A new high-performance heterologous fungal expression system based on regulatory elements from the *Aspergillus terreus* terrein gene cluster.

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Abstract

Recently, the *Aspergillus terreus* terrein gene cluster was identified and selected for development of a new heterologous expression system. The cluster encodes the specific transcription factor TerR that is indispensable for terrein cluster induction. To identify TerR binding sites, different recombinant versions of the TerR DNA-binding domain were analyzed for specific motif recognition. The high affinity consensus motif TCGGHHWYHCGGH was identified from genes required for terrein production and binding site mutations confirmed their essential contribution to gene expression in *A. terreus*. A combination of TerR with its *terA* target promoter was tested as recombinant expression system in the heterologous host *Aspergillus niger*. TerR mediated target promoter activation was directly dependent on its transcription level. Therefore, *terR* was expressed under control of the regulatable amylase promoter P*amyB* and the resulting activation of the *terA* target promoter was compared with activation levels obtained from direct expression of reporters from the strong *gpdA* control promoter. Here, the coupled system outcompeted the direct

expression system. When the coupled system was used for heterologous polyketide synthase expression high metabolite levels were produced. Additionally, expression of the *Aspergillus nidulans* polyketide synthase gene *orsA* revealed lecanoric acid rather than orsellinic acid as major polyketide synthase product. Domain swapping experiments assigned this depside formation from orsellinic acid to the OrsA thioesterase domain. These experiments confirm the suitability of the expression system especially for high-level metabolite production in heterologous hosts.

Involved units

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