

Regulation of safracin biosynthesis and transport in *Pseudomonas poae* PMA22

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Figure S1. Hyper-production and purification of MexT regulator. A) Cloning of the *mexT* gene into the pET29a(+) expression vector. The pETmexT_F/R oligonucleotides were used to PCR-amplify the *mexT* gene. Plasmid pETmexT allows the expression of a MexT-(His)₆ fusion protein under the control of LacI^q in *E. coli* BL21 (DE3). B) SDS-PAGE gel (12.5%) of the purified fractions of MexT-(His)₆ fusion protein using a Ni-NTA affinity chromatography. Lane E: protein extract excluded on the affinity column. Lanes 20, 50, 100, 100, 250, 500 and 750 represent the fractions obtained after the successive washes of the affinity column by adding 20 mM to 750 mM imidazole to elute the MexT-(His)₆ protein.

Figure S2. A) Mass spectrometric identification of the chromatographic profile of safracin production in *P. poae* PMA22. The chromatogram corresponds to the strain grown on MS medium with 4% mannitol. The peaks indicated in the chromatogram were isolated by semi-preparative HPLC analysis and the chemical species were identified by mass spectrometry, as shown in the upper part of the figure. B) A comparative chromatogram in three blocks shows the effect of MexT and MexS on the efficiency of safracin production. The first chromatograms show the effect of MexT and MexS when overexpressed in *trans* in PMA22 strains. The second chromatogram shows the production compound profile of PMA22, PMA22Δ*mexT* and PMA22NfxC1 strains.

The quantification of the areas is detailed over the peaks previously identified by mass spectrometry.

Table S1.

Strains	Genotype/Phenotype	Reference
<i>Escherichia coli</i>		
DH10B	F ⁻ , $\Delta(ara-leu)7697$, $\Delta(lac)X74$, <i>galK</i> $\phi 80dlacZ\Delta M15$, <i>recA1</i> , <i>endA1</i> , <i>rpsL</i> , $\Delta(mrr-$ <i>hsdRMS-mcrBC), λ^-, <i>deoR</i>, <i>galU</i>, <i>nupG</i></i>	Invitrogen
BL21(DE3)	F ⁻ <i>ompT gal dcm lon hsdS_B(r_B-m_B⁻)</i> λ (DE3 [<i>lacI lacUV5-T7p07 ind1 sam7 nin5</i>]) [<i>malB</i> ⁺] _{K-12} (λ^S)	(Green et al., 2012)
S17-1 λ pir	Tp ^R , Sm ^R , <i>recA</i> , <i>thi</i> , <i>hsdRM</i> ⁺ , <i>RP4-2-</i> <i>Tc::Mu::Km</i> , <i>Tn7</i> , λ pir lysogenic	(de Lorenzo and Timmis, 1994)
<i>Pseudomonas poae</i>		
PMA22	Wild type	PharmaMar
PMA22NfxC1	PMA22 derivative coding a nonfunctional <i>mexS</i> gene due to a 14 bp deletion from position 237	This work
PMA22NfxC2	PMA22 derivative coding a nonfunctional <i>mexS</i> gene due to a 12 pb deletion from position 237	This work
PMA22NfxC3	PMA22 derivative coding a nonfunctional <i>mexS</i> gene due to an insertion of 32 bp at position 256	This work
PMA22 $\Delta mexT$	PMA22 derivative with a deletion on <i>mexT</i> gene	This work
PMA22 $\Delta mexEF-oprN$	PMA22 derivative with a deletion on <i>mexEF-oprN</i> genes	This work
PMA22:: <i>Ptac-mexEF-oprN</i>	PMA22 derivative carrying an insertion in the chromosome coding the MexEF-OprN efflux pump under the control of <i>P_{tac}</i> promoter	This work
PMA22 $\Delta mexEF-$ <i>oprN</i> :: <i>Ptac-mexEF-oprN</i>	PMA22 $\Delta mexEF-oprN$ derivative carrying an insertion in the chromosome coding the	This work

	MexEF-OprN efflux pump under the control of P_{tac} promoter	
PMC-3	PMA22 derivative safracin hyperproducer	PharmaMar

Table S2.

Plasmids	Description	References
pTnS-1	Amp ^R , <i>ori</i> -R6K, containing <i>tnsABC-tnsD</i> operon	(Choi et al., 2005)
pRK600	Cm ^R , <i>ori</i> -ColE1, RK2 Mob ⁺ , Tra ⁺	(Knudsen and Karlstrom, 1991)
pSEVA637	Gm ^R , <i>ori</i> -pBBR1, <i>gfp</i> .	https://seva-plasmids.com/
pPa	pSEVA637 derivative carrying the P_a promoter region of BGC <i>sac</i> from <i>P. poae</i> PMA22	This work
pPi	pSEVA637 derivative carrying the P_i promoter region of BGC <i>sac</i> from <i>P. poae</i> PMA22	This work
pSEVA254	Km ^R , <i>ori</i> -RSF1010, P_{trc}	https://seva-plasmids.com/
pmexT	pSEVA254 derivative containing <i>mexT</i> gene	This work
pmexS	pSEVA254 derivative containing <i>mexS</i> gene	This work
pmexEF-oprN	pSEVA254 derivative containing <i>mexEF-oprN</i> operon	This work
pET29a(+)	Km ^R , Cloning and expression vector, <i>ori</i> -ColE1, P_{T7}	Novagen
pET29mexT	pET29a(+) derivative containing the <i>mexT</i> gene	This work
pK18mobsacB	Km ^R , <i>ori</i> -ColE1, Mob ⁺ , <i>lacZα</i> , <i>sacB</i> ; vector for allelic exchange homologous recombination mutagenesis	(Schäfer et al., 1994)
pΔmexT	Km ^R , pK18mobsacB derivative containing fragments UP and DOWN flanking <i>mexT</i> gene,	This work
pΔmexEF-oprN	Km ^R , pK18mobsacB derivative containing fragments UP and DOWN flanking <i>mexEF-oprN</i> operon	This work

pBG		Km ^R , Gm ^R , <i>ori</i> -R6K, Tn7L and Tn7R extremes, BCD2–msfgfp fusion	Zobel et al., 2015
pBG_ <i>Ptac-mexEF-oprN</i>		Km ^R , Gm ^R , pBG derivative carrying <i>mexEF-oprN</i> operon flanked by Tn7L and Tn7R	This work
pCR-Blunt TOPO	II-	Km ^R , Neo ^R , PCR cloning vector, <i>ori</i> -pBR322	Thermofisher

Table S3.

Primer	Sequence ¹ (5'→3')	Use ²
mexT_F	GAATCTCTAGATGACCTAAGGAGGT AAATAATGAACCGTAACGACCTGC	Amplification of <i>mexT</i> for cloning in pSEVA254 (<i>Xba</i> I/ <i>Hind</i> III)
mexT_R	TTAGCAAGCTTTTAAAGGCTATCGGG GTCGC	
pETmexT_F	ATCGAACATATGATGAACCGTAACG ACCTGC	Amplification of <i>mexT</i> for cloning in pET29a(+) (<i>Nde</i> I/ <i>Hind</i> III)
pETmexT_R	GACTGGAAGCTTAAGGCTATCGGG TCGC	
mexS_F	GGCATAGAATCTGACCTAAGGAGG TAAATAATGTCCCGCACGATCCGTTT	Amplification of <i>mexS</i> for cloning in pSEVA254 (<i>Eco</i> RI/ <i>Hind</i> III)
mexS_R	ACCGATTAAAGCTTGGTTTGGAACT CCTTTGCG	
Pa_F	TTAGCATGCCTTGACTTTTAATCTGA ACGGGCA	Amplification of 220 pb covering <i>Pa</i> promoter for cloning in pSEVA637 (<i>Sph</i> I/ <i>Hind</i> III).
Pa_R	TTGACGTAAAGAAGCTTGCACC	

Pi_F	TTAGCATGCCCCGAAATGATGGAC AAGGA	Amplification of 240 pb covering <i>P_i</i> promoter for cloning in pSEVA637 (<i>Sph</i> I/ <i>Hind</i> III)
Pi_R	GGCAAGCTTTGAACATCCATATATCA ACAGTTATCC	
mexT_UP_F	TTAGAGAATTCTCAGCAGCGGCGTGT AGTGC	Amplification of ~600 pb fragment upstream and downstream <i>mexT</i> for cloning in pk18mobsacB (<i>Eco</i> RI/ <i>Bam</i> HI/ <i>Sph</i> I)
mexT_UP_R	TGAACTGGATCCCCTGGAAGAGTCA GCTTGCT	
mexT_DOWN_F	TGAACTGGATCCGACCCCGATAGCCT TTAATT	
mexT_DOWN_R	CGAATTGCATGCTGCTTTTCCGATTC AATACG	
mexS_Sec_F	GGTTACTTTGACGAGGTTTGCG	<i>mexS</i> sequencing
mexS_Sec_R	GGGTTTGGAACACTCCTTTGC	
ΔmexT_Sec_F	CAAATTGCCCAGGCTTGACC	<i>mexT</i> sequencing
ΔmexT_Sec_R	AGGTTTGCAGATGGGGATCG	
ΔmexEF-oprN_Sec_F	GTCTTTGGGGCTCAGGTCC	Cheking <i>mexEF-oprN</i> deletion
ΔmexEF- oprN_Sec_R	CGCAGCAAGGCAACCTCG	

¹Restriction sites are underlined. ²Restriction enzymes used for cloning are indicates in brackets.

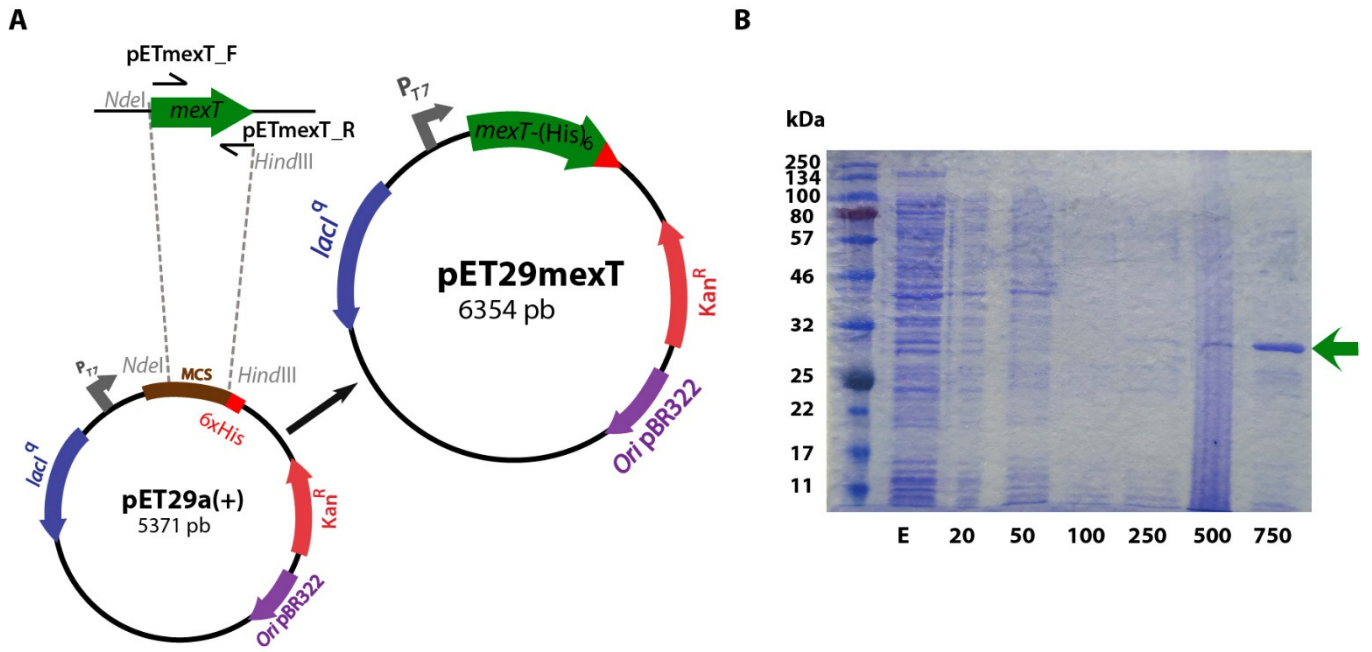
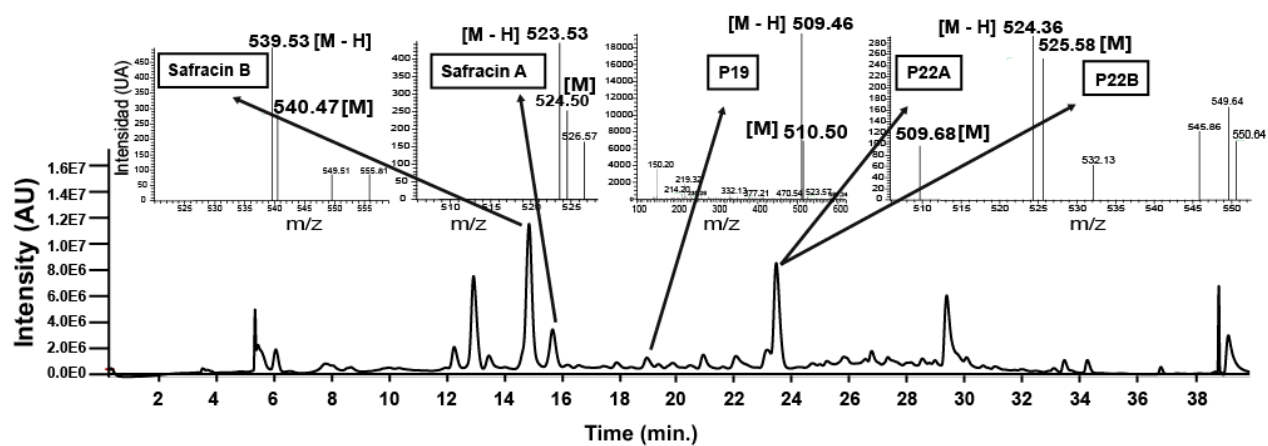


Fig S1.

A



B

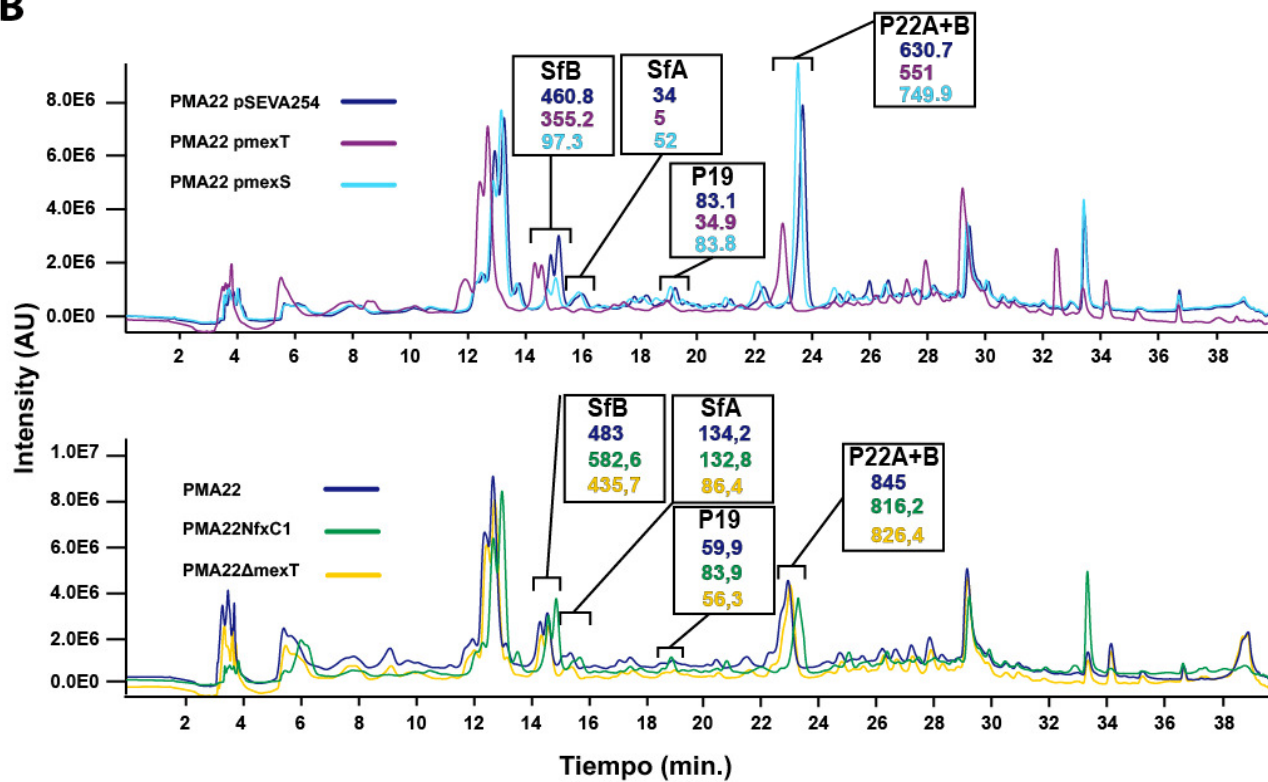


Figure S2.