“眠っている”生合成遺伝子を起こす別の方法

加藤直樹

微生物ポストゲノム研究の主要なトピックスの一つとして二次代謝を挙げることができる。数百に上る微生物ゲノムが解読され、その結果、我々の予想を遙かに凌ぐ数の二次代謝産物生合成遺伝子群の存在が明らかになっている。しかしながら、その大半は通常の実験室の培養条件では“眠った”状態にあることが知られている。そういった遺伝子にアクセスする様々なアプローチが試みられ、最近、遺伝子発現の制御系を改変することにより、化合物の同定に成功した例が報告されている（参照；JC #460, 462)。今回はそれらとは全く異なる方法で、眠った遺伝子の活性化に成功しているので取り上げる。微生物の共培養による、化合物の生産誘導を分子レベルで証明したのは初めての例であり、拡散性のシグナル分子ではなく、物理的な相互作用がその誘導に関わっていることが示唆されており、非常に興味深い。

紹介論文

“Intimate bacterial–fungal interaction triggers biosynthesis of archetypal polyketides in *Aspergillus nidulans*” Schroeckh, V., et al., Hertweck, C., & Brakhage, A. A. (Leibniz Institute for Natural Product Research and Infection Biology–HKI, Jena, Germany)

PNAS, 106(34), 14558-14563 (2009)

要旨

カビは多様な生物活性を有する多数の小分子化合物を生産する。しかしながら、カビの全ゲノム配列の解読により、それらの有する二次代謝産物生産能を過小評価していたことが明らかになった。それは、多くの生合成遺伝子群が通常の実験室で用いられる培養条件では“眠っている”ためであり、それらの遺伝子発現が活性化する生理的な条件を突き止めることは、重要な課題の一つであると言える。我々はそこで、モデル糸状菌である *Aspergillus nidulans* を 58 種類の土壌放線菌と共培養し、発現解析により、カビと細菌の相互作用がカビの二次代謝遺伝子を特異的に活性化することを実証した。驚くべきことに、透析実験と電子顕微鏡観察の結果は、細菌とカビの菌糸の密接した物理的な相互作用が、特異的な応答を誘発するのに必要であることを示唆している。遺伝子破壊実験により、発現が誘導された遺伝子クラスターのひとつが、orsellinic acid とその関連化合物の生合成を担っていることを確認した。以上の結果は、ドメインの異なる微生物間での特異的な相互作用の存在を証明しており、拡散性のシグナル分子だけでなく、物理的な相互作用もまた、微生物間のコミュニケーションならびに“眠った”生合成遺伝子の発現誘導に一役買っている可能性を支持している。

Another way to activate silent gene clusters

"Intimate bacterial-fungal interaction triggers biosynthesis of archetypal polyketides in *Aspergillus nidulans*"

Schroeckh, V., Scherlach, K., Nutzmann, H.-W., Shelest, E., Schmidt-Heck, W., Schuemann, J., Martin, K., Hertweck, C.* & Brakhage, A. A.*
Leibniz Institute for Natural Product Research and Infection Biology–HKI

PNAS, **106**, 14558-14563 (2009)

Many biosynthetic genes, few products

Thousands of micobial genome sequences

Genes typically involved in secondary metabolite production (PKS, NRPS, etc.)

>>

Known microbial secondary metabolites

Most of the genes remain silent under standard laboratory conditions…

ex.) a model fungus *Aspergillus nidulans*

46 predicted biosynthetic gene clusters >> Products of 9 gene cluster identified

Ectopic expression of pathway regulators
Epigenetic methods (histone MT, HDAC, SUMO)

It is important to understand the physiological conditions under which such dormant genes are activated.

What are the triggers to elicit silent biosynthetic pathways?

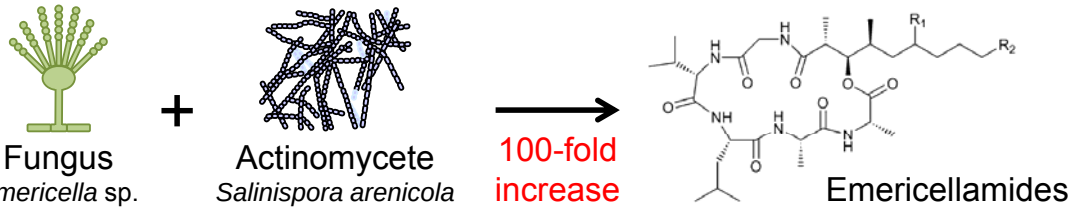
Communication between organisms could be the trigger for the activation.

A common language in the microbial world is probably "chemistry"-based.

Huge natural product diversity

pheromones, predator-prey molecules, metabolites of symbiotic associations, etc.

This concept has been applied to screen natural products.

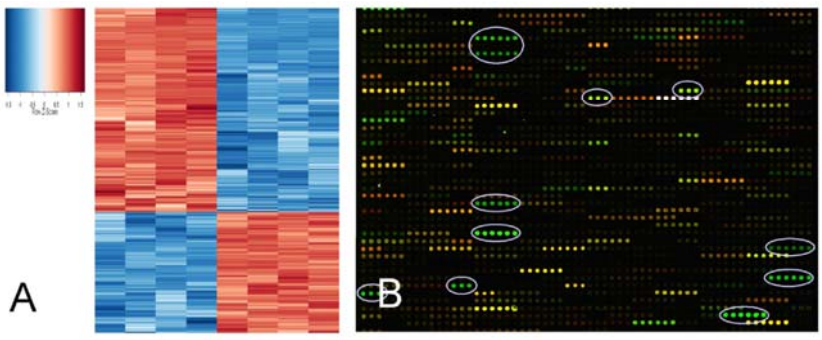


J. Nat. Prod. **70**, 515-520 (2007)

However, most of such studies are empirical and at chemical level.

Objectives

To monitor the induced expression of silent biosynthetic gene clusters in the model fungus *A. nidulans* systematically by microarray-based approaches.



To examine whether the induction is triggered by the interaction between the fungus and actinomycetes sharing the same habitat.

Cocultivation experiments

Preculture

Conidia of *A. nidulans* wild-type strain
Inoculated into 100-mL minimum medium (10^8 conidia per mL)

o/n

Collected by filtration
Transfer to 100-mL fresh minimum medium

Co-culture

Added 5 mL of a freshly prepared actinomycete culture
Incubated at 37˚C with shaking

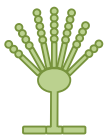
→ 3 h **Transcriptome analyses**

Microarray (ASMA, full genome), Northern blotting, & qPCR

→ 24 h ~~HPLC analysis of metabolites~~

Transcriptome analyses (1)

Cocultivation



A. nidulans

×

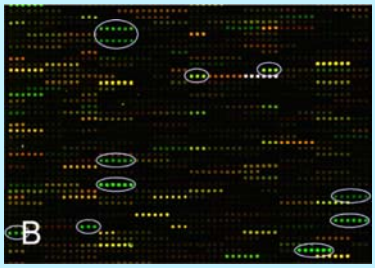
58 soil actinomycetes

(→See page 16 for list of actinomycetes tested)

Expression analysis using ASMA

ASMA (*A. nidulans* secondary metabolism array)

- Comprising genes encoding backbone & tailoring enzymes + regulatory genes
- 25-mers corresponding to 3' half of each gene were spotted in hexaplicates



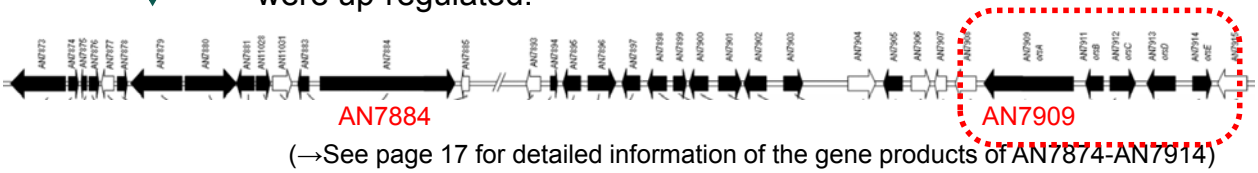
Only a single strain specifically induced the fungal gene expression.
└ *Streptomyces hygroscopicus* ATCC 29253

2 backbone genes, AN7909 (PKS) and AN7884 (NRPS), were up-regulated.

Transcriptome analyses (2)

Full-genome microarray

- 248 genes were up-regulated and 147 down.
- Most of the genes located near AN7909 and AN7884 were up-regulated.



(→See page 17 for detailed information of the gene products of AN7874-AN7914)

Northern blotting

- Induced expression of the whole region with some exceptions

(→See page 18 for Northern blot analyses of this region)

qRT-PCR

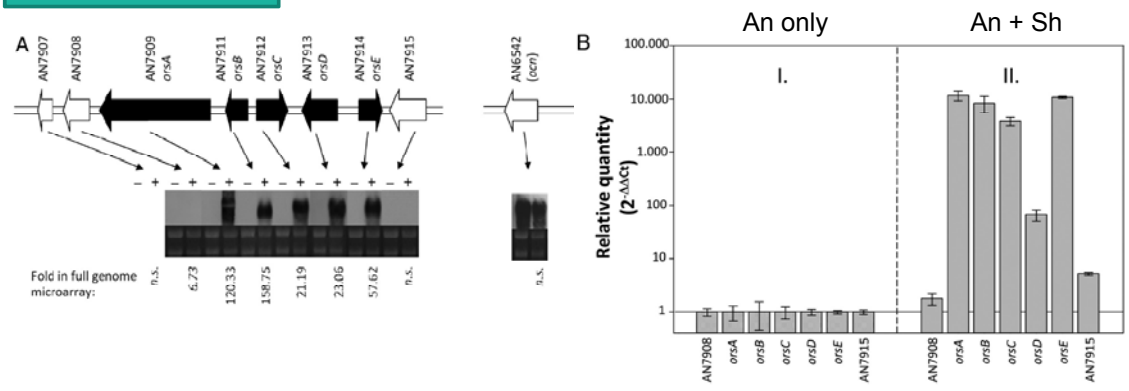
- The specific induction of the biosynthetic gene cluster from AN7909 to AN7914

Activation of the biosynthetic gene cluster from AN7909 to AN7914

Full-genome microarray

Northern blotting

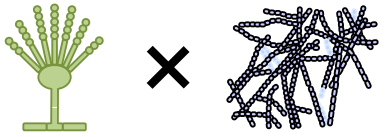
qRT-PCR



A distinct fungal-bacterial interaction leads to the specific activation of fungal gene clusters.

Next question is …

1



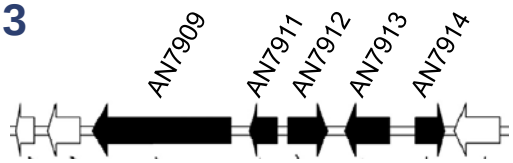
A. nidulans × *S. hygroscopicus*

Only a single strain specifically induced the fungal gene expression.

2

What causes the specific activation?

3



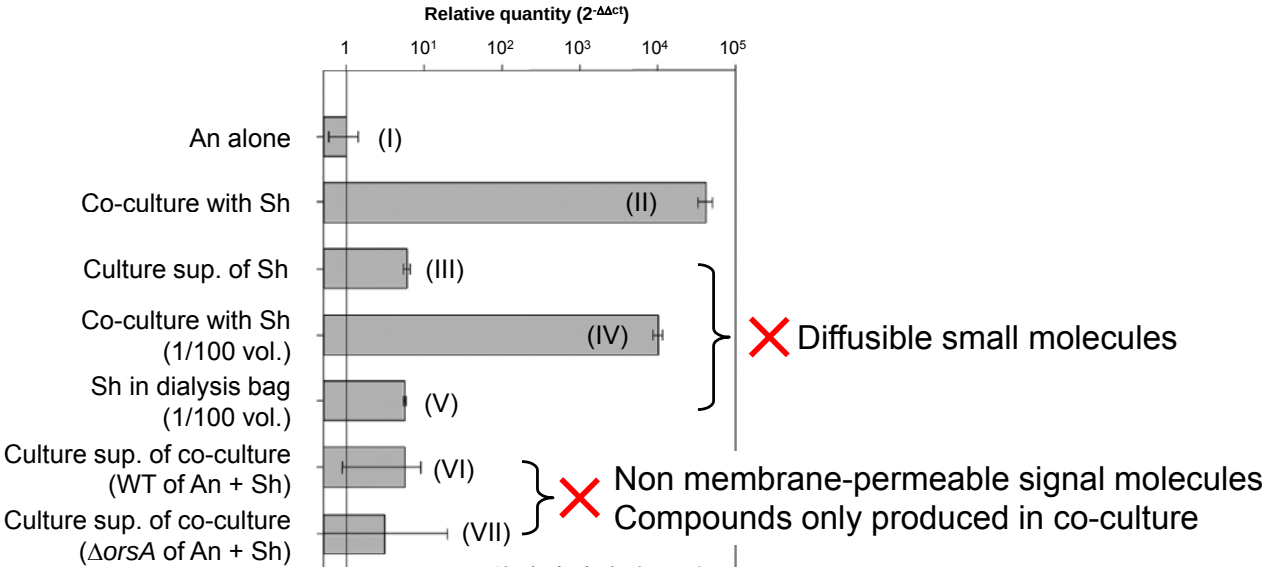
The biosynthetic gene cluster from AN7909 to AN7914 was clearly up-regulated by co-cultivation.

4

New metabolites?

What caused the specific activation?

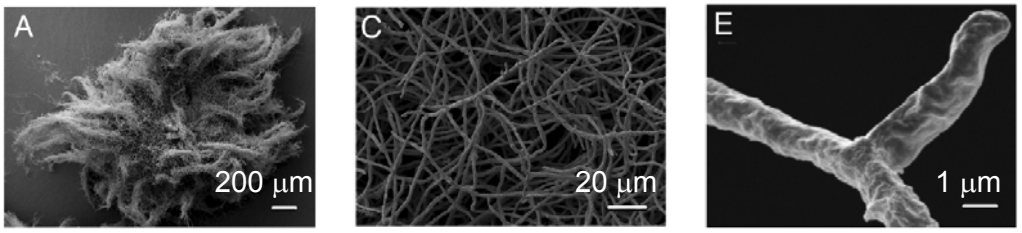
Expression analysis of the PKS gene AN7909 (*orsA*) by qRT-PCR



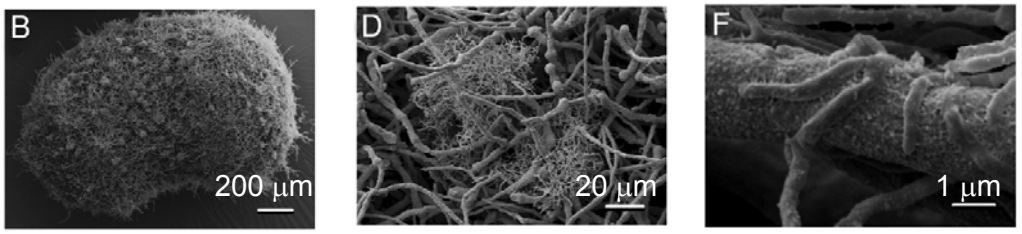
The fungal response appeared to depend on the direct contact with the bacterium.

Scanning electron microscopy of the co-culture

A. nidulans alone



A. nidulans cocultivated with *S. hygroscopicus*

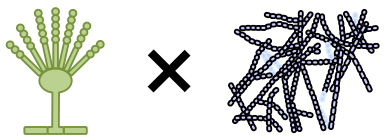


Magnify

Intimate physical interaction of the bacterium and fungal mycelia was observed.

Next question is …

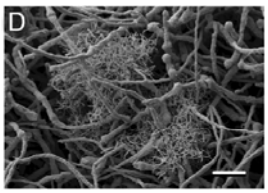
1



A. nidulans × *S. hygroscopicus*

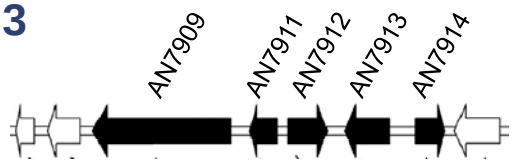
Only a single strain specifically induced the fungal gene expression.

2



The intimate physical interaction was required for the induction.

3



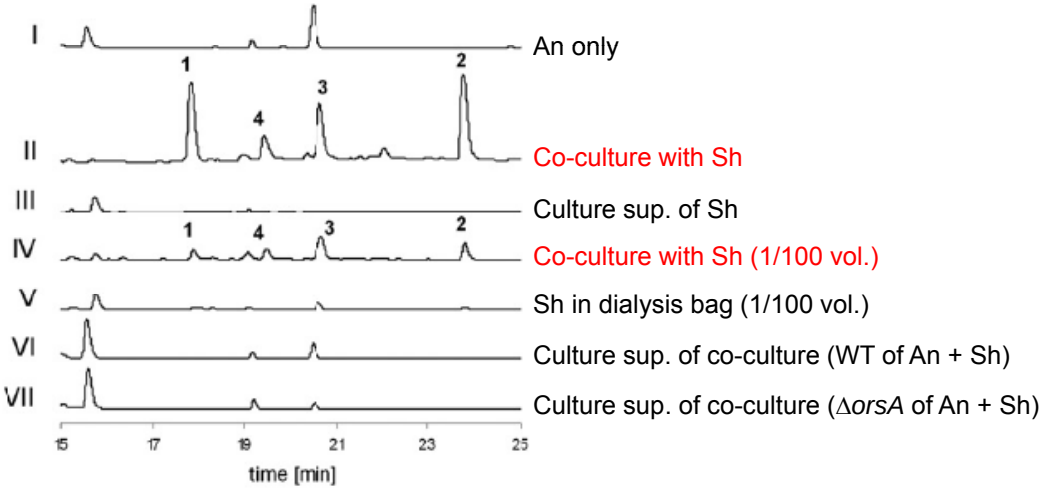
The biosynthetic gene cluster from AN7909 to AN7914 was clearly up-regulated by co-cultivation.

4

New metabolites?

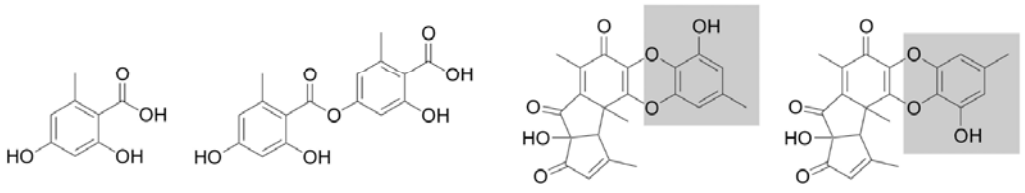
New metabolites?

HPLC profiles of the culture extracts under the conditions I-VII



→ New peaks (**1**-**4**) were detected only when *A. nidulans* could interact physically with *S. hygroscopicus*.

Orsellinic acid and related compounds

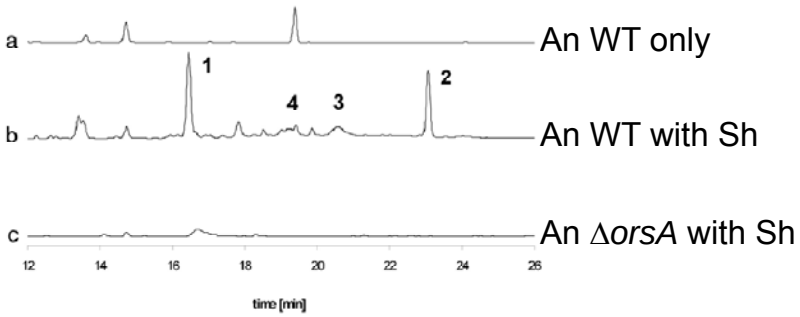


Orsellinic acid (**1**) Lecanoric acid (**2**) F-9775A (**3**) F-9775B (**4**)

A typical lichen metabolite (地衣類)

Cathepsin K inhibitors

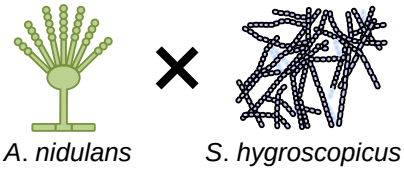
Knockout experiment of the PKS gene AN7909 (*orsA*)



The PKS gene is required for the biosynthesis of **1**.
Compound **1** serves as a biosynthetic building block for **2**-**4**.

Summary

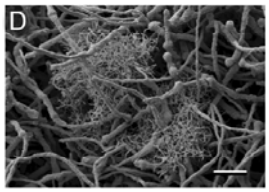
1



A. nidulans × *S. hygroscopicus*

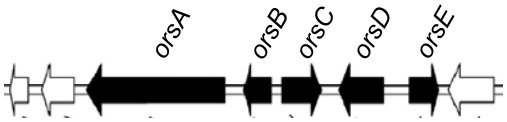
Only a single strain specifically induced the fungal gene expression.

2



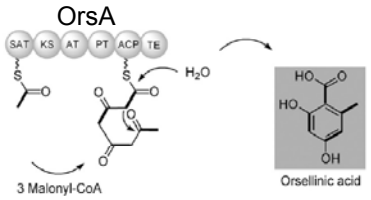
The intimate physical interaction was required for the induction.

3



The *ors* gene cluster was clearly up-regulated by co-cultivation.

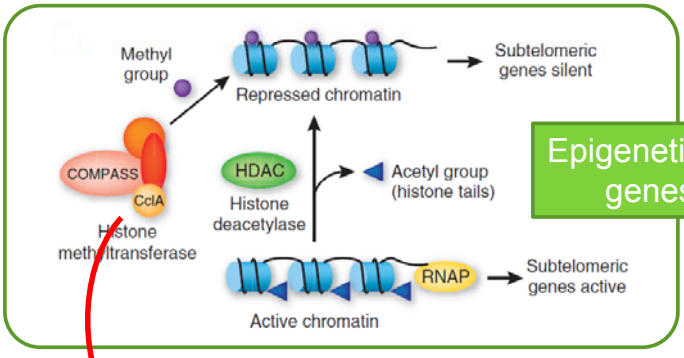
4



Orsellinic acid and related compounds

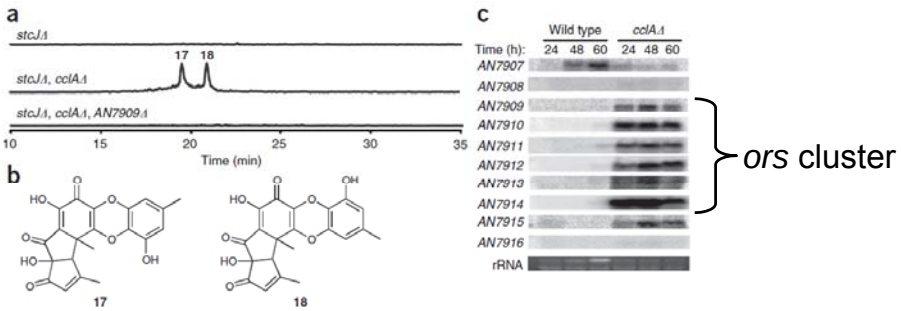
Co-cultivation systems will provide a new strategy for activation of silent biosynthetic gene clusters. *…is it true?*

Is this a coincidence, or...?



Epigenetic regulation of biosynthetic genes at the chromatin level

Knockout of the *cclA* gene



ors cluster

F-9775A/B were also detected in the Δ*cclA* mutant

Nat. Chem. Biol. **5**, 450-452, 462-464 (2009)

Actinomycetes tested for induction of silent gene clusters

Taxon and strain		
Actinomadura hibisca ATCC 53646	*Streptomyces antibioticus* ATCC 8663	*Streptomyces nogalater* DSM 40546
Amycolatopsis mediterranei ATCC 13685	*Streptomyces antibioticus* ATCC 8663	*Streptomyces olivaceus* ATCC 11626
Amycolatopsis sulphurea DSM 46092	*Streptomyces aurantiacus* JA 04570	*Streptomyces pactum* DSM 40530
Kitasatospora azatica DSM 41650	*Streptomyces aureofaciens* ATCC 10762	*Streptomyces ramulosus* ATCC 19802
Kitasatospora griseola DSM 43859	*Streptomyces chartreusis* DSM 41255	*Streptomyces rimosus* JA 04696
Kitasatospora mediocidica ATCC 49055	*Streptomyces clavuligerus* DSM 738	*Streptomyces rochei* ATCC 10739
Kitasatospora phosalacinea DSM 43860	*Streptomyces collinus* DSM 40733	*Streptomyces* sp. D-82
Kitasatospora setae DSM 43861	*Streptomyces eurythermus* DSM 40014	*Streptomyces* sp. D-58
Micromonospora olivasterospora IFO 14303	*Streptomyces fradiae* ATCC 10745	*Streptomyces* sp. (*avermitilis*) ATCC 31267
Micromonospora sp. (*fusca*) CBS 253.48	*Streptomyces galilaeus* ATCC 31649	*Streptomyces* sp. (*brunneogriseus*) ATCC 29772
Micromonospora sp. (*inyoensis*) NRRL 3292	*Streptomyces gelaticus* DSM 40065	*Streptomyces* sp. (*erythrochromogenes*) JA 04400
Nonomuraea pusilla DSM 43357	*Streptomyces gougerotii* DSM 40324	*Streptomyces* sp. (*humifer*) DSM 40602
Saccharopolyspora erythrea ATCC 11635	*Streptomyces griseus* NCIB 9004	*Streptomyces* sp. (*luteolutescens*) JA 04654
Saccharopolyspora hirsuta ATCC 27875	*Streptomyces hygroscopicus* ATCC 53730	*Streptomyces* sp. (*viridifaciens*) DSM 40239
Saccharothrix mutabilis subsp. *capreolus* DSM 40225	*Streptomyces hygroscopicus* ATCC 29253	*Streptomyces thioluteus* DSM 40027
Saccharothrix mutabilis subsp. *mutabilis* ATCC 31520	*Streptomyces kanamyceticus* DSM 40500	*Streptomyces tuirus* ATCC 19007
Saccharothrix syringae INA 2240	*Streptomyces laurentii* ATCC 31255	*Streptomyces zaomyceticus* DSM 40196
Streptomyces aburaviensis DSM 40033	*Streptomyces lavendulae* DSM 40708	*Streptosporangium* sp. (*sibiricum*) ATCC 29053
Streptomyces albus DSM 41398	*Streptomyces lincolnensis* DSM 40355	
Streptomyces ambofaciens JA 04927	*Streptomyces matensis* DSM 40188	

Strains were received from the ATCC; The Netherlands Culture Collection of Bacteria (CBS); German Collection of Microorganisms and Cell Cultures (DSM); own isolates (D); Institute for Fermentation (IFO), Osaka, Japan; Culture Collection of the Institute for New Antibiotics (INA), Russian Academy of Medical Sciences; former collection of Jena Actinomycetes Germany (JA); National Collection of Industrial, Marine, and Food Bacteria (NCIMB), Aberdeen, United Kingdom; and Agricultural Research Service Culture Collection (NRRL), United States.

Proposed functions of the gene products from AN7874 to AN7914

a. Putative NRPS gene cluster (echinocandin B?)

Protein	Size (bp/aa)	Deduced function
AN7874.4	921/306	hp
AN7875.4	378/125	hp
AN7876.4	1,047/348	Branched-chain amino-acid transaminase
AN7877.4	1,214/383	RTA1 domain protein
AN7878.4	1,191/396	Branched-chain amino-acid transaminase
AN7879.4	4,674/1,557	ABC transporter
AN7880.4	4,980/1,659	Fatty acid synthase subunit-a
AN7881.4	1,626/518	Cytochrome P450
AN11028.4	945/314	hp
AN11031.4	1,518/505	MFS multidrug transporter
AN7883.4	471/156	hp
AN7884.4	21,778/7,214	NRPS

b. Putative PKS gene cluster?

Protein	Size (bp/aa)	Deduced function
AN7894.4	333/110	hp
AN7895.4	1,072/351	Similar to CipB protein
AN7896.4	1,755/584	C6 transcription factor
AN7897.4	1,182/393	FAD-binding domain protein
AN7898.4	1,335/444	hp
AN7899.4	837/278	hp
AN7900.4	1,404/467	hp
AN7901.4	1,263/420	C6 transcription factor
AN7902.4	1,389/462	hp
AN7903.4	723/240	PKS
AN7904.4	978/325	hp
AN7905.4	882/293	Oxidoreductase
AN7906.4	636/211	hp
AN7907.4	489/162	hp
AN7908.4	1,435/325	Arabinoxylan arabinofuranohydrolase

c. Orsellinic acid gene (*ors*) cluster

Protein	Size (bp/aa)	Deduced function
OrsA/AN7909.4	6,312/2,103	PKS, orsellinic acid synthase
OrsB/AN7911.4	1,127/331	Amidohydrolase
OrsC/AN7912.4	1,110/369	Tyrosinase
OrsD/AN7913.4	1,562/501	C2H2 transcription factor
OrsE/AN7914.4	1,118/348	Zinc-binding dehydrogenase

Abbreviations:
hp, hypothetical protein
ABC, ATP-binding cassette
MFS, major facilitator superfamily
NRPS, nonribosomal peptide synthetase
PKS, polyketide synthase

Expression analysis of the region from AN7874 to AN7914