

Figure 3. Genomic map and biosynthetic pathway of the *spn* gene cluster. (A) Genomic map showing ORFs 1-6 and sub-modules A-M. (B) Biosynthetic pathway showing the conversion of precursors (Phe, Trp, Pyruvate) into SPNs or ANTs via various Spn enzymes.

Table 1. Deduced Functions of ORFs in *spn* Biosynthetic Gene Cluster (Accession Number KP719128)

ORF	Start	End	Length	Identity (%)	Function
1	10	201	191	22	...
2	1	22	21	100	...
3	113	223	110	3	...
4	13	21	9
5	3	11	9

...

(3) ...

...

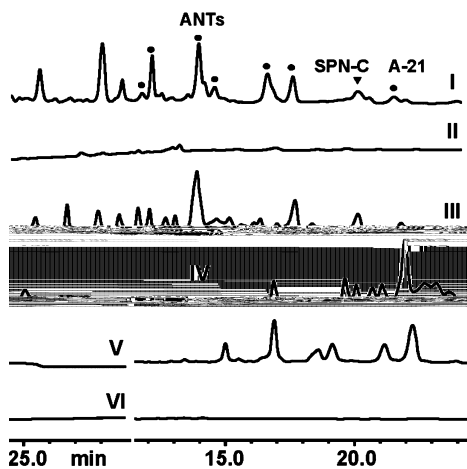


Figure 4. HPLC chromatograms of ANTs, SPN-C, and A-21. The x-axis represents time in minutes, with markers at 25.0, 15.0, and 20.0. The chromatograms are labeled I, II, III, V, and VI.

Characterization of Benzylmalonyl-CoA as an Extender Unit and the selectivity of the AT Domain.

The α,β -unsaturated structure of benzylmalonyl-CoA is a key feature for its recognition by the AT domain. The AT domain shows high selectivity for this extender unit, as demonstrated by the HPLC analysis of the products. The chromatograms show distinct peaks for the different products, indicating that the AT domain can discriminate between different extender units. The selectivity of the AT domain is further supported by the mass spectrometry data, which shows a characteristic mass shift of 176.0516 Da, corresponding to the addition of the benzylmalonyl group. This selectivity is crucial for the synthesis of the target polyketide, as it ensures that the correct extender unit is incorporated into the growing chain.

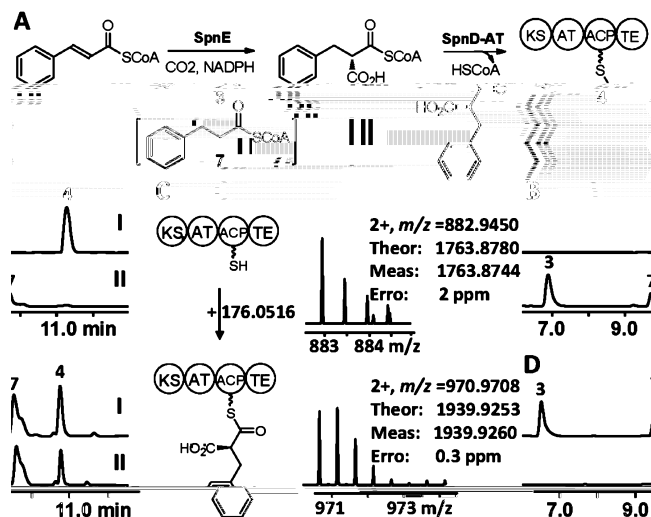


Figure 5. Reaction scheme and mass spectrometry data. The reaction scheme shows the conversion of benzylmalonyl-CoA to benzylmalonyl-SH by SpnE, and then to benzylmalonyl-S-C(=O)-R by SpnD-AT. The mass spectrometry data shows peaks at 883 and 884 m/z for the benzylmalonyl-SH product, and at 971 and 973 m/z for the benzylmalonyl-S-C(=O)-R product. The x-axis is labeled with 11.0 min, 7.0, and 9.0. The chromatograms are labeled I, II, III, and D.

The correlation of amino acid origin to benzylmalonyl-CoA synthesis and crosstalk of *enc_c* and *spn*. The amino acid origin of the benzylmalonyl-CoA extender unit is a critical factor in determining the selectivity of the AT domain. The amino acid origin is determined by the presence of specific amino acid residues in the AT domain, which are known to be involved in the recognition and binding of the extender unit. The crosstalk between *enc_c* and *spn* is a complex process that involves the coordinated action of these two domains. The amino acid origin of the extender unit is a key factor in determining the outcome of this crosstalk, as it influences the binding of the extender unit to the AT domain and the subsequent reaction. This correlation is essential for the synthesis of the target polyketide, as it ensures that the correct extender unit is incorporated into the growing chain.

Correlation of Amino acid Origin to Benzylmalonyl-CoA Synthesis and Crosstalk of *enc_c* and *spn*.

The amino acid origin of the benzylmalonyl-CoA extender unit is a critical factor in determining the selectivity of the AT domain. The amino acid origin is determined by the presence of specific amino acid residues in the AT domain, which are known to be involved in the recognition and binding of the extender unit. The crosstalk between *enc_c* and *spn* is a complex process that involves the coordinated action of these two domains. The amino acid origin of the extender unit is a key factor in determining the outcome of this crosstalk, as it influences the binding of the extender unit to the AT domain and the subsequent reaction. This correlation is essential for the synthesis of the target polyketide, as it ensures that the correct extender unit is incorporated into the growing chain.

Broad Selectivity of the AT Domain and Potential to Introduce Structural Diversity into the Polyketide Scaffold.

The AT domain of the polyketide synthase (PKS) is a key component in the biosynthesis of polyketides. It is responsible for the formation of the polyketide chain and the introduction of structural diversity. In this study, we have investigated the broad selectivity of the AT domain and its potential to introduce structural diversity into the polyketide scaffold. We have shown that the AT domain can accept a wide range of substrates and can catalyze a variety of reactions, including the formation of branched polyketide chains. This suggests that the AT domain is a versatile enzyme that can be used to generate a diverse array of polyketide products. The results of this study have important implications for the design of novel polyketide synthases and for the synthesis of new polyketide derivatives.

CONCLUSION

The AT domain of the polyketide synthase (PKS) is a key component in the biosynthesis of polyketides. It is responsible for the formation of the polyketide chain and the introduction of structural diversity. In this study, we have investigated the broad selectivity of the AT domain and its potential to introduce structural diversity into the polyketide scaffold. We have shown that the AT domain can accept a wide range of substrates and can catalyze a variety of reactions, including the formation of branched polyketide chains. This suggests that the AT domain is a versatile enzyme that can be used to generate a diverse array of polyketide products. The results of this study have important implications for the design of novel polyketide synthases and for the synthesis of new polyketide derivatives.

ASSOCIATED CONTENT

Supporting Information

Supporting Information is available for this article. See the Supporting Information for details.

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