Supporting material

tRNA-dependent aminoacylation of an amino-sugar intermediate in the biosynthesis of a streptothricin-related antibiotic

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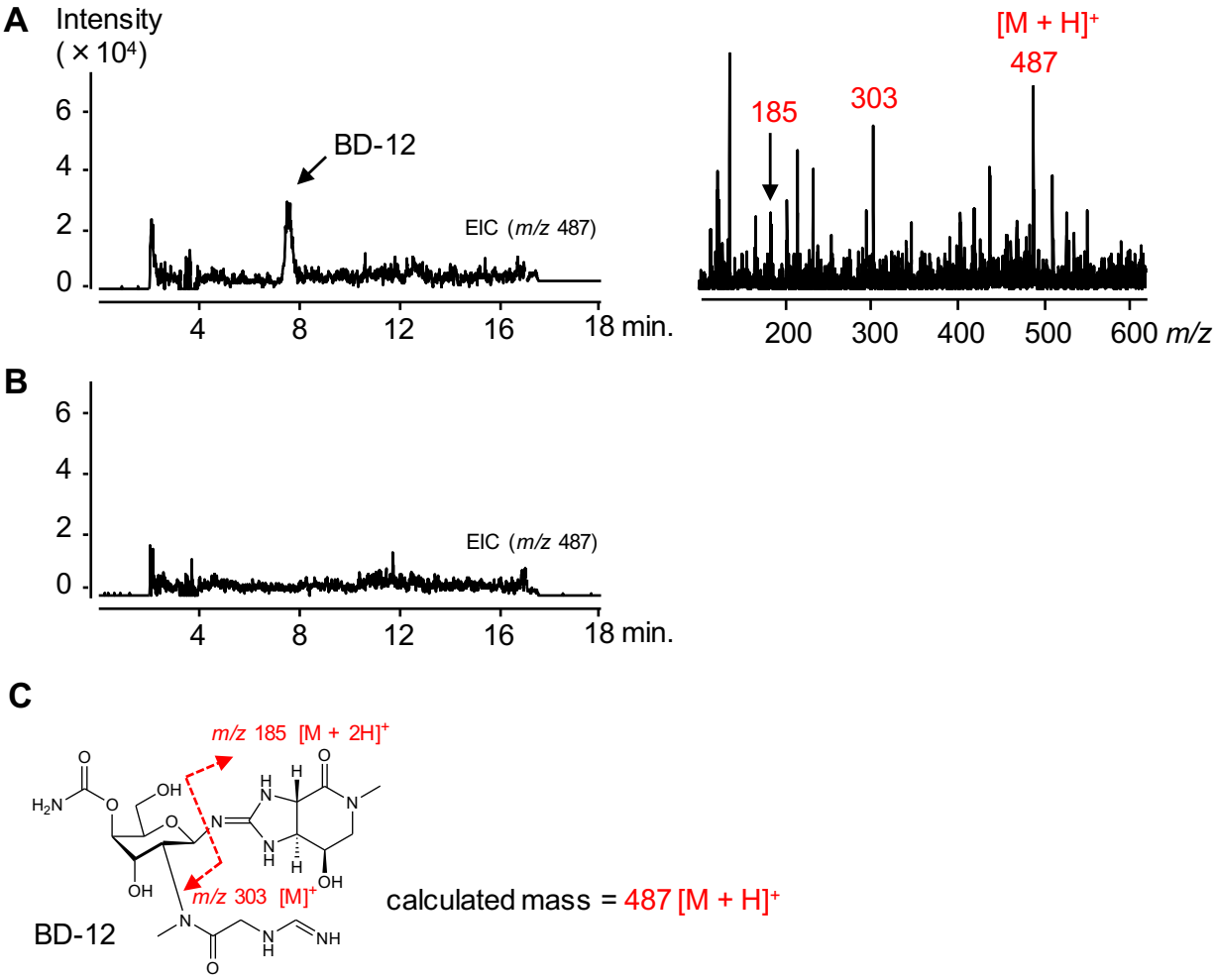

Fig. S1 HPLC/ESI-MS analysis of BD-12 produced by the *Streptomyces* strains. *S. avermitilis* SUKA17 harboring pKU493A-BD12 (A) and *S. avermitilis* SUKA17 harboring the empty vector (pKU493A_aac(3)IV) (B) were cultivated, and the culture broths were analyzed by HPLC/ESI-MS (Esquire 4000; Bruker, Billerica, MA, USA) using a reversed-phase column (Sunniest RP-AQUA, 3 μm, 100 × 2.0 mm; ChromaNik Technologies) at 30 °C at a flow rate of 0.3 ml min^{-1} and with an initial gradient of 8% (v/v) acetonitrile in water to 16% (v/v) acetonitrile over 11 min, which was then ramped to 40% (v/v) acetonitrile over 5 min. Both acetonitrile and water contained 0.05% (v/v) HFBA and 0.05% (v/v) FA. Extracted ion chromatograms (EICs) for BD-12 (m/z 487) are shown. The BD-12 chemical structure with the fragmentation pattern is also shown (C).

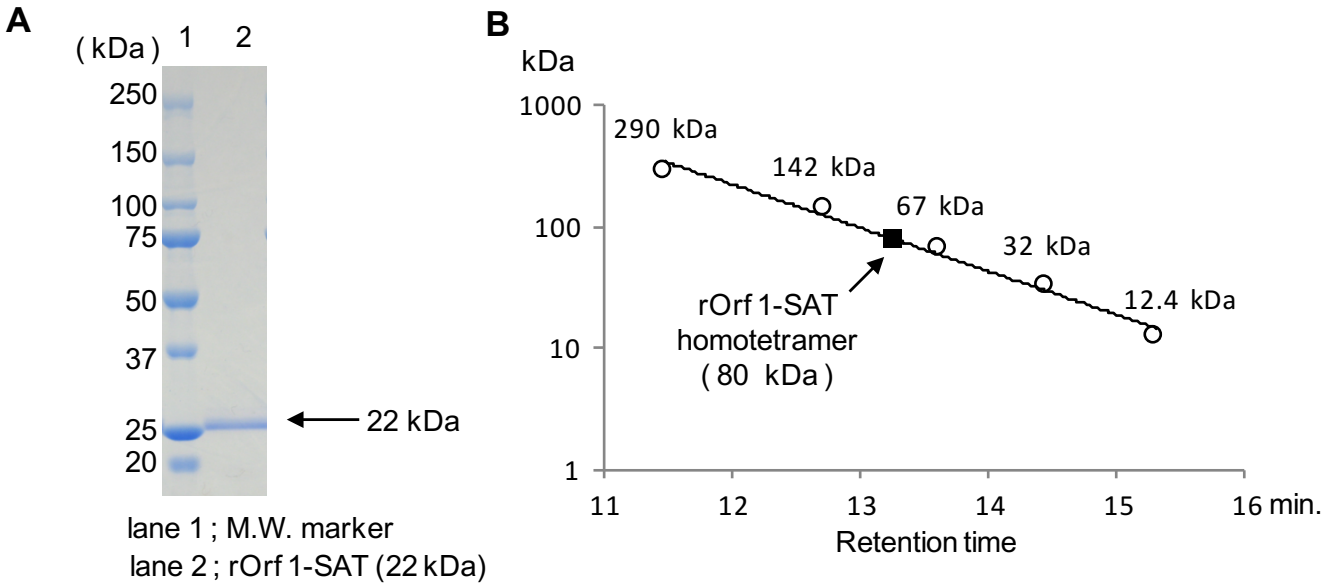

Fig. S2 Enzymatic characterization of rOrf 1-SAT. (A) The purified rOrf 1-SAT was subjected to SDS-PAGE. Proteins were stained with CBB R-250. (B) The relative molecular mass of rOrf 1-SAT was estimated by gel-filtration chromatography (SunSec Diol, 4 μm, 300 4.6 mm, ChromaNik Technologies, Osaka, Japan). Glutamate dehydrogenase (290 kDa), lactate dehydrogenase (142 kDa), enolase (67 kDa), myokinase (32 kDa), and cytochrome C (12.4 kDa) were used as the standard molecular masses.

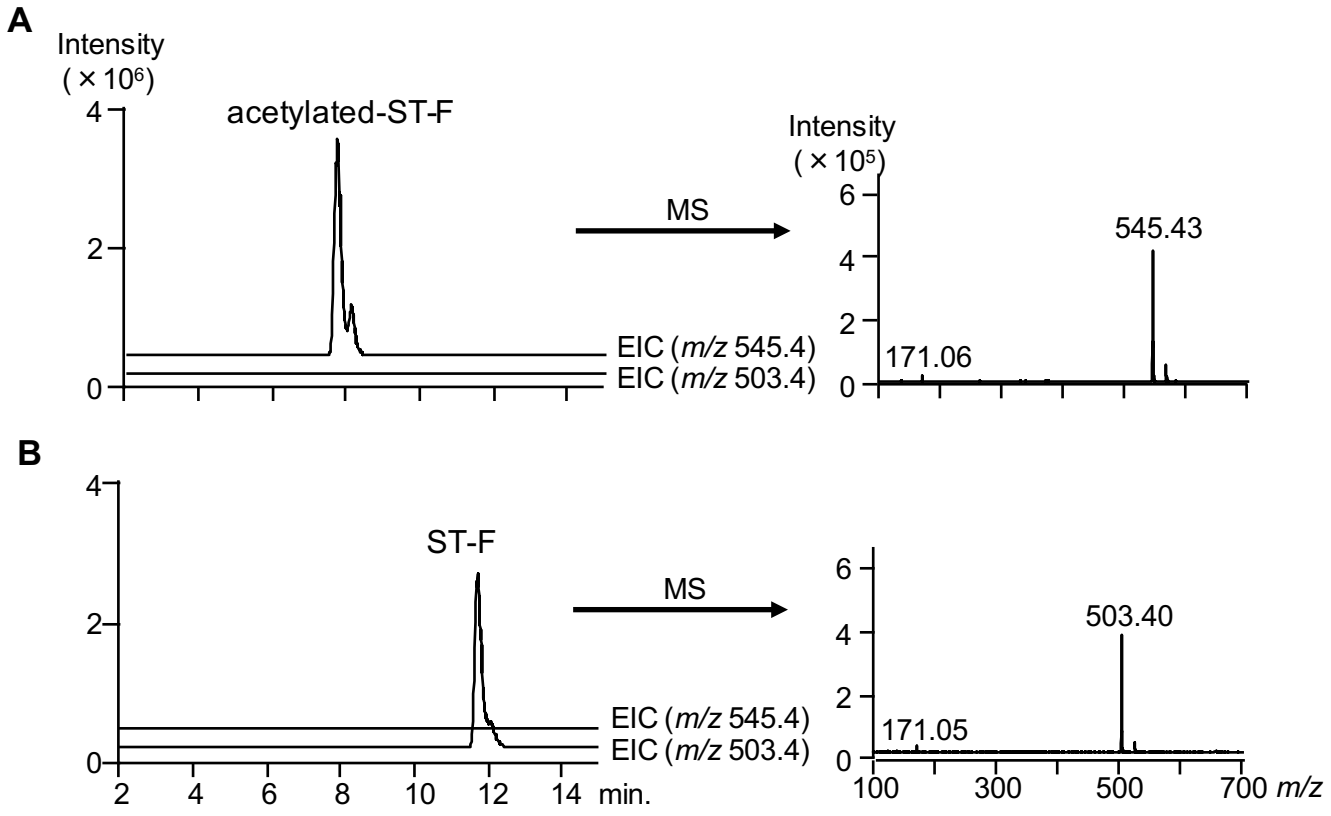

Fig. S3 Acetylation of ST-F by rOrf 1-SAT. (A) ST-F was incubated with rOrf 1-SAT and acetyl-CoA. (B) ST-F was incubated only with rOrf 1-SAT (no acetyl-CoA). These two reaction mixtures were analyzed by HPLC/ESI-MS. The extracted ion chromatograms (EICs) for ST-F (*m/z* 503.4) and acetylated-ST-F (*m/z* 545.4) are shown.