

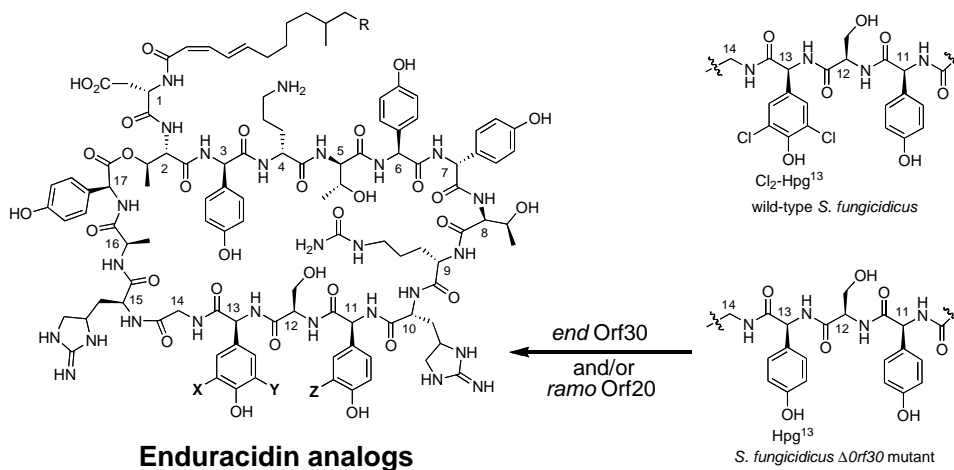
Supporting Information

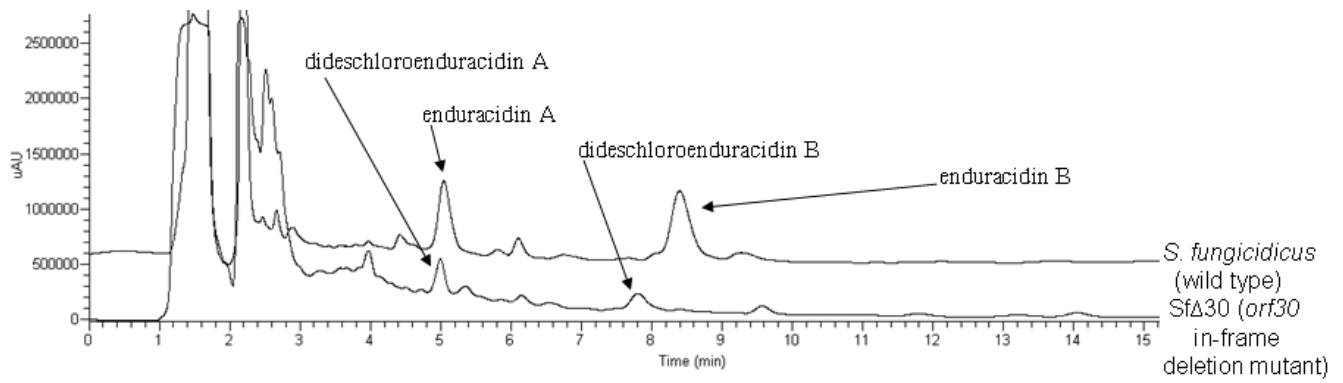
Enduracidin Analogues with Altered Halogenation Patterns Produced by Genetically Engineered Strains of *Streptomyces fungicidicus*

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S1. HPLC analysis comparing wild-type *S. fungicidicus* metabolites with the extract from mutant SfΔ30.

Orf30 VPGGRMSTG**QHEEFDV**VVVG**GGPSG**STLSTLVAM**QGH**S**VLLLEKET**FPRYQIGESLLPST 60
Orf20 -----VAA**QPEEFDV**IVV**GGPGG**STAAAL**TAKQ**AKV**LLLE**RE**KET**FPRYQIGESLLPST 54
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Orf30 **IHGIC**HL**LGVT**DE**LAAAG**F**PHKR**GG**TFR**WGAS**PKP**WN**FS**SVSS**KV**SG**PTS**FAY**Q**VERSK 120
Orf20 **VHGVC**N**LLG**V**DEI**AK**AGF**MR**KH**GG**TFK**WG**T**ST**EP**W**TF**F**AT**SP**RM**AG**PT**SHAF**Q**VERRR 114
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Orf30 **FDKILL**DNA**ARK**G**VVVR**Q**DRT**V**T**D**V**DD**DAD**GR**AR**GL**RY**T**DP**D**G**TE**HE**VS**ARY**V**VD**AS**G**NT 180
Orf20 **FDQILL**EN**ARR**L**GVD**V**REN**HP**VTE**AI**AD**DE-**RVR**G**V**R**FT**Q-**DG**Q**TR**T**AL**AR**FV**VD**AS**GNR 172
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Orf30 **SRIH**K**R**V**G**GS**R**T**Y**S**DF**FK**SL**AL**F**GY**F**ENG**K**RM**P**AP**Y**AG**N**IL**C**V**A**F**G**SG**W**F**W**Y**I**PL**S**ST**L**T 240
Orf20 **STLHT**T**V**GG**TRE**Y**S**P**FF**R**N**L**AL**F**GY**F**ENG**R**RL**P**AP**NS**G**N**IL**C**V**A**F**SG**W**F**W**Y**I**PL**S**ET**L**T 232
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Orf30 **SVGAV**V**R**RE**DA**AK**VQ**GD**P**ES**AL**R**GL**I**D**EC**PM**I**K**E**Y**L**A**D**A**T**R**V**T**T**G**Q**Y**Q**L**R**V**R**K**D**Y**S**Y**H**H** 300
Orf20 **SVGAV**V**R**RE**MA**H**K**V**Q**GD**Q**E**K**AL**F**E**L**I**A**EC**PM**I**A**D**F**L**G**D**A**T**R**V**T**E**G**D**Y**Q**I**R**V**R**K**D**Y**S**Y**S**S** 292
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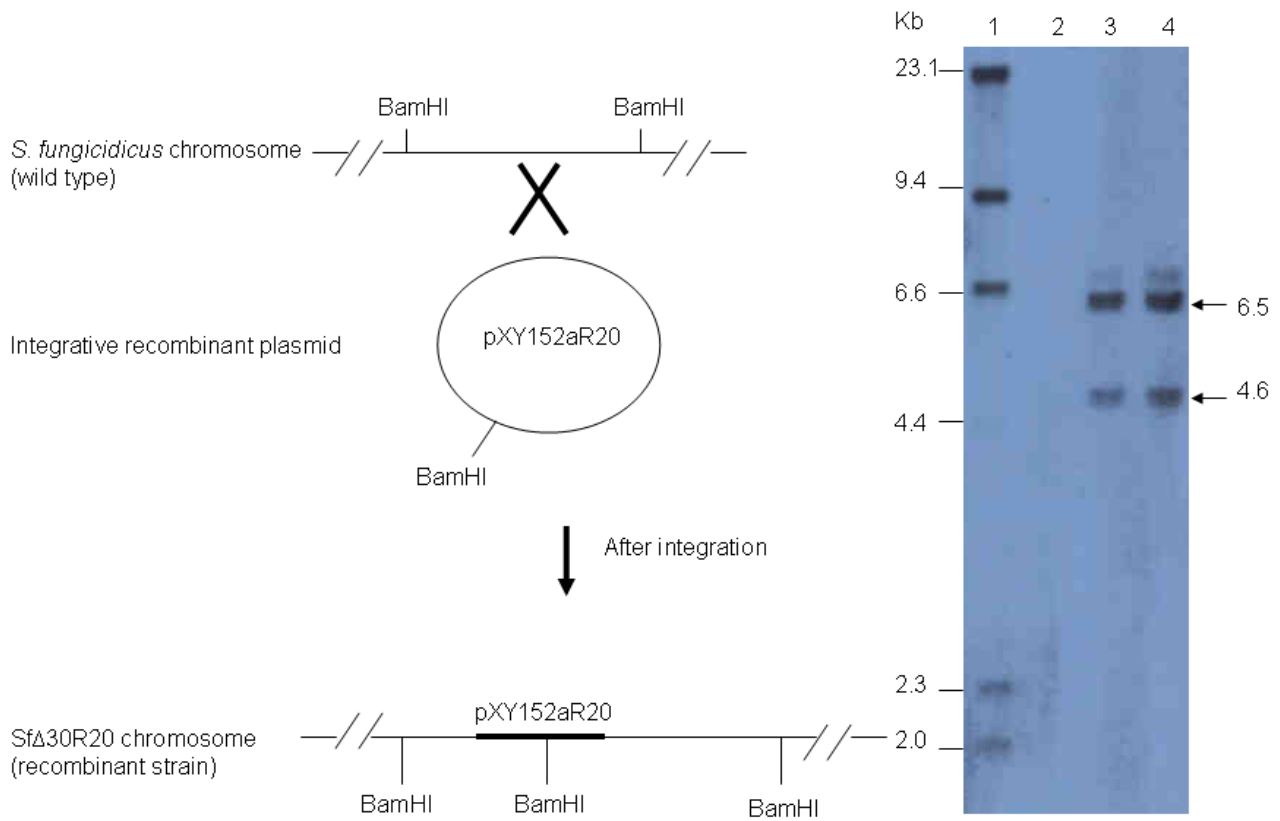
Orf30 **TTFWR**PG**M**VL**V**G**DA**AC**F**VD**P**V**F**SS**G**V**H**L**A**T**Y**S**A**LL**A**AR**S**L**N**SV**L**AG**R**I**D**ER**R**A**F**DE**F**E**A**R 360
Orf20 **TSYWR**PG**M**CL**V**G**DA**AC**F**ID**P**V**F**SS**G**V**H**L**A**T**Y**S**G**LL**A**AR**S**I**N**SV**L**AG**T**V**D**E**D**R**A**F**T**E**F**E**Q**R 352
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Orf30 **YRRE**Y**G**V**F**Y**E**FL**T**S**F**Y**D**M**H**V**D**E**S**Y**F**W**T**A**K**K**V**T**R**S**S**H**A**E**L**E**S**F**V**E**L**V**A**G**M**S**S**T**D**F**D**L**S**D**A** 420
Orf20 **YRRE**F**G**V**F**H**D**FL**V**S**F**Y**D**M**H**V**D**E**S**Y**F**W**A**A**R**K**V**T**E**S**S**A**P**A**M**E**S**F**T**E**L**V**G**G**I**A**S**G**E**D**A**L**T**G**S** 412
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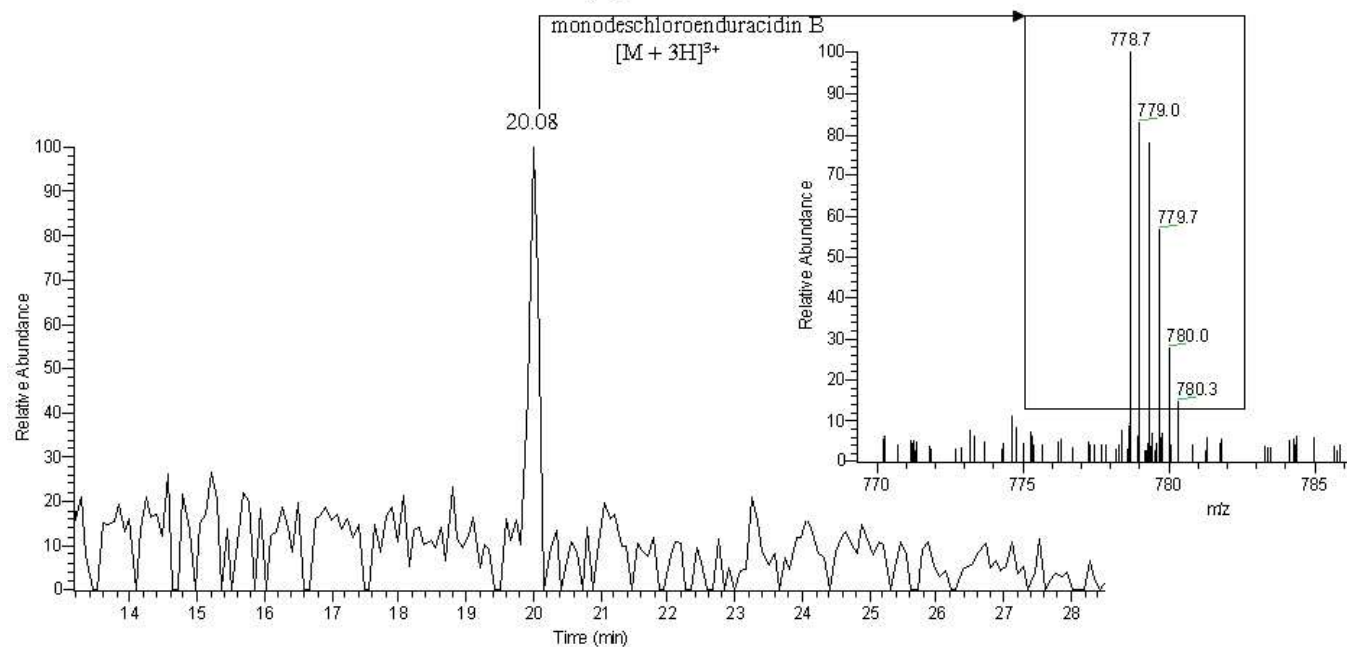
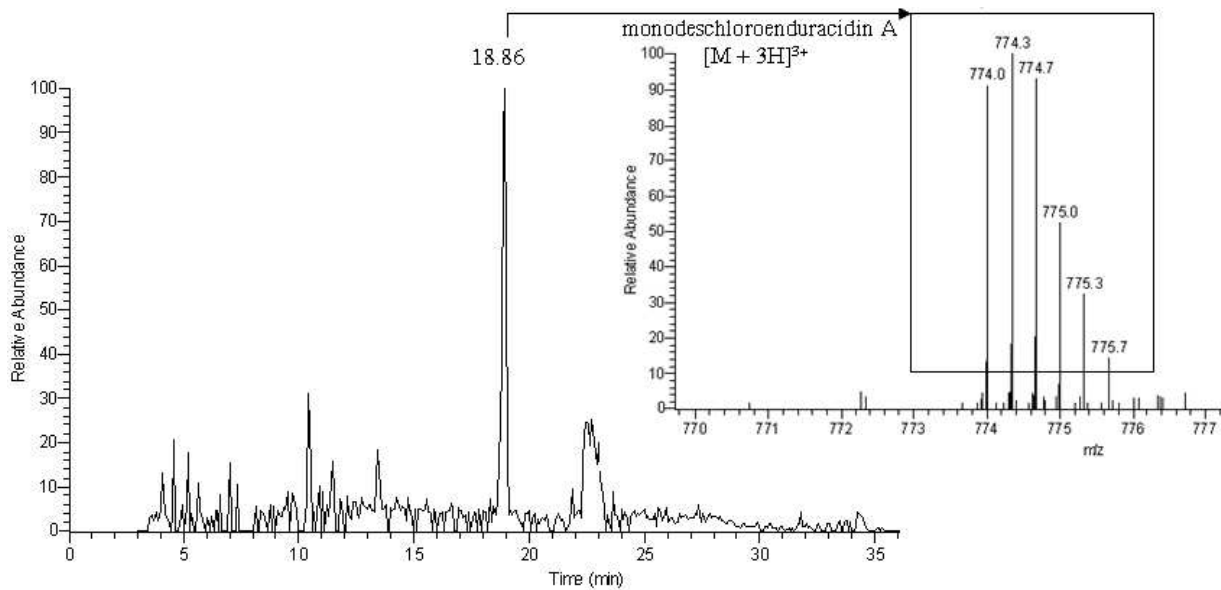
Orf30 **ESSVL**RL**K**Q**Q**SA**E**F**A**D**A**V**D**D**M**AG**R**Q**E**EN**M**AP**L**FR**S**SA**V**S**R**AM**Q**E**G**S**Q**V**Q**T**R**A**Q**L**G**E**Y**A**G**E 480
Orf20 **TELVR**R**H**S**R**Q**T**A**E**L**G**Q**AV**--**AG**L**E**E**G**G**T**G**F**L**R**G**S**S**V**V**A**Q**A**M**F**E**G**S**Q**I**Q**A**G**A**I**L**G**P**E**G**T**Q 469
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Orf30 **DVPL**F**D**G**G**L**V**AS**S**D**S**M**F**W**E**E**P**H**S** 504
Orf20 **EQPL**F**E**G**G**L**T**P**S**G**N**L**T**W**V**A**A**D-- 491
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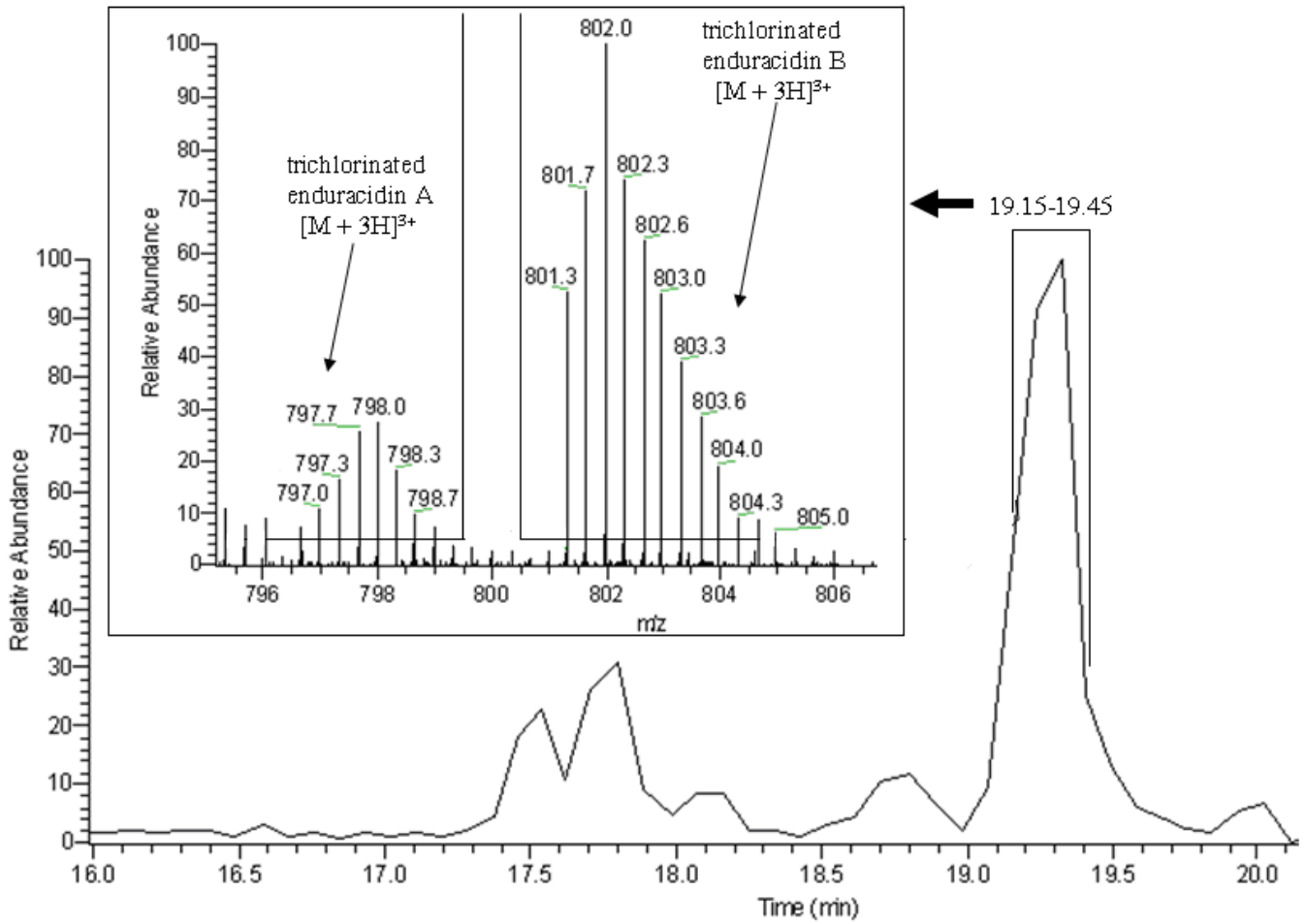
S2. Alignment of enduracidin halogenase Orf30 from *Streptomyces fungicidicus* ATCC21013 with ramoplanin halogenase Orf20 from *Actinoplanes* strain ATCC33076. Identical amino acid sequences are in bold.



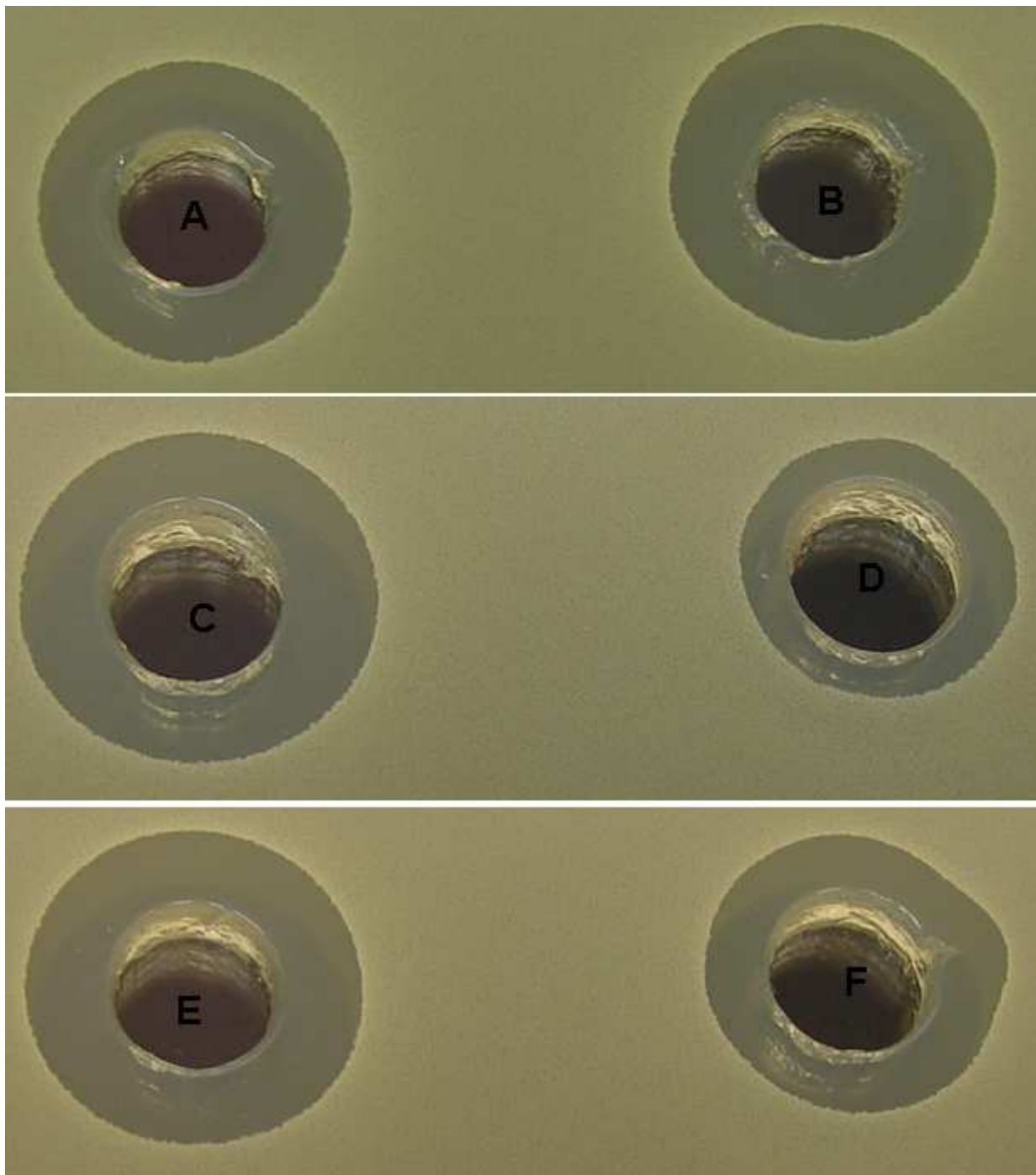
S3. Southern blot analysis confirming chromosomal integration of plasmid pXY152aR20 into *S. fungicidicus* enduracidin halogenase in-frame deletion mutant strain Sf Δ 30. Genomic DNA was prepared from the wild type and recombinant strain Sf Δ 30R20, respectively, and digested with BamHI. pXY152aR20 contains a unique BamHI site and was Dig-labeled and used as probe. Lane 1, molecular markers; Lane 2, wild-type *S. fungicidicus*; Lanes 3 and 4: independent integrant strains.



S4. Confirming the triply charged monodeschloroenduracidins A and B by LC-ESI-MS analysis of the extract from the recombinant SfΔ30R20.



S5. Confirming the triply charged trichlorinated enduracidins A and B by LC-ESI-MS analysis of the extract from the recombinant Sfr20.



S6. The zones of inhibition produced by the enduracidin standards and purified enduracidin analogues. (**A**, **C** and **E**) enduracidin standards, (**B**) partial purified mixture of enduracidins plus trichlorinated enduracidin A, (**D**) purified dideschloroenduracidin A, (**F**) purified monodeschloroenduracidin A. 100 μ L 50% aqueous MeOH containing 2 μ g enduracidins or analogues were loaded into each well. Pictures were taken after 16 h incubation at 37 $^{\circ}$ C.