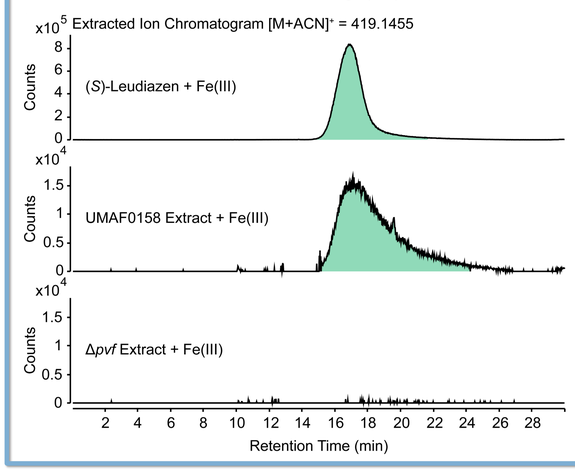


Mean ± SD (n = 4)
Letters denote significant differences (P < 0.05) by Tukey's Honestly Significant Difference Test

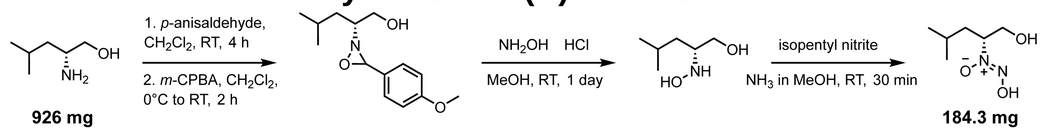
Iron(III) coordination enables detection of leudiazen







Synthesis of (*R*)-leudiazen



926 mg D-leucinol

184.3 mg (*R*)-leudiazen 14.4% yield

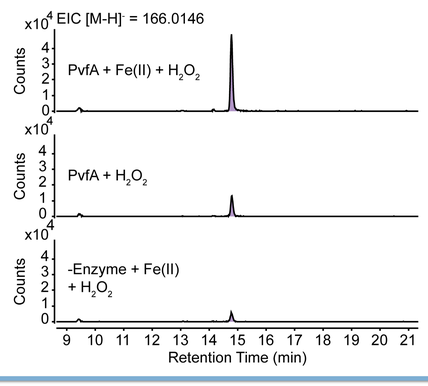
Sieber et al. (2021); Breuning et al. (2009)

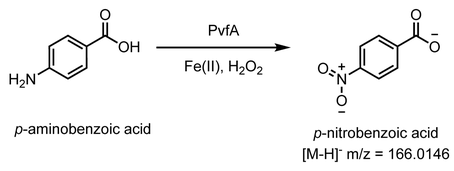
PvfA is a heme oxygenase-like domain containing oxidase (HDO)

X = Conserved iron-binding residue

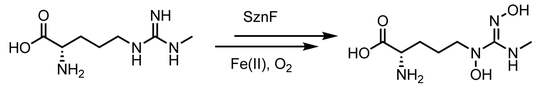
PvfA	73	L	I	N	**E**	I	V	L	G	E	E	S	D	D	R	L	D	H	G	H	Y	S	**H**	94	164	G	R	**E**	S	V	168	195	Q	R	**H**	I	E	V	**D**	S	E	D	**H**	205
AetD	68	I	I	N	K	H	T	Y	–	–	–	–	–	–	–	–	–	E	D	D	H	**H**	79	141	A	T	G	N	V	145	170	K	H	**H**	F	R	V	**E**	T	G	**H**	A	180
CADD	74	L	M	D	**E**	E	I	G	–	–	–	–	–	–	–	–	–	N	P	N	H	84	136	T	Y	**E**	S	Q	140	168	S	V	**H**	Q	D	V	**D**	V	R	**H**	A	178
UndA	94	L	M	R	**N**	I	R	V	–	–	–	–	–	–	–	–	–	E	L	N	H	104	157	A	I	**E**	G	A	161	192	K	M	**H**	A	Q	Y	**D**	D	A	**H**	P	202
FlcD	178	L	Y	G	**E**	L	G	E	E	D	E	–	–	–	–	–	–	K	G	S	H	191	242	I	T	**E**	L	V	246	272	K	V	**H**	I	S	L	V	P	P	R	A	282
FlcE	191	L	Y	G	**E**	A	G	A	E	T	P	–	–	–	–	–	–	E	R	S	H	204	255	A	V	**E**	S	V	259	285	R	L	**H**	G	T	Q	**D**	E	I	D	T	295
SznF	212	V	I	D	**E**	Y	G	Y	G	V	H	–	–	–	–	–	–	D	T	K	H	225	279	Y	T	**E**	S	S	283	309	T	E	**H**	I	H	I	**D**	Q	H	**H**	G	319
RohS	143	L	I	D	**E**	F	G	C	G	N	L	–	–	–	–	–	–	R	Q	A	H	156	210	Y	L	**E**	A	S	214	238	T	E	**H**	L	H	I	**D**	T	F	**H**	M	248
BesC	109	V	A	D	**E**	V	G	S	–	–	–	–	–	–	–	–	–	D	P	T	H	119	171	A	D	**E**	T	M	175	201	Q	L	**H**	I	D	V	**E**	V	G	**H**	S	211
BelK	202	Y	W	D	**E**	M	G	N	G	E	F	–	–	–	–	–	–	A	D	V	H	215	267	V	T	**E**	F	L	271	297	D	I	**H**	I	N	V	**D**	A	H	**H**	A	397

PvfA exhibits *N*-oxygenation activity



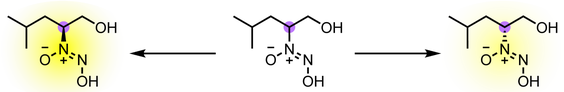


SznF, Another *N*-Oxygenating HDO[5]

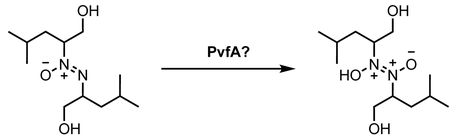


Future Directions

- Determine stereochemistry of biological leudiazen, using the difference in bioactivity of (*R*)- and (*S*)-leudiazen as a guide



- Determine how PvfA fits into the biosynthetic route to leudiazen and related diazeniumdiolate natural products



- Determine the role of PvfD in biosynthesis of the diazeniumdiolate in leudiazen and related natural products

Acknowledgments

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Former Members:
Dr. Qiang Guo

THE DAVID & LUCILE Packard Foundation

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