

Figure S1. Physical map of plasmid pRFHU2-veA.

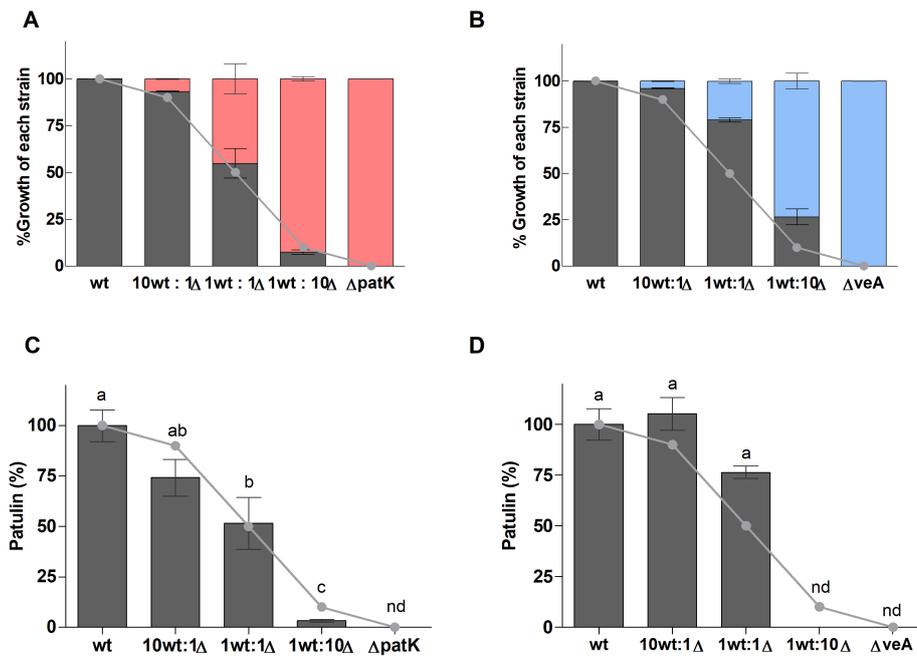


Figure S2. Competitiveness of $\Delta patK$ (A) (red bars) and ΔveA (B) (blue bars) knockout mutants against the patulin producer *P. expansum* (grey bars) on PDA after 4 days of incubation. Competitive assays were conducted at ratios of 10:0, 10:1, 1:1, 1:10, and 0:10 (WT vs. Δ). The percentage of each strain at 4 days post-inoculation was determined using qPCR. The expected values for the WT strain are represented by gray lines. Values represent the mean \pm the standard error of the mean of at least three biological replicates. Statistical analyses are presented in Table S3. Patulin production at 4 days post-inoculation during competition assays of WT vs. $\Delta patK$ (C) and ΔveA (D) knockout mutants analyzed by HPLC. Letters in the same panel show significant differences (one-way ANOVA and Tukey's HSD test, $p < 0.05$). nd—not detected during the testing conditions.

Table S1. List of primers used in this study.

Name	Sequence (5' → 3')	Description
PeveA mutant		
veA_O1	GGTCTTAAUTGACGTGGCTTCCAATACA	Amplification of upstream region
veA_O2	GGCATTAAUGGTGGTCTGTTTGCCATTTT	Amplification of upstream region
veA_A3	GGACTTAAUCACAATGGCGCAAATACAC	Amplification of downstream region
veA_A4	GGGTTTAAUATCATGGGCTCCGTAATCAG	Amplification of downstream region
veA-1F	ACAGCACAACCTCCTCCAAGG	Screening gene deletion; T-DNA copy number
veA-2R	TAGGGCTGGAGTAGCCTCAA	Screening gene deletion
veA-6R	GGCAGCTTGTATGCTAACT	Screening gene deletion and qPCR (<i>in vitro</i> competition assay) – screening gene of interest
veA – 7R	TATTTTGCCGGTTCGAGTAT	T-DNA copy number
veA-8F	CCGGCTGTATCGCAAGGTAT	Screening gene deletion
veA-5F	TGAAGATACTTTCGGCCTTG	qPCR (<i>in vitro</i> competition assay) – screening gene of interest
Other primers		
RF-1	AAATTTTGTGCTCACCGCCTGGAC	<i>E. coli</i> colonies selection and DNA sequencing
RF-2	TCTCCTGCATGCACCATTCCTTG	<i>E. coli</i> colonies selection and DNA sequencing
RF-5	GTTTGCAGGGCCATAGAC	<i>E. coli</i> colonies selection and DNA sequencing
RF-6	ACGCCAGGGTTTCCAGTC	<i>E. coli</i> colonies selection and DNA sequencing
HMBF1	CTGTGAGAAGTTTCTGATCG	Screening insertion resistant marker
HMBR1	CTGATAGAGTTGGTCAAGACC	Screening insertion-resistant marker
HPH3F	TATGTCTGCGGGTAAATAGCTG	qPCR (<i>in vitro</i> competition assay) – screening mutants
HPH4R	GAGATGCAATAGGTCAGGCTCTC	qPCR (<i>in vitro</i> competition assay) – screening mutants
PepatK_Li-F	GACGCTGGGCTACTGGATTG	qPCR (<i>in vitro</i> competition assay) – screening gene of interest (<i>patK</i>) (1)
PepatK_Li-R	TCGTGCGTGAGGCCAGTAT	qPCR (<i>in vitro</i> competition assay) – screening gene of interest (<i>patK</i>) (1)
Pe37-S	GCTCTGGTCTACGACTCCTC	37s ribosomal protein s24 as reference gene
Pe37-R	GGAAGCCTTCTCGATCTTGC	37s ribosomal protein s24 as reference gene
PeTub-1F	AGCGGTGACAAGTACGTTC	β-tubulin as protein as reference gene
PeTub-2R	ACCCTTGGCCAGTTGTTAC	β-tubulin as protein as reference gene

¹Li, B., Chen, Y., Zong, Y., Shang, Y., Zhang, Z., Xu, X., Wang, X., Long, M., & Tian, S. Dissection of patulin biosynthesis, spatial control and regulation mechanism in *Penicillium expansum*. *Environ. Microbiol.* **2019**, *21*(3), 1124–1139. <https://doi.org/10.1111/1462-2920.14542> [50]

Table S2. Statistical analysis of the results presented in Figure 5. A one-way analysis of variance (ANOVA) with Tukey's honestly significant difference (HSD) procedure was performed. Similar letters in the same sample/ experiments indicate that there are no statistically significant differences ($p < 0.05$). nd: non detected.

	Figure 5A		Figure 5B	
COUNTING	t0 WT vs $\Delta patK$		t0 WT vs ΔveA	
	WT exp.1	WT exp.2	WT exp.1	WT exp.2
wt	A	A	A	A
10wt:1 Δ	A	A	A	A
1wt:1 Δ	B	B	B	B
1wt:10 Δ	B	C	C	B
Δ	nd	nd	nd	nd
	t0 WT vs $\Delta patK$		t0 WT vs ΔveA	
	$\Delta patK$ exp.1	$\Delta patK$ exp.2	ΔveA exp.1	ΔveA exp.2
wt	nd	nd	nd	nd
10wt:1 Δ	B	C	C	B
1wt:1 Δ	A	B	B	A
1wt:10 Δ	A	A	A	A
Δ	A	A	A	A
	Figure 5C		Figure 5D	
COUNTING	t7 WT vs $\Delta patK$		t7 WT vs ΔveA	
	WT exp.1	WT exp.2	WT exp.1	WT exp.2
wt	A	A	A	A
10wt:1 Δ	A	A	A	AB
1wt:1 Δ	B	B	A	B
1wt:10 Δ	C	C	B	C
Δ	nd	nd	nd	nd
	t7 WT vs $\Delta patK$		t7 WT vs ΔveA	
	$\Delta patK$ exp.1	$\Delta patK$ exp.2	ΔveA exp.1	ΔveA exp.2
wt	nd	nd	nd	nd
10wt:1 Δ	C	D	C	C
1wt:1 Δ	B	C	C	C
1wt:10 Δ	A	B	B	B
Δ	A	A	A	A
	Figure 5E		Figure 5F	
qPCR	t7 WT vs $\Delta patK$		t7 WT vs ΔveA	
	WT exp.1	WT exp.2	WT exp.1	WT exp.2
wt	A	A	A	A
10wt:1 Δ	A	A	A	AB
1wt:1 Δ	B	B	B	B
1wt:10 Δ	C	C	C	C
Δ	nd	nd	nd	nd
	t7 WT vs $\Delta patK$		t7 WT vs ΔveA	
	$\Delta patK$ exp.1	$\Delta patK$ exp.2	ΔveA exp.1	ΔveA exp.2
wt	nd	nd	nd	nd
10wt:1 Δ	C	C	D	C
1wt:1 Δ	B	B	C	C
1wt:10 Δ	A	A	B	B
Δ	A	A	A	A

Table S3. Statistical analysis of the results presented in Figure S2. A one-way analysis of variance (ANOVA) with Tukey's honestly significant difference (HSD) procedure was performed. Similar letters in the same sample/ experiments indicate that there are no statistically significant differences ($p < 0.05$). nd: non detected.

	Figure S2A	Figure S2B
qPCR	t4 WT vs $\Delta patK$	t4 WT vs ΔveA
	WT exp.1	WT exp.1
wt	A	A
10wt:1Δ	A	A
1wt:1Δ	B	B
1wt:10Δ	C	C
Δ	nd	nd
	t4 WT vs $\Delta patK$	t4 WT vs ΔveA
	$\Delta patK$ exp.1	ΔveA exp.1
wt	nd	nd
10wt:1Δ	C	D
1wt:1Δ	B	C
1wt:10Δ	A	B
Δ	A	A