Method Details

Bacterial strains, plasmids, media, and conjugations

Strains, plasmids, and oligonucleotides used in this study are listed in Table S3. *Escherichia coli* strain DH5α was used for plasmid and cosmid propagation. Disruption cosmids were generated using *E. coli* strain BW25113 (Datsenko and Wanner, 2000) carrying a λ RED plasmid, pIJ790. The *dam dsm hsdS E. coli* strain ET12576 containing pUZ8002 (Paget et al., 1999) was used to conjugate cosmids and plasmids into *S. venezuelae* (Kieser et al., 2000, Bibb et al., 2012). *E. coli* strains were grown on LB or LB agar at 37°C. *S. venezuelae* strains were typically grown in liquid or solid MYM media supplemented with trace element solution (Bibb et al., 2012) but the hypersporulation phenotype was examined on Difco Nutrient Agar (DNA) or in Difco Nutrient Broth (DNB), where this phenotype is strongest. Where required for selection, the following antibiotics were added to growth media: 50 μg/mL apramycin, 100 μg/mL carbenicillin, 25 μg/mL chloramphenicol, 25 μg/mL hygromycin, and/or 50 μg/mL kanamycin.

Construction and complementation of S. venezuelae null mutants

The *whiG*, *vnz15005*, and *rsiG* null mutants were generated using the Redirect PCR targeting system (Gust et al., 2003, Gust et al., 2004). A cosmid library that covers > 98% of the *S. venezuelae* genome has been constructed and is fully documented at http://strepdb.streptomyces.org.uk/. Cosmid Sv-6_E12 (contains *whiG*), Pl2_H19 (contains *vnz15005*), or Sv-2_C01 (contains *rsiG*) was introduced to *E. coli* strain BW25113 containing pIJ790 and *whiG*, *vnz15005*, or *rsiG* was replaced with the *apr-oriT* cassette amplified from pIJ773 using primer pairs vnz26215_redi_F and vnz26215_redi_R, vnz15005_redi_F and vnz15005_redi_R, or vnz19430_redi_F and vnz19430_redi_R. Null mutants were confirmed by PCR analysis using the flanking primer sets vnz26215_check_F and vnz26215_check_R, vnz15005_check_F and vnz15005_check_R, or vnz19430_check_F and vnz19430_check_R. Complementation of the *whiG* and *rsiG* null mutants was achieved by amplifying the coding region and native promoter of each gene using the primer sets vnz26215_comp_F and vnz26215_comp_R, or vnz19430_comp_F and vnz19430_comp_R and cloning each fragment into HindIII/Asp718-cut pIJ10770 (Schlimpert et al., 2017) to generate plasmids pIJ10900 and pIJ10901. Complementation of the *rsiG* null mutant with the *rsiG** allele was accomplished by gene synthesis of *rsiG** (GenScript), with identical promoter and coding sequence to that found in pIJ10901 except for the four mutated codons. The *rsiG** allele was subsequently cloned into HindIII/Asp718-cut pIJ10770 to generate pIJ10913. Complementation plasmids were introduced into *S. venezuelae* null mutants by conjugation.

Chromatin immunoprecipitation, library construction, sequencing, and ChIP-seq data analysis

WT *S. venezuelae* and the *ΔwhiG* null mutant (negative control) were grown in 30 mL volumes of MYM. During mid-sporulation, formaldehyde was added to cultures at a final concentration of 1% (v/v) and incubation was continued for 30 min. Glycine was then added to a final concentration of 125 mM to stop the cross-linking. Cultures were left at room temperature (RT) for 5 min before the mycelium was harvested and washed twice in PBS buffer (pH 7.4). Each mycelial pellet was

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Commented [MF3]: Note how the methods section is written in past tense and 3rd person (NOT "I used the strains…." – rather,"Strains used in this study")

Commented [MF4]: Note how briefly and succinctly the bacterial growth conditions are described. You should maybe go into a little more detail than this (an error here is that it doesn't give a reference/formulation for LB), but this is otherwise a good example of how to write this.

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resuspended in 0.75 mL lysis buffer (10 mM Tris HCl pH 8.0, 50 mM NaCl) containing 10 mg/mL lysozyme and protease inhibitor (Roche Applied Science) and incubated at 37°C for 25 min. An equal volume of IP buffer (100 mM Tris HCl pH 8, 250 mM NaCl, 0.5% Triton X-100, 0.1% SDS) containing protease inhibitor was added and samples were chilled on ice. Samples were sonicated for 8 cycles of 20 s each at 8 microns to shear the chromosomal DNA into fragments ranging from 300-1000 bp in size. Samples were centrifuged twice at 13,000 rpm at 4°C for 10 min to clear the cell extract. The supernatant was incubated with 10% (v/v) protein A-Sepharose (Sigma) for 1 h on a rotating wheel to remove non-specifically binding proteins. Samples were then centrifuged for 15 min at 4°C and 13,000 rpm to remove the beads. Supernatants were incubated with 10% (v/v) anti-σ^{WhiG} polyclonal antibody overnight at 4°C with rotation. Subsequently, 10% (v/v) protein A-Sepharose was added to precipitate σ^{WhiG} and incubation was continued for 4 hr. Samples were centrifuged at 4°C and 3500 rpm for 5 min and the pellets were washed twice with 0.5x IP buffer, and then twice with 1x IP buffer. Each pellet was incubated overnight at 65°C in 150 µl IP elution buffer (50 mM Tris HCl pH 7.6, 10 mM EDTA, 1% SDS) to reverse cross-links. Samples were centrifuged at 13,000 rpm for 5 min to remove the beads. Each pellet was re-extracted with 50 µl TE buffer (10 mM Tris HCl pH 7.4, 1 mM EDTA) and the supernatant incubated with 0.2 mg/mL Proteinase K (Roche) for 2 h at 55°C. The resulting samples were extracted with phenol-chloroform and further purified using QiaQuick columns, eluting in 50 µL EB buffer (QIAGEN). Library construction and sequencing were performed by the Earlham Institute, Norwich Research Park, Norwich, United Kingdom.

The reads in the fastq files received from the sequencing contractor were aligned to the *S. venezuelae* genome (GenBank: CP018074) using the bowtie2 software (version 2.2.9), which resulted in one SAM (.sam) file for each fastq file (single ended sequencing). For each sam file, the *depth* command of *samtools* (version 1.8) was used to arrive at the depth of sequencing at each nucleotide position of the *S. venezuelae* chromosome (https://www.sanger.ac.uk/science/tools/samtools-bcftools-htslib). From the sequencing depths at each nucleotide position determined in step 2, a local enrichment was calculated in a moving window of 30 nucleotides moving in steps of 15 nucleotides as (the mean depth at each nucleotide position in the 30-nucleotide window) divided by (the mean depth at each nucleotide position in a 3000-nucleotide window centered around the 30-nucleotide window).This results in an enrichment ratio value for every 15 nucleotides along the genome. Local enrichment in total (non-IP) samples were subtracted from those in IP samples. Enrichment for the control samples (*whiG* deletion strain) was subtracted from the enrichment in corresponding WT samples. Significance of enrichment values were calculated assuming normal distribution of the enrichment values. Genomic positions were ordered from low to high P values. Association of regions of enrichment with P values below 1e-4 with genes on the chromosome was done by simply listing genes left and right of the region. Rows of lower significance with the same context of genes were removed to leave the most significant row for each combination of left, right and *within* genes. Genes had to be in the right orientation and within 500 nucleotides of the enriched region for association with the region.

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Better practice would be to take this one step further and deposit the code/data on github or a similar repository, and provide a link here (which many of you have done.)

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