# Construction of a novel meroterpenoid biosynthetic pathway in *Aspergillus oryzae*

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**Research aims**

Fungal meroterpenoids are a structurally complex class of compounds that include pharmaceutically important molecules, such as pyripyropene A and mycophenolic acid[1,2]. The study of the enzymes involved in the biosynthetic pathway of meroterpenoids will provide a knowledge for future enzyme engineering aiming to the production of the system to yield these compounds easily. This research focuses on sequential oxidations catalyzed by non-heme iron dependent oxygenases[2] involved in fungal meroterpenoid biosynthesis. The goal of the project is to answer the following questions:

1. How diverse are the reactions of non-heme iron dependent oxygenases in fungal meroterpenoid biosyntheses?
2. How do non-heme iron dependent oxygenases control their reactivity?

Once we delineate the biosynthetic reactions that produce meroterpenoids, a heterologous expression system to produce diverse fungal meroterpenoids will be constructed.

**Methods**

We have previously reported two enzymes of non-heme iron dependent oxygenase family, including AusE, an enzyme that catalyzes the addition of a spiro-lactone ring preaustinoid A1 to produce austinol. Structural similarities between austinol and paraherquonin, derived from the different oxidation reactions from preaustinoid A1, suggest that both compounds are derived from preaustinoid A1. Therefore, we set out to investigate AusE homologs that accept the same substrate, preaustinoid A1, but catalyze different types of oxidation. First, we sequenced the genome of *Penicillium brasilianum* NBRC 6234, a paraherquonin producer. The biosynthesis genes were found using a BLAST search with known meroterpenoid terpene cyclase genes as a query. The resultant genes were expressed in *Aspergillus oryzae* NSAR1, a fungi for a multigene expression[3]. The compounds that accumulated in the transformants will be structurally determined via NMR and MS analyses. From the structures, we will be able to deduce the reaction of each biosynthetic enzyme and to use this knowledge to construct a production system of fungal meroterpenoid compounds.

**Results**

From the genome sequence of *P. brasilianum* NBRC 6234, we searched for a genomic region that encodes enzymes homologous to those encoded by the *aus* cluster and found a possible biosynthetic gene cluster for paraherquonin, designated as the *prh* cluster, that consisted of 14 genes altogether. Of these, four genes encoding oxidative enzymes, PrhI, PrhJ, PrhK, and PrhA, were chosen from the fourteen genes, and introduced into the heterologous host *Aspergillus oryzae* NSAR1, and the resulting transformants were cultivated in the presence of the substrate protoaustinoid A. The transformant produced from the introduction of *prhI* alone transformed protoaustinoid A into the new metabolite protoaustinoid B (Figure 1). The two or three gene-expression system consisting of *prhI* and *prhJ*, with or without *prhK*, were subsequently analyzed, with the result that the transformants produced preaustinoid A and preaustinoid A1, respectively (Figure 1). Finally, the strain harboring all four genes converted protoaustinoid A into berkeleydione (Figure 1). Hence, it was demonstrated that PrhA has a different activity than AusE and that PrhA produces berkeleydione from protoaustinoid A1 via berkeleyone B by constructing a cycloheptadiene system. These data indicate that we successfully constructed a new system to produce fungal meroterpenoids[4].

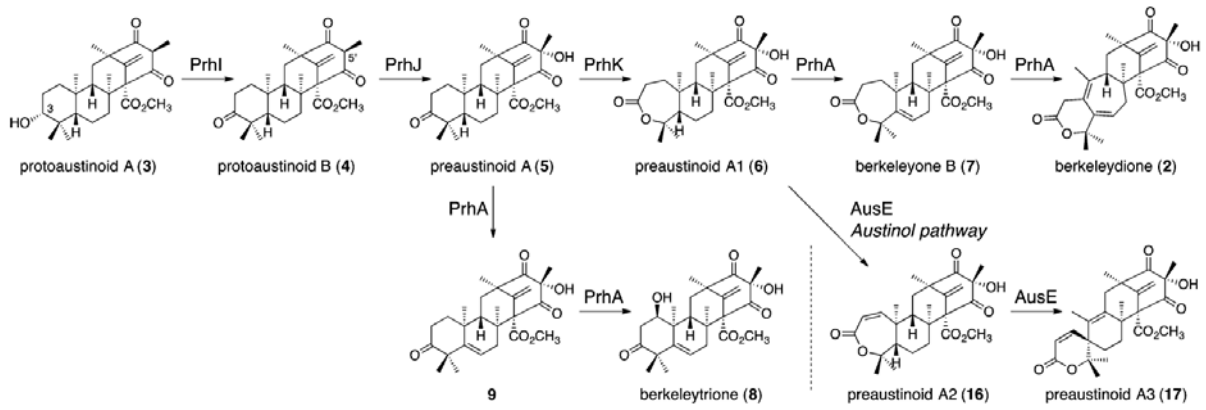


Figure 1. The production of fungal meroterpenoids using paraherquonin biosynthetic enzymes

We proposed that the conversion of berkeleyone B to berkeleydione could occur according to the following mechanism (Figure 2). Initially, α-KG and molecular oxygen are incorporated into the active site of the enzyme, where subsequent oxidative decarboxylation of α-KG leads to the generation of the highly active Fe(IV)–oxo species. The enzyme then abstracts the hydrogen atom from C-1 to yield the radical species **11**, which is followed by a C-C bond formation between C-1 and C-5 to give **12**. The C-C bond cleavage between C-5 and C-10 then achieves the expansion of the B-ring, providing **13** with a radical at the C-10 position. Finally, the abstraction of the hydrogen atom from C-1 finalizes the reaction to

afford the cycloheptadiene skeleton of berkeleydione. At this point, we analyzed the enzyme structure of PrhA via X-ray crystallization in order to compare it to that of AusE.

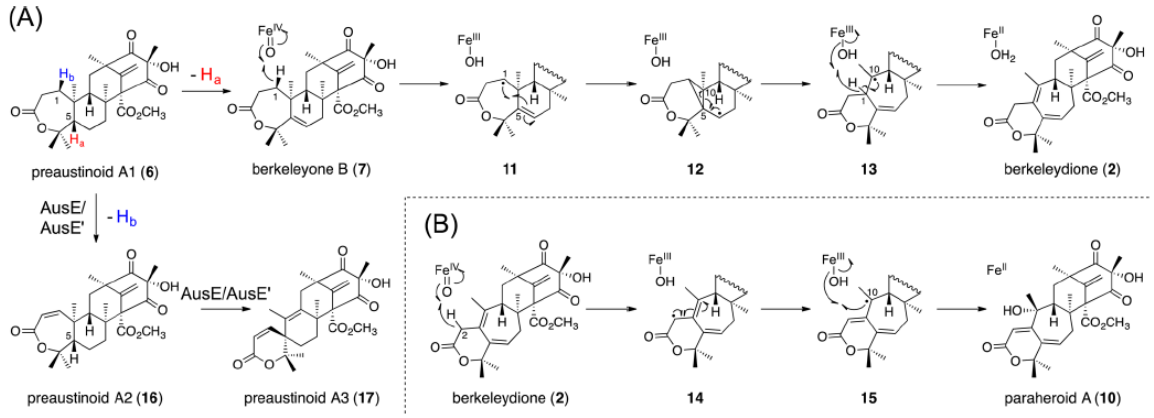


Figure 2. The reaction mechanism of PrhA

## Conclusion

In this study, we identified the biosynthetic paraherquonin gene cluster and revealed the molecular pathway leading to berkeleydione. Fascinatingly, we found that the dioxygenase PrhA catalyzes not only multistep oxidations but also a structural rearrangement to afford the cycloheptadiene moiety. To further clarify the factors that define their versatility, protein crystallization and mutational studies of PrhA and AusE are ongoing in our laboratory. The knowledge obtained in this research will be applied to produce unnatural fungal meroterpenoids. We truly appreciate the “Young Investigator Research Grant from Noda Institute for Scientific Research” that was used to fund this research.

## References

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