Exploring the Chemistry of Uncultivated Bacterial Symbionts: Antitumor Polyketides of the Pederin Family

ARTICLE *in* JOURNAL OF NATURAL PRODUCTS · APRIL 2005

Impact Factor: 3.8 · DOI: 10.1021/np049612d · Source: PubMed

7 AUTHORS, INCLUDING:

Nobuhiro Fusetani
Fisheries and Oceans Hakodate
412 PUBLICATIONS **12,355** CITATIONS
SEE PROFILE

Matthias Platzer
Leibniz Institute on Aging - Fritz Lipmann Inst…
260 PUBLICATIONS **23,807** CITATIONS
SEE PROFILE

Shigeki Matsunaga
The University of Tokyo
412 PUBLICATIONS **14,069** CITATIONS
SEE PROFILE

Reviews

Exploring the Chemistry of Uncultivated Bacterial Symbionts: Antitumor Polyketides of the Pederin Family[#]

Jörn Piel,[*,†,‡] Daniel Butzke,[†,‡] Nobuhiro Fusetani,[⊥] Dequan Hui,[‡] Matthias Platzer,[∥] Gaiping Wen,[∥] and Shigeki Matsunaga[⊥]

Kekulé Institute of Organic Chemistry and Biochemistry, University of Bonn, Gerhard-Domagk-Strasse 1, 53121 Bonn, Germany, Department of Bioorganic Chemistry, Max Planck Institute for Chemical Ecology, Hans-Knöll-Strasse 8, Beutenberg Campus, 07745 Jena, Germany, Graduate School of Agricultural and Life Sciences, University of Tokyo, Bunkyo-ku, Tokyo, 113-8657, Japan, and Genome Analysis, Institute of Molecular Biotechnology, Beutenbergstrasse 11, 07745 Jena, Germany

Received November 30, 2004

Symbiotic bacteria have long been proposed as being responsible for the production of numerous natural products isolated from invertebrate animals. However, systematic studies of invertebrate−symbiont associations are usually associated with serious technical challenges, such as the general resistance of symbionts to culturing attempts and the complexity of many microbial consortia. Herein an overview is provided on the culture-independent, metagenomic strategies recently employed by our group to contribute to a better understanding of natural product symbiosis. Using terrestrial *Paederus* spp. beetles and the marine sponge *Theonella swinhoei* as model animals, the putative genes responsible for the production of pederin-type antitumor polyketides have been isolated. In *Paederus fuscipes*, which uses pederin for chemical defense, these genes belong to an as-yet unculturable symbiont closely related to *Pseudomonas aeruginosa*. To study the extremely complex association of *T. swinhoei* and its multispecies bacterial consortium, we used a phylogenetic approach that allowed the isolation of onnamide/theopederin polyketide synthase genes from an uncultured sponge symbiont. Analysis of the biosynthesis genes provided unexpected insights into a possible evolution of pederin-type pathways. Besides revealing new facets of invertebrate chemical ecology, these first gene clusters from uncultivated symbiotic producers suggest possible biotechnological strategies to solve the supply problem associated with the development of most marine drug candidates.

Introduction

The similarity of many natural products isolated from invertebrates to those from bacteria has intrigued scientists for several decades.[2,3] Examples are the numerous complex polyketides and nonribosomal peptides discovered in marine sponges, tunicates, and bryozoans during pharmaceutical screening programs.[4,5] These compound classes are otherwise almost exclusively known from microorganisms, where they are synthesized by multifunctional megaenzymes called polyketide synthases (PKSs) and nonribosomal peptide synthetases (NRPSs), respectively.[6] Many attempts have been made to determine whether symbiotic bacteria are the actual producers of animal-derived natural products. The symbiont hypothesis is supported by sometimes large numbers of prokaryotic cells colonizing the animal tissues, but cultivation of these bacteria has been largely unsuccessful.[2] Several groups have studied the secondary metabolism of some of these microorganisms by separating symbiont from animal cells and analyzing extracts of the individual cell preparations.[2,3] By showing that several natural products were localized in bacteria, Faulkner and co-workers have provided the first convincing evidence for symbiotic producers in sponges.[7−9] However, these results do not rule out the possibility that substances might be transported between different cell types via export or sequestration mechanisms. If the symbiont cannot be cultivated, an ultimate proof for a symbiotic origin therefore requires the localization of biosynthetic pathways instead of substances. One example of such an approach is the detection of putative bryostatin biosynthesis genes in a symbiont of the bryozoan *Bugula neritina* by Haygood and co-workers.[10] To subsequently clone the genes, a screening of the entire animal metagenome was performed, i.e., the combined genomes of all organisms present in the specimen. This approach led to the isolation of the PKS gene *bryA* with relatively good architectural agreement to the polyketide starter moiety of the bryostatins, suggesting its possible involvement in bryostatin biosynthesis.[11] Expression of *bryA* and the remaining parts of the gene cluster in a culturable bacterium should provide the ultimate proof for their function and allow the generation of a sustainable bryostatin source for drug development.

Metagenomic studies on marine animals are currently impeded by a number of technical challenges. Many bacteria contain more than one pathway for a specific type of natural product. This is exemplified by the eight modular PKS clusters discovered in the sequenced genome of

[#] Based on a Matthew Suffness Award lecture presented at the International Congress on Natural Products Research (45th Annual Meeting of the American Society of Pharmacognosy), Phoenix, AZ (July 21−August 4, 2004).

* Address correspondence to this author at the Kekulé Institute of Organic Chemistry and Biochemistry. Tel: +49-228-732652. Fax: +49-228-739608. E-mail: joern.piel@uni.bonn.de.

[†] University of Bonn.

[‡] Max Planck Institute for Chemical Ecology.

[⊥] University of Tokyo.

[∥] Institute of Molecular Biotechnology.

10.1021/np049612d CCC: $30.25
Published on Web 03/08/2005

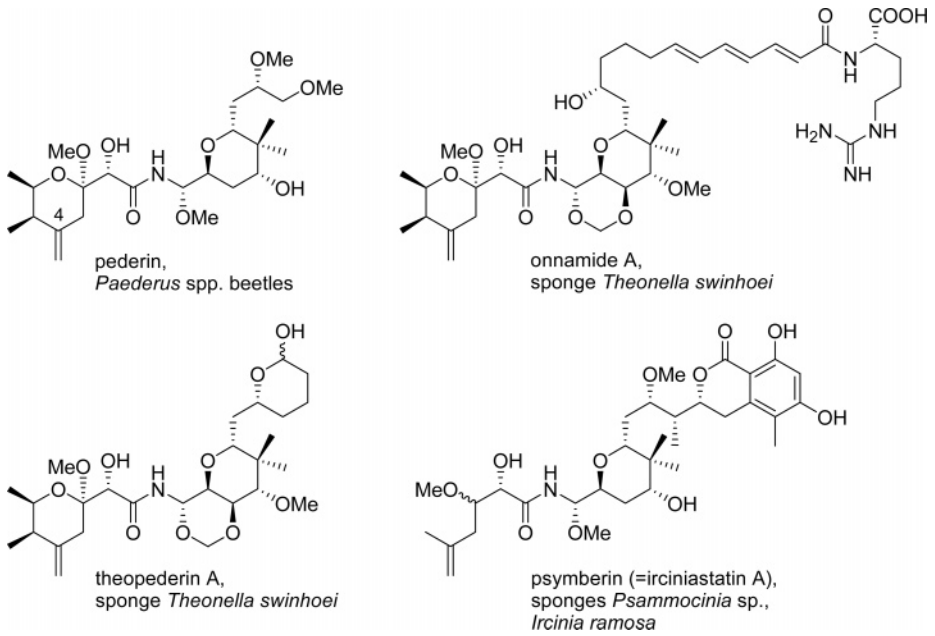

Figure 1. Some members of the pederin group of antitumor polyketides from terrestrial beetles and marine sponges.

Streptomyces avermitilis.[12] A common obstacle in molecular biosynthetic studies is therefore to identify the correct genes among multiple homologues from different pathways. This task is even more demanding in invertebrates, which usually contain multiple prokaryotic genomes. The record-holders in terms of microbial diversity seem to be sponges, of which some specimens have been shown by 16S rDNA to harbor several hundred bacterial species.[13,14] With this problem in mind, we selected *Paederus* spp. beetles and the sponge *Theonella swinhoei* (Gray) (Theonellidae) as model systems. Since these animals differ in their biology and bacterial diversity but contain closely related metabolites, it was hoped that the potential similarity of the biosynthetic pathways would make the corresponding genes identifiable in both metagenomes.

Antitumor Agents of the Pederin Family

Beetles of the genera *Paederus* Fabricius and *Paederidus* Mulsant & Rey (Staphylinidae) contain a hemolymph toxin that causes blistering inflammations on the human skin.[15] In countries with warm climates the insects can swarm in large numbers and represent a considerable nuisance, the symptoms being known to locals under names such as "night burn", "Nairobi eye", or "Dracula". In 1953, Pavan and co-workers isolated the active principle, named pederin, from 25 million collected *Paederus fuscipes* beetles (Figure 1).[16] The structure of this compound suggests that it is derived from polyketide metabolism, which is also supported by feeding studies.[17] Pederin most likely acts as a chemical defense agent, as it has been shown to protect beetle larvae against spiders.[18] Unexpectedly, more than 30 years later closely related substances, among them the mycalamides, onnamides, and theopederins, were discovered in several marine sponges during antitumor screening programs (Figure 1).[19] Today, 36 members constitute the pederin family, which all contain almost identical core regions with variable polyketide or amino acid termini. Most of these natural products exhibit highly potent cytotoxic actitivities due to their ability to inhibit protein biosynthesis.[19] Among the most promising candidates for anticancer drug development is the very recently reported psymberin from a *Psammocinia* sp. sponge[20] (simultaneously described as irciniastatin A from the sponge *Ircinia ramosa*[21]), which displays an impressive selectivity toward solid tumor cells. Unfortunately, nothing is known about the role pederin-type compounds play in sponge biology. As many members exhibit a similar dermatotoxicity to pederin, they might serve as defensive agents against predators or epibionts. The occurrence of almost identical compounds in animals from different phyla has prompted speculation that the actual producers are symbiotic bacteria.[22] This issue has first been addressed experimentally by Kellner and Dettner.[23] They observed that within each examined *Paederus* species pederin is only produced in about 90% of the females, which alone confer the pederin-producing trait to their offspring. The trait can be established artificially in a nonproducing line by feeding eggs of pederin-positive beetles to negative females.[24] Kellner subsequently showed that treatment of the eggs with antibiotics prior to feeding inhibited this trait transfer and proposed the presence of bacterial pederin producers.[25] However, all cultivation attempts to further characterize the suspected symbiont failed.

Paederus spp. beetles offer several important advantages for the study of uncultivated symbionts. They can be reared in the laboratory, do not feed on bacteria, and occur in two different chemotypes that can be compared genetically. Moreover, the presence of pederin-type compounds in sponges provided the unique opportunity for a comparative study of natural product symbiosis in two animal groups with a similar chemistry but entirely different biology. We therefore believed that the beetles represented an ideal starting point to learn about natural product symbioses and at the same time provided a possible access to the more complex marine sponges by comparing PKS genes.

Isolation of Structurally Unusual Polyketide Synthase Genes from the *Paederus fuscipes* Metagenome

PKSs involved in the biosynthesis of bacterial complex polyketides are giant, multifunctional enzymes with modular structure.[26] Each of the PKS modules is usually responsible for the incorporation of a single building block into the polyketide chain. These units are provided by malonyl-CoA or simple derivatives, which are connected by decarboxylative Claisen-type condensations. During

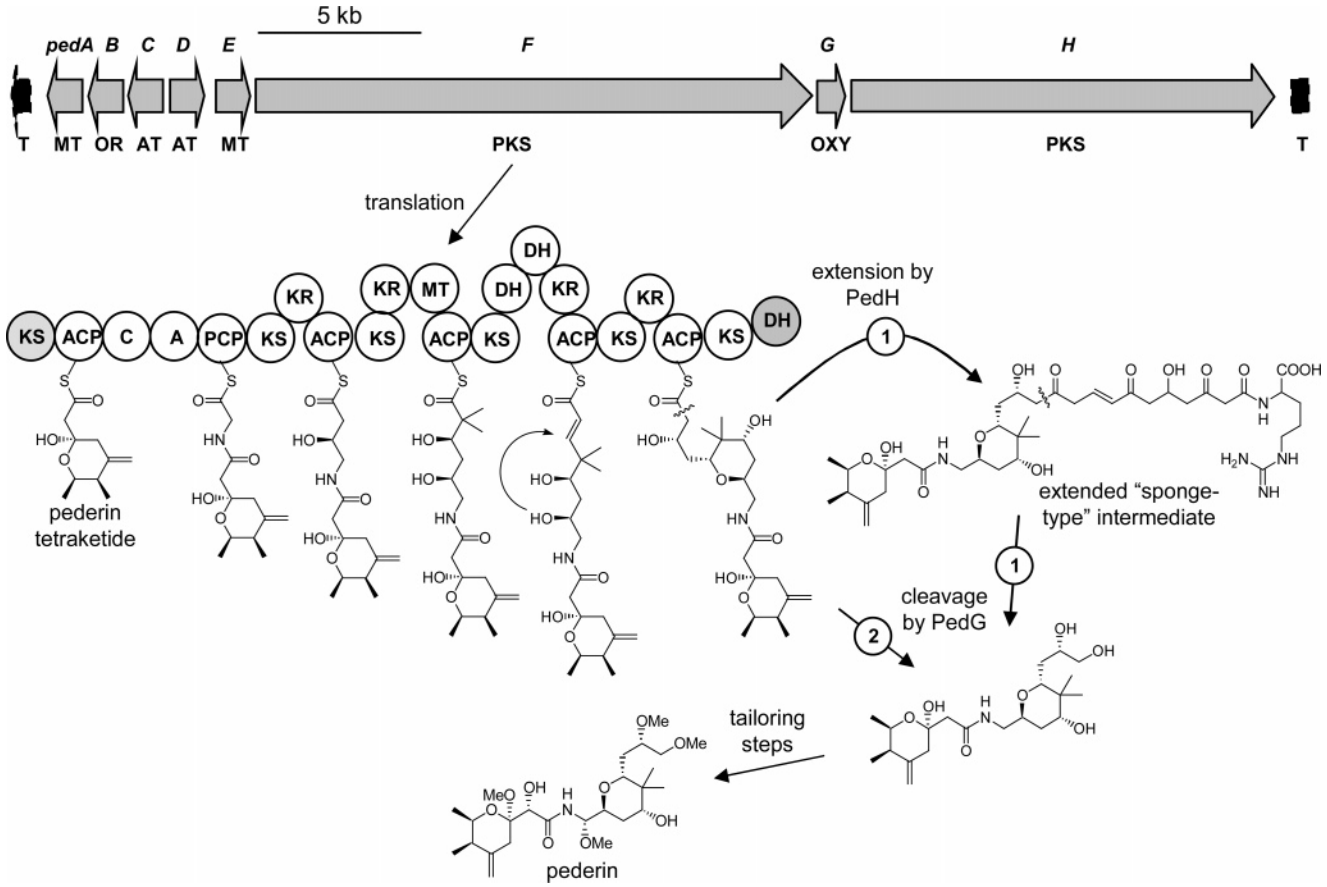

Figure 2. Map of the pederin genes *pedA* to *pedH* and proposed biosynthetic pathway. Gray arrows represent genes of the *ped* cluster; solid black arrows and boxes are gene fragments not belonging to the cluster. The translated protein product PedF contains multiple catalytic domains represented by circles. Domains in gray are predicted to be nonfunctional due to the lack of conserved motifs. Two alternative pathways, 1 and 2, are shown. A, adenylation domain; ACP, acyl carrier protein domain; AT, acyltransferase; C, condensation domain; DH, dehydratase domain; KR, ketoreductase domain; KS, ketosynthase domain; MT, methyltransferase; OR, oxidoreductase; OXY, oxygenase. PCP, peptidyl carrier protein domain; T, transposase.

biosynthesis, variable sets of catalytic domains present on each module determine the outcome of each chain elongation step. The usual minimal equipment is a ketosynthase (KS) domain that performs the condensation reaction, an acyltransferase (AT) domain that selects the corresponding acyl-CoA unit, and an acylcarrier protein (ACP) domain that covalently binds the intermediates. Various further modifications, such as reductions of keto groups or double bonds, dehydrations, or methylations, can be introduced by optional additional domains. Thus, the domain organization of modular PKSs in most cases mirrors the final structure of the natural product. This "collinearity rule" allows basic structure predictions from an analysis of PKS gene sequences and vice versa.

In modular PKSs, several regions of the KS domains are sufficiently well conserved for the design of degenerate oligonucleotide PCR primers. Such primers can therefore be used to survey DNA samples for the presence of PKS genes. When we compared total DNA extracted from several *Paederus* and *Paederidus* species that had been collected at different localities, we observed a strict correlation between the presence of large pederin amounts and the amplification of PKS gene fragments.[27] These fragments therefore belonged either to the pederin PKS itself or to an organism that is present only in pederin-producing beetles. To obtain insights into the modular architecture of the biosynthetic machinery, a corresponding 106 kb genomic region was isolated from a metagenomic library constructed from the total DNA of *Paederus fuscipes* beetles.[27,28] When we sequenced this region, we found a large PKS system containing the 11 genes *pedA* to *pedH*, most of which architecturally match precisely the structure of pederin (Figure 2). A surprising feature of the *ped* system was that none of the PKS modules contains an AT domain that usually selects the extension units during polyketide biosynthesis. Instead, two small, isolated AT genes were found that apparently complement this activity *in trans*. Fragments of other PKSs lacking AT domains had been published earlier,[29,30] and a GenBank search revealed that the *pksX* system with unknown function from *Bacillus subtilis* features a similar "trans-AT" architecture.[31] Subsequent reports of several additional trans-AT PKSs suggest that these enzymes belong to a larger group, whose members are widespread in bacteria.[32,33] Of these, only the leinamycin PKS from *Streptomyces atroolivaceus* has been functionally studied to some extent, which confirmed the trans-acting mechanism of its single AT.[33]

Insights into Pederin Biosynthesis

Not all regions of the isolated *ped* cluster agree with the pederin structure.[27] At the downstream end a large gene, *pedH*, was identified that could not be assigned to any portion of the pederin molecule and would rather correspond to a polyketide with a considerably longer chain (Figure 2). The pederin terminus consists of an unusual oxygenated single-carbon unit, which suggests that it might be generated by oxidative cleavage of an extended precursor. An oxygenase gene, *pedG*, was indeed found in the *ped* cluster, precisely inserted between the two PKS genes *pedF* and *pedH* at a position that corresponds to the cleavage

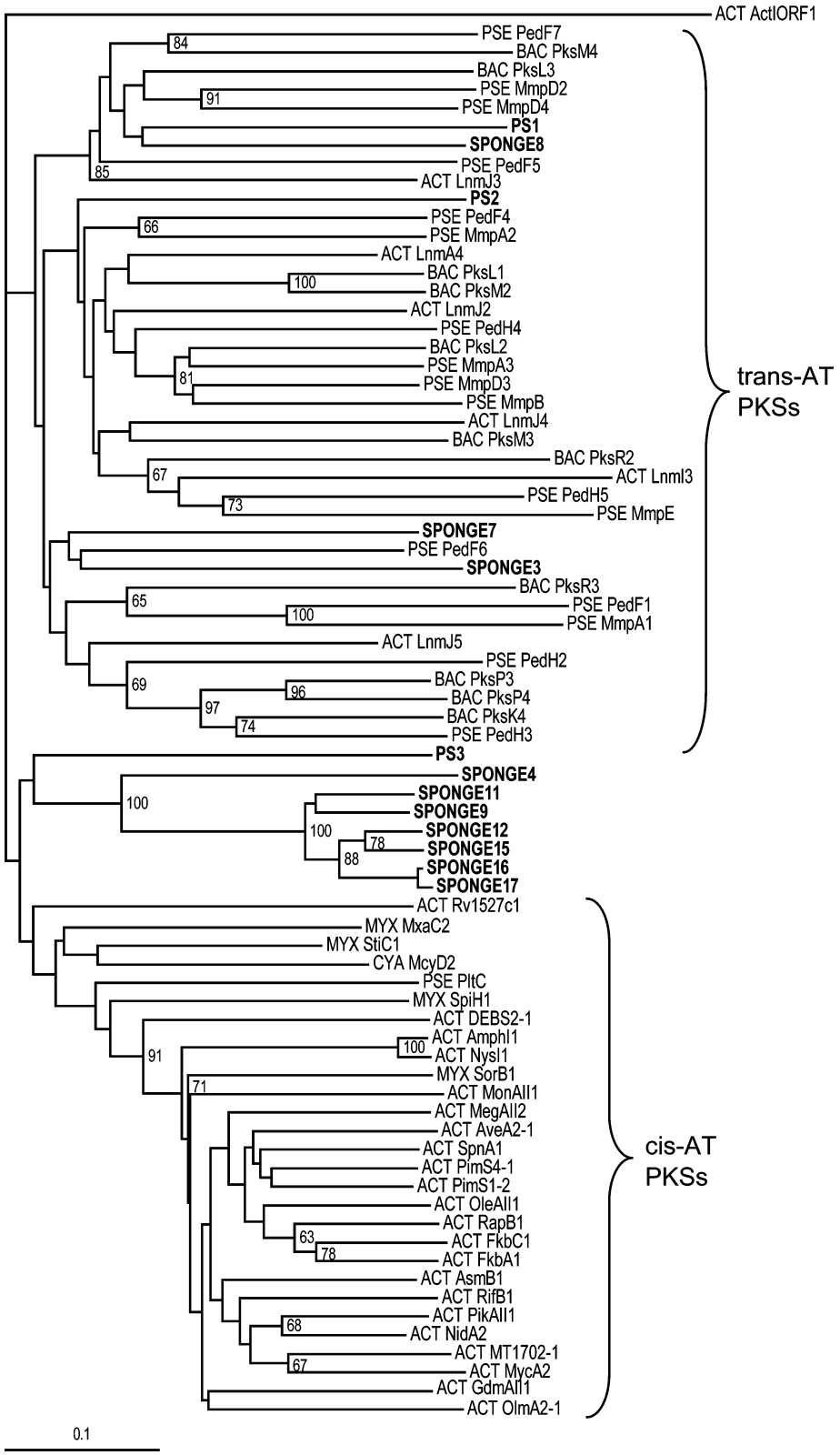

Figure 3. Phylogenetic analysis of bacterial type I KS domains. Sequences corresponding to amino acid residues 118 and 341 of the DEBS2 protein from *Saccharopolyspora erythraea* were aligned. The bacterial origin of the sequence is designated as ACT, Actinobacteria; BAC, Bacilli; PSE, Pseudomonadales; CYA, Cyanobacteria; MYX, Myxococcales. The last digit of each entry specifies the module number from which the KS sequence was derived. Bootstrap values larger than 60% are shown. The type II KS ActII ORF1 was used as outgroup. Entries starting with PS are translated PCR products from beetle bacteria; those starting with SPONGE are derived from the sponge *Theonella swinhoei*. Of sponge sequences with very high sequence identity (>95%) to each other, only one member was included in the tree diagram.

point. The predicted protein product of *pedG* belongs to the FAD-dependent oxygenase family, of which several members have been shown to catalyze Baeyer−Villiger-type cleavage reactions.[34] If the collinearity rule applies to *pedH*, the hypothetical extended precursor with its terminal arginine residue bears a considerable similarity to a large number of sponge-derived compounds, such as onnamide A (Figure 1).[35−37] It is unclear at this point whether indeed

a cleavage of a fully extended intermediate occurs (Figure 2, route 1) or whether *pedH* is merely an evolutionary remnant that plays no role in pederin biosynthesis anymore (route 2). Expression studies of pederin genes in a culturable bacterium, which are currently underway in our laboratory, will clarify the function of *pedH* and could provide some interesting insights into the evolution of pederin biosynthesis.

The second apparently noncollinear feature of the *ped* cluster was the lack of modules at the upstream region that would perform the initial elongation steps in pederin biosynthesis to generate the tetraketide intermediate shown in Figure 2.[27] This suggested to us that some missing genes of the pathway were located elsewhere in the genome. We addressed this problem by analyzing a number of KS PCR products that did not originate from the isolated DNA region and might have been amplified from additional PKS clusters.[38] To avoid the laborious screening of our complex metagenomic library for multiple PKS systems, we attempted to group the unassigned KS fragments into categories. Our rationale was that if the individual modules of the pederin PKS are functionally related to each other or arose from a recent common protein ancestor by module duplication, PCR products belonging to the missing part of the *ped* PKS would group together with the remaining *ped* sequences in a phylogenetic tree and could be recognized. Indeed, when we performed a tree calculation with KS sequences from PKSs of the GenBank database, all trans-AT systems formed a distinct subgroup, while KSs of the more common "cis-AT" type with integrated ATs fell into an independent clade (Figure 3).[38] This implicated that the functional types of PKS systems present in a DNA sample can be rapidly determined by placing PCR-amplified KS gene fragments into a phylogenetic tree. By this strategy, we identified two of our unassigned PCR products (PS1 and PS2 in Figure 3) as belonging to a new trans-AT PKS. Isolation of the genes from the metagenomic library and subsequent sequence analysis confirmed this prediction.[39]

One of the newly identified regions encodes a tetramodular PKS, designated as PedI, that is devoid of AT domains and agrees well with the structure of the pederin tetraketide (Figure 4). Unexpectedly, several features unprecedented in PKSs were found in the deduced protein. The first module, which should be responsible for the incorporation of the acetyl starter unit of pederin, harbors a novel domain with similarity to enzymes of the GCN5-related *N*-acetyltransferase superfamily[40] that likely selects the acetyl group. Other unprecedented components were two domains resembling members of the crotonase (CR) superfamily[41] in the third module. According to the PKS organization, this module corresponds to an unusual exomethylene bond branching off the C-4 position in pederin. Similar carbon branches are known from a small number of other polyketides and have been proposed to arise from a nucleophilic attack of acetyl-CoA or an equivalent on a keto group, followed by a decarboxylative Grob-type fragmentation of the resulting β-hydroxy acid (Figure 4).[32,42] The gene clusters of such branched polyketides invariably contain a 3-hydroxy-3-methylglutaryl-CoA synthase-like gene that is usually grouped with genes encoding free-standing KS, ACP, and CR homologues (the latter usually annotated as enoyl-CoA hydratases, a subgroup of the CR superfamily).[32,33,42] The unusual CR domains in the *ped* system might therefore participate in the biosynthesis of the exomethylene group. The fact that CRs can also be integrated as domains into modules documents an impressive biosynthetic versatility of PKSs. Further studies will show whether these features could be exploited to generate novel branched polyketides by engineering CR domains into other PKS modules.

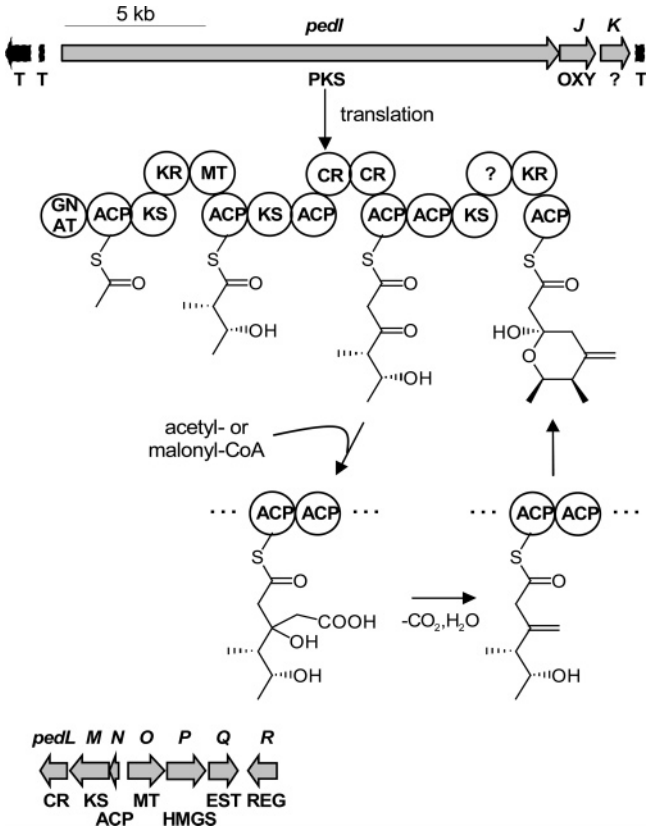

Figure 4. Map of the remaining *ped* genes and proposed biosynthesis of the pederin tetraketide. The two gene clusters *pedIJK* and *pedLMNOPQR* were isolated from different genome regions. GNAT, GCN5-related *N*-acetyltransferase superfamily; CR, crotonase superfamily; EST, esterase; HMGS, 3-hydroxy-3-methylglutaryl-CoA synthase; REG, regulator.

Remarkably, the symbiont genome contains a third isolated set of *ped* genes, *pedL*−*pedP* (Figure 4), which were found during an ongoing genome project after the discovery of homologues in the marine sponge *Theonella swinhoei* (see below).[43] The genes likely participate in exomethylene bond formation, regulation and attachment of an *O*-methyl group. To our knowledge this is the only reported PKS system, the genes of which are distributed on three distinct genomic regions. The reasons for this unusually disjointed architecture are unknown, but one possible explanation is suggested by the large number of transposase gene fragments located at both sides of each *ped* region.[28,39] As transposases catalyze the translocation of DNA to different genome regions,[44] such enzymes could have caused a fragmentation of a formerly intact gene cluster.

Identification of the Polyketide Producer

On both sides outside the individual *ped* subclusters, we found large regions unrelated to polyketide biosynthesis.[28,39] These contain numerous genes with prokaryotic architectures and exceptionally high nucleotide similarities to homologues from the bacterium *Pseudomonas aeruginosa*, an opportunistic pathogen that also infects insects.[45] Many genes belong to primary metabolism and are involved in amino acid and vitamin biosynthesis according to their homologies. Particularly suggestive of a bacterial origin are several genes from pathways that are absent in animals,

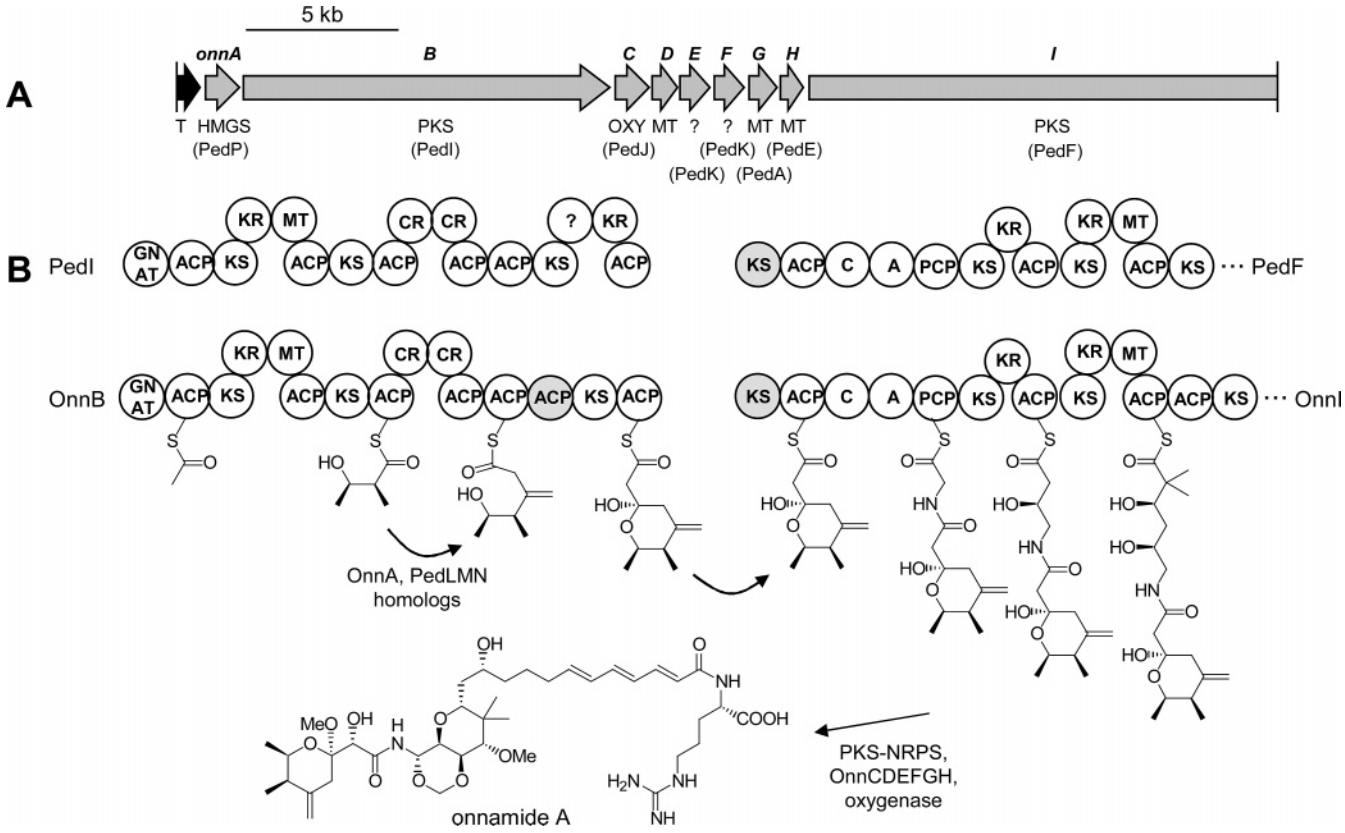

Figure 5. (A) Map of the isolated *onn* genes from the *T. swinhoei* symbiont. Proteins with closest homologies are shown in parentheses. (B) Comparison of the translated PKS-NRPS protein from the beetle and sponge symbiont and predicted onnamide/theopederin biosynthesis.

such as the biosynthesis of vitamins B_6 and B_{12}. To obtain independent evidence for the existence of a polyketide-producing bacterium, we mechanically separated extracellular bacteria from insect tissues by differential centrifugation. This procedure resulted in an enrichment of PKS and *Pseudomonas*-type genes by several orders of magnitude, which is consistent with a bacterial origin of the *ped* genes.[28] Our results are consistent with a 16S rDNA study that has been reported by Kellner and showed that only pederin-positive beetles contain a bacterium with closest relationship to *P. aeruginosa*.[46] To obtain specific insights into the biology of this as-yet unculturable symbiont, we have recently initiated a genome project. The data obtained so far reveal a remarkable overall similarity to *P. aeruginosa*, but also the presence of numerous rearrangements, deletions, and several interspersed islands specific to the symbiont. The picture that is beginning to emerge suggests that the beetle symbiont evolved from a free-living *Pseudomonas* ancestor that already contained pederin-type genes and was presumably an insect pathogen. The protective function of pederin may have provided a mechanism of selection for infected insect specimens that fostered the evolution of a mutualistic relationship. The benefits of this association for the symbiont might be the availability of a nutrient-rich habitat free of competing microorganisms. It is currently unknown why some beetle specimens do not harbor the symbiont. *Paederus* spp. belong to the few members of the Staphylinid family that are brightly colored, which may serve as warning to predators. The coexistence of symbiotic and aposymbiotic females may be the result of several factors that outbalance themselves, such as metabolic costs of high symbiont loads, benefits of aposematic color mimicry for symbiont-free specimens, bottlenecks of symbiont transfer at the egg stage, and lower survival rates of aposymbiotic beetle lines. Genomic studies and the development of cultivation techniques for the pederin producer will likely help to dissect the mechanisms underlying this unique symbiosis.

Cloning of Pederin-Type Genes from the Metagenome of the Sponge *Theonella swinhoei*

Japanese specimens of the marine sponge *T. swinhoei* with a yellow interior are the source of most reported marine members of the pederin family. Compounds with a terminal arginine residue were described as onnamides, and those with a polyketide terminus as theopederins.[19] Interestingly, a second, entirely different chemotype of *T. swinhoei* with a white interior that is devoid of pederin-type natural products grows in the same habitats. This offers the opportunity to compare specimens at the chemical and the genetic level. A PCR-based survey of PKSs encoded on the metagenome of the yellow sponge revealed a large number of different homologues.[38] This finding had been expected, as *T. swinhoei* is known for its tremendous bacterial and metabolic diversity. We anticipated that the onnamide PKS would be closely related to the *ped* system and therefore likely exhibit a trans-AT architecture. A phylogenetic tree calculation from the sponge-derived PCR products and PKS sequences from GenBank indeed predicted that three of the *T. swinhoei* sequences should belong to the trans-AT type (Figure 3).[38] The vast majority of PCR products could therefore be neglected in our search for the onnamide/theopederin genes. The trans-AT sequences were not found in an onnamide-negative sponge, which supported our assumption that we had selected the correct PCR products.[43] To isolate the corresponding gene cluster, we constructed a metagenomic cosmid library from the sponge DNA and screened 60 000 clones using a clone pooling strategy. This resulted in a single positive cosmid for one trans-AT sequence.[43]

A comparison of the PKS cluster present on the isolated DNA region (designated as *onn* cluster) with the *ped* system (Figure 5) shows that both are strikingly similar to each other. Although nucleotide homologies are low, both PKSs contain almost identical domains, including previously unique PKS components such as the GNAT and the two CR domains. The two clusters should therefore be involved in the biosynthesis of polyketides with closely related structures, which strongly argues for their role in the production of pederin-type compounds. Minor differences between the two PKSs are the number of repeated ACP domains and the lack in the *onn* PKS of a KR domain preceded by a region with unknown function, both of which could not be reasonably correlated with any structural counterpart. According to the collinearity rule, the cloned *onn* genes should correspond to the region of the onnamide/theopederin molecule that is largely identical with pederin. Since the sponge metabolites differ from pederin almost exclusively in the polyketide terminus, differences between the *onn* and the *ped* PKS genes would therefore be expected only for the genes downstream of the cloned *onn* region. However, the enormous complexity of the sponge DNA library has so far prevented the isolation of neighboring genome sections to gain insight into the structure of these genes. An interesting feature of the isolated *onn* region is that it combines genes whose homologues are distributed on three different regions on the *Paederus* symbiont genome. This supports the idea that the pederin genes once constituted a similar intact cluster that was subsequently fragmented by genomic rearrangement processes, such as gene transposition.

How are the sponge polyketides produced? The clustered arrangement of the genes, the presence of purine-rich motifs resembling bacterial ribosome-binding sites in front of each gene, and the lack of eukaryotic features such as promoter regions, introns, and polyadenylation sites all point to a bacterial origin.[43] However, no genes outside of the *onn* system have yet been isolated that could provide detailed taxonomic information. Since a part of the symbiont genome is now known, genome walking using a larger library will yield adjacent genome regions that should be useful to identify the symbiont. Then the interesting question can be answered as to whether the pederin and onnamide producer both belong to the genus *Pseudomonas* or whether they are unrelated. Genetic data indicate that the pederin genes have been introduced into the beetle symbiont by horizontal transfer from an unknown source.[28] In this case, a distant relationship between both symbionts is not unlikely. Horizontal transfer would also explain the phenomenon that animals from entirely different phyla and habitats contain almost identical metabolites.

Concluding Remarks

Circumstantial evidence suggests that biosynthetically active symbionts might have a considerable impact on the natural product chemistry and biology of many animals. Our studies on the pederin-type antitumor polyketides have provided the first deeper insights on a genetic basis into the chemistry and biology of these elusive bacteria. The fact that biosynthetic genes from the sponge are clustered together and can be isolated from the metagenome suggests that gene cloning and their heterologous expression in a culturable bacterial host could be a general strategy to develop renewable sources of otherwise inaccessible drug candidates. An important goal of the future is therefore to develop efficient strategies that permit the rapid cloning of target genes from highly complex metagenomic libraries. The results also raise a number of intriguing questions in chemical ecology: How widespread is natural product symbiosis? What is the function of the metabolites for the host? Which factors of each partner are involved in establishment and maintenance of symbioses? Can cultivation methods be developed? Our model organisms *P. fuscipes* and *T. swinhoei* represent ideal systems to address these issues.

Acknowledgment. The authors gratefully acknowledge generous financial support from the Deutsche Forschungsgemeinschaft (Pi430/1-1, Pi430/1-2, and Pi430/2-1), the Japanese Foundation for the Promotion of Science (S02275), and the DECHEMA e.V. (Young Investigator Award in Natural Products Research) to J.P.

References and Notes

(1) Piel, J. *Nat. Prod. Rep.* **2004**, *21*, 519−538.
(2) Bewley, C. A.; Faulkner, D. J. *Angew. Chem., Int. Ed.* **1998**, *37*, 2163−2178.
(3) Hildebrand, M.; Waggoner, L. E.; Lim, G. E.; Sharp, K. H.; Ridley, C. P.; Haygood, M. G. *Nat. Prod. Rep.* **2004**, *21*, 122−142.
(4) Faulkner, D. J. *Nat. Prod. Rep.* **2000**, *17*, 1−6.
(5) Newman, D. J.; Cragg, G. M. *J. Nat. Prod.* **2004**, *67*, 1216−1238.
(6) Cane, D. E.; Walsh, C. T. *Chem. Biol.* **1999**, *6*, R319−R325.
(7) Unson, M. D.; Faulkner, D. J. *Experientia* **1993**, *49*, 349−353.
(8) Unson, M. D.; Holland, N. D.; Faulkner, D. J. *Mar. Biol.* **1994**, *119*, 1−11.
(9) Bewley, C. A.; Holland, N. D.; Faulkner, D. J. *Experientia* **1996**, *52*, 716−722.
(10) Davidson, S. K.; Allen, S. W.; Lim, G. E.; Anderson, C. M.; Haygood, M. G. *Appl. Environ. Microbiol.* **2001**, *67*, 4531−4537.
(11) Hildebrand, M.; Waggoner, L. E.; Liu, H.; Sudeck, S.; Allen, S.; Anderson, C.; Sherman, D. H.; Haygood, M. G. *Chem. Biol.* **2004**, *11*, 1543−1552.
(12) Ikeda, H.; Ishikawa, J.; Hanamoto, A.; Shinose, M.; Kikuchi, H.; Shiba, T.; Sakaki, Y.; Hattori, M.; Omura, S. *Nat. Biotechnol.* **2003**, *21*, 526−531.
(13) Webster, N. S.; Wilson, K. J.; Blackall, L. L.; Hill, R. T. *Appl. Environ. Microbiol.* **2001**, *67*, 434−444.
(14) Hentschel, U.; Hopke, J.; Horn, M.; Friedrich, A. B.; Wagner, M.; Hacker, J.; Moore, B. S. *Appl. Environ. Microbiol.* **2002**, *68*, 4431−4440.
(15) Borroni, G.; Brazzelli, V.; Rosso, R.; Pavan, M. *Am. J. Dermatopathol.* **1991**, *13*, 467−474.
(16) Pavan, M.; Bo, G. *Physiol. Comput. Oecol.* **1953**, *3*, 307−312.
(17) Cardani, C.; Fuganti, C.; Ghiringhelli, D.; Grasselli, P.; Pavan, M.; Valcurone, M. D. *Tetrahedron Lett.* **1973**, 2815−2818.
(18) Kellner, R. L. L.; Dettner, K. *Oecologia* **1996**, *107*, 293−300.
(19) Narquizian, R.; Kocienski, P. J. In *The Role of Natural Products in Drug Discovery*; Mulzer, R., Bohlmann, R., Eds.; Springer-Verlag: Heidelberg, 2000; Vol. 32.
(20) Cichewicz, R. H.; Valeriote, F. A.; Crews, P. *Org. Lett.* **2004**, *6*, 1951−1954.
(21) Pettit, G. R.; Xu, J. P.; Chapuis, J. C.; Pettit, R. K.; Tackett, L. P.; Doubek, D. L.; Hooper, J. N. A.; Schmidt, J. M. *J. Med. Chem.* **2004**, *47*, 1149−1152.
(22) Fusetani, N.; Matsunaga, S. *Chem. Rev.* **1993**, *93*, 1793−1806.
(23) Kellner, R. L. L.; Dettner, K. *J. Chem. Ecol.* **1995**, *21*, 1719−1733.
(24) Kellner, R. L. L. *Chemoecology* **2001**, *11*, 127−130.
(25) Kellner, R. L. L. *J. Insect Physiol.* **2001**, *47*, 475−483.
(26) Rawlings, B. J. *Nat. Prod. Rep.* **2001**, *18*, 231−281.
(27) Piel, J. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 14002−14007.
(28) Piel, J.; Höfer, I.; Hui, D. *J. Bacteriol.* **2004**, *186*, 1280−1286.
(29) Paitan, Y.; Alon, G.; Orr, E.; Ron, E. Z.; Rosenberg, E. *J. Mol. Biol.* **1999**, *286*, 465−474.
(30) Huang, G. Z.; Zhang, L. H.; Birch, R. G. *Microbiol.-UK* **2001**, *147*, 631−642.
(31) Albertini, A. M.; Caramori, T.; Scoffone, F.; Scotti, C.; Galizzi, A. *Microbiol.-UK* **1995**, *141*, 299−309.
(32) El-Sayed, A. K.; Hothersall, J.; Cooper, S. M.; Stephens, E.; Simpson, T. J.; Thomas, C. M. *Chem. Biol.* **2003**, *10*, 419−30.
(33) Cheng, Y. Q.; Tang, G. L.; Shen, B. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 3149−3154.
(34) Fraaije, M. W.; Kamerbeek, N. M.; van Berkel, W. J. H.; Janssen, D. B. *FEBS Lett.* **2002**, *518*, 43−47.
(35) Kobayashi, J.; Itagaki, F.; Shigemori, H.; Sasaki, T. *J. Nat. Prod.* **1993**, *56*, 976−981.
(36) Matsunaga, S.; Fusetani, N.; Nakao, Y. *Tetrahedron* **1992**, *48*, 8369−8376.
(37) Sakemi, S.; Ichiba, T.; Kohmoto, S.; Saucy, G.; Higa, T. *J. Am. Chem. Soc.* **1988**, *110*, 4851−4853.
(38) Piel, J.; Hui, D.; Fusetani, N.; Matsunaga, S. *Environ. Microbiol.* **2004**, *921−927*.
(39) Piel, J.; Wen, G.; Platzer, M.; Hui, D. *ChemBioChem* **2004**, *5*, 93−98.
(40) Neuwald, A. F.; Landsman, D. *Trends Biochem. Sci.* **1997**, *22*, 154−155.

(41) Holden, H. M.; Benning, M. M.; Haller, T.; Gerlt, J. A. *Acc. Chem. Res.* **2001**, *34*, 145−157.
(42) Edwards, D. J.; Marquez, B. L.; Nogle, L. M.; McPhail, K.; Goeger, D. E.; Roberts, M. A.; Gerwick, W. H. *Chem. Biol.* **2004**, *11*, 817−833.
(43) Piel, J.; Hui, D.; Wen, G.; Butzke, D.; Platzer, M.; Fusetani, N.; Matsunaga, S. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 16222−16227.
(44) Mahillon, J.; Chandler, M. *Microbiol. Mol. Biol. Rev.* **1998**, *62*, 725−774.
(45) Jander, G.; Rahme, L. G.; Ausubel, F. M. *J. Bacteriol.* **2000**, *182*, 3843−3845.
(46) Kellner, R. L. L. *Insect Biochem.* **2002**, *32*, 389−395.

NP049612D