Microbial Chemical Ecology:
Bacterial Hormone-sensing Rediscovered in
Applied Microbiology

Kenji Ueda
麹 Koji
Aspergillus oryzae カビ  黄麹菌 Koji-kin
Aspergillus sojae ショウユコウジカビ
Rice (starch) → amylase → glucose → Sake (alcohol)

Aspergillus sp. → protease → amino acids peptides

Soy bean (protein) → protease → amino acids peptides

Lactic bacteria → Lactic acid

Yeast → alcohol
秋里騒島『撰津名所図会』(寛政10年)より。伊丹の酒造りのありさまが一目でわかる。
本文中の『日本山海名産図会』と同時代の出版
唐尼二刀をさした選吉少年(右)、長崎留学中の写真で、一緒に留学した安達松太郎(左)とのスタジオ。お付きの加賀藩士(中央)はちんまげを結っているが、二人の少年は穴に分けている。

1891年6月、ピオニアで撮影された長男・選吉(左:3歳)、選吉(37歳)、および次男・エベネレ(右:2歳)。婦白盛りの二人の息子もネクタイをして、おとなしくママのほうを見ている。

三共合資会社時代に作られた強力消化酵素・タカジアスターゼの薬瓶。ラベルに東京市日本橋区室町3丁目10番地とある。

塩化アドリナリン
ADRENALIN

塩酸エピレナミン注射液

三共合資会社

三共株式会社で販売していた塩化アドレナリンのアンプル箱。

Jokichi Takamine
1854-1922
Prof Kin-ichiro Sakaguchi

坂口謹一郎先生 1897-1994
Penicillin G

Alexander Fleming (1928)
Jackson W Foster’s Visit in 1946

Taken after the Three-day Symposium on Penicillin Production by J.W. Foster, Nov. 15, 1946
Traditional Fermentation Industry

Penicillin Streptomycin

Industrial Applications

Microbial Screening

Bases of ‘Chemical Biology’
歴代教授

古在由直  1900（明治33）～

高橋倩造  1924（大正13）～

坂口謹一郎  1939（昭和14）～

有馬 啓  1958（昭和33）～

別府輝彦  1977（昭和52）～

堀之内未治  1994（平成6）～

大西康夫  2010（平成22）～
有馬 啓先生 1916-1988

Prof Kei Arima
kanamycin
Masa Hamada-Kuroya
The 167 compounds she discovered:
カスガミシンの効果に関する研究

カスガミシンは、稲病原に対する効果を示す。図に示されている試験結果により、カスガミシンは稲病原の発生を抑制することが確認されている。図の左側は、稲の成長状況を示しており、右側はカスガミシン処理後の稲の発育状態を比較している。カスガミシンは、稲の健康を維持し、稲病原の増殖を抑制する効果があることが示されている。
新規物質発見の鍵

浜田 雅

1) 微生物の能力を信じて

2) スクリーニング系の確立

3) 基礎研究を大切に

4) 機械的にならぬよう

5) 人間関係を大切に
<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collecting soils</td>
<td>Collect soil samples as sources for actinomycetes.</td>
</tr>
<tr>
<td>Drying soil samples</td>
<td>Dry the samples at room temperature for 5-7 days. This process promotes spore formation and decreases bacterial cell number.</td>
</tr>
<tr>
<td>Heating samples</td>
<td>Heat at 100°C for 30 min. Non-spore forming bacteria are killed, while many actinomycete spores survive.</td>
</tr>
<tr>
<td>Inoculating the treated samples</td>
<td>Sprinkle (or spread) the treated soil samples on HV-agar, directly. Incubate at 28°C, ~1 week.</td>
</tr>
<tr>
<td>Isolating actinomycetes colonies</td>
<td>Pick up actinomycete colonies by toothpick and transfer them to new HV-agar (4 strains / Petri dish). Incubate at 28°C, ~1 week.</td>
</tr>
<tr>
<td>Purifying the isolates</td>
<td>Transfer the isolates from HV-agar to YS-agar in order to purify the colonies (1 strain / Petri dish). Incubate at 28°C for 3 days.</td>
</tr>
<tr>
<td>Preserving the isolates</td>
<td>Transfer purified and selected isolates to slant culture. Remove duplicate strains.</td>
</tr>
</tbody>
</table>
HV agar

0.05 % humic acid*
0.05 % Na$_2$HPO$_4$$\cdot$12H$_2$O
0.17 % KCl
0.005 % MgSO$_4$$\cdot$7H$_2$O
0.001 % FeSO$_4$$\cdot$7H$_2$O
0.002 % CaCO$_3$
0.1 % Yeast extract
( pH7.2 with 1N HCl)
1.5 % Agar

Autoclaving at 121 ºC, 20 min

+ 50 γ Cycloheximide
+ 25 γ Nalidixic acid

*predissolve in 0.2 N NaOH
(100 ºC, 30 min)
Streptomycin: Background, Isolation, Properties, and Utilization

Selman A. Waksman

Institute of Microbiology, Rutgers University, New Brunswick, New Jersey

The highest scientific award and honor presented to me today gives me the opportunity to summarize briefly the discovery and utilization of streptomycin for disease control, notably in the treatment of tuberculosis, the "Great White Plague" of man.

Historical Background

Streptomycin belongs to a group of compounds, known as antibiotics, which are produced by microorganisms and possess the property of inhibiting the growth of, and even of destroying, other microorganisms. Antibiotics vary greatly in their chemical nature, mode of action upon different organisms, and effect upon the animal body. The selective action of antibiotics upon bacteria and other microorganisms is known as the antibiotic spectrum. Some antibiotics are characterized by a very narrow spectrum, whereas others possess a wide range of activity. Some are active only against certain bacteria and not against others, whereas some are active against fungi, and some against viruses. There is not only considerable qualitative variation in the activity of different antibiotics, but also wide quantitative differences. Antibiotics are produced by bacteria, fungi, actinomycetes, and, to a limited extent, by other groups of microorganisms.

It has been known for more than six decades that certain fungi and bacteria are capable of producing chemical substances which have the capacity to inhibit the growth of, and even to destroy, pathogenic organisms. Only within the last twelve or thirteen years, however, have antibiotics begun to find extensive application as chemotherapeutic agents. Among these, penicillin and streptomycin have occupied a prominent place. Penicillin is largely active against gram-positive bacteria, gram-negative enteric, anaerobic bacteria, spirochetes and actinomycetes; streptomycin is active against a variety of gram-negative and acid-fast bacteria, as well as against gram-positive organisms which have become resistant to penicillin. Neither of these antibiotics is active upon rickettsias, viruses, and fungi. They differ too in their physical and chemical properties and in their toxicity to animals.

Since the discovery of streptomycin, the production and clinical application of this antibiotic have had a

Selman A. Waksman
The first streptomycin manufactured in Japan (July, 1950)
Sweet Products of Meiji in 1950’s
< A syrup bottle containing penicillin
Streptomyces griseus
Frequent occurrence of Sm\(^{-}\) mutant in *Streptomyces griseus*

<table>
<thead>
<tr>
<th>Acridine Orange (µg/ml)</th>
<th>Cultivation temp (°C)</th>
<th>No. of colonies tested</th>
<th>Sm (-) mutants</th>
<th>Sm(-)</th>
<th>Sm(-)/Spore(-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>30</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>30</td>
<td>100</td>
<td>11</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>37</td>
<td>100</td>
<td>8</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>
Cosynthesis tests between Sm⁻ mutants

<table>
<thead>
<tr>
<th>Inside colonies</th>
<th>Outside colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 S. griseus parent</td>
<td>-</td>
</tr>
<tr>
<td>2 Mutant No.55</td>
<td>-</td>
</tr>
<tr>
<td>3 Mutant No.2</td>
<td>-</td>
</tr>
<tr>
<td>4 Parent</td>
<td>No. 55</td>
</tr>
<tr>
<td>5 Parent</td>
<td>No. 2</td>
</tr>
<tr>
<td>6 No. 55</td>
<td>No. 2</td>
</tr>
</tbody>
</table>
Case I

Mutant 55

Mutant 2

A → B → C → D → Sm

A → B → C → D → Sm
Cosynthesis of streptomycin between 95 Sm(-) mutants
Case II

Factor X

Mutant A

Sm

Factor X

Mutant B

A-factor
A-фактор, обеспечивающий биосинтез стрептомицина мутантным штаммом Actinomycos streptomyces

<table>
<thead>
<tr>
<th>Вариант</th>
<th>Сутки ферmentation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Штамм № 1439</td>
<td>0</td>
</tr>
<tr>
<td>Штамм № 751</td>
<td>7</td>
</tr>
<tr>
<td>Совместный посев шт. № 1439 и 751</td>
<td>860</td>
</tr>
<tr>
<td>Ферментация шт. № 751 (3 суток) + культуральная жидкость шт. № 1439</td>
<td>0</td>
</tr>
<tr>
<td>Ферментация шт. № 1439 (3 суток) + культуральная жидкость шт. № 751</td>
<td>800</td>
</tr>
</tbody>
</table>

очень небольшие количества последней. В то же время присутствие различных фрагментов молекулы стрептомицина не приводит к образованию штаммом № 1439 самого антибиотика (табл. 2).

Если эти данные позволили предположить, что в культуральной жидкости штамма № 751 содержится вещество, которое даже в очень небольших количествах обеспечивает биосинтез стрептомицина штаммом № 1430; таким образом, по характеру действия оно подобно стимуляторам или кофакторам. Это вещество названо нами А-фактором (аддитивным фактором). 

A-factor ('Additional' factor)
A-factor

(‘Auto-regulatory’ factor)
A-factor $\rightarrow ?$ Sm $\rightarrow$ Aerial mycelium
Gel filtration fraction

High MW → Low MW

+protease
+cold A-factor

Radioactivity (10^3 dpm)

<table>
<thead>
<tr>
<th>Gel filtration fraction</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>⬇️</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>⬆️</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Two strategies…

A-factor Receptor protein (Arp)

Reverse genetics (protein purification)

Genetics (mutant isolation)
Arp = positive regulator?

Vegetative growth

Aerial mycelium
Arp = negative regulator?

Vegetative growth → Aerial mycelium

Sm → Aerial mycelium↑
The A-Factor-Binding Protein of *Streptomyces griseus* Negatively Controls Streptomycin Production and Sporulation

KATSUHIDE MIYAKE, TOMOHISA KUZUYAMA, SUEHARU HORINOUCHI,* and TERUHIKO BEPPU

Department of Agricultural Chemistry, Faculty of Agriculture,
The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

Received 11 December 1989/Accepted 12 March 1990

[Graph showing streptomycin production over days of growth.]

(a) Wild-type

(b) KM7

(c) HH1
Virginiae Butanolide Binding Protein from *Streptomyces virginiae*

EVIDENCE THAT VbrA IS NOT THE VIRGINIAE BUTANOLIDE BINDING PROTEIN AND REIDENTIFICATION OF THE TRUE BINDING PROTEIN*

(Received for publication, May 2, 1994, and in revised form, February 9, 1995)

Susumu Okamoto†, Kenji Nakamura, Takuya Nihira, and Yasuhiro Yamada§

From the Department of Biotechnology, Faculty of Engineering, Osaka University, 2-1 Yamada-oka, Suita-shi, Osaka 565, Japan

[Chemical structures of VB-C (S. virginiae) and A-factor (S. griseus)]

Susumu Okamoto  Hiroyasu Onaka
Cloning and Characterization of the A-Factor Receptor Gene from *Streptomyces griseus*

HIROYASU ONAKA,¹ NORIKO ANDO,¹ TAKUYA NIHIRA,² YASUHIRO YAMADA,² TERUHIKO BEPPU,¹,* and SUEHARU HORINOUCHI¹

Department of Biotechnology, Division of Agriculture and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113,¹ and Department of Biotechnology, Faculty of Engineering, Osaka University, Yamada-oka, Suita-shi, Osaka 565,² Japan

Received 10 July 1995/Accepted 18 August 1995

**TABLE 1. Purification of the A-factor receptor protein from *S. griseus* IFO 13350**

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Total protein (mg)</th>
<th>Total activity (10⁶ dpm)</th>
<th>Sp act (dpm/µg)</th>
<th>Purification (fold)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>12,262.6</td>
<td>61.8</td>
<td>5.04</td>
<td>1.0</td>
<td>100</td>
</tr>
<tr>
<td>(NH₄)₂SO₄ fractionation</td>
<td>2,022.6</td>
<td>61.1</td>
<td>30.2</td>
<td>6.0</td>
<td>99</td>
</tr>
<tr>
<td>DEAE-Sephacel</td>
<td>849.8</td>
<td>58</td>
<td>68.1</td>
<td>13.5</td>
<td>94</td>
</tr>
<tr>
<td>Mono Q</td>
<td>129.8</td>
<td>29.8</td>
<td>229.4</td>
<td>45.5</td>
<td>48</td>
</tr>
<tr>
<td>Superdex 75</td>
<td>37.25</td>
<td>15.7</td>
<td>422.6</td>
<td>83.8</td>
<td>25</td>
</tr>
<tr>
<td>Heparin</td>
<td>0.95</td>
<td>9.8</td>
<td>10,356</td>
<td>2,054.8</td>
<td>16</td>
</tr>
<tr>
<td>Phenyl-Superose</td>
<td>0.34</td>
<td>3.6</td>
<td>10,863</td>
<td>2,155.4</td>
<td>6</td>
</tr>
</tbody>
</table>

**FIG. 4.** Amino acid sequence alignment between the A-factor and virginase butanolide receptor proteins. Identical amino acids are marked by asterisks.
The A-factor Cascade
3D structure of CprB, a γ-butyrolactone autoregulator receptor homolog of *S. coelicolor* A3(2)

R. Natsume et al.
Probable ligand-binding pocket

The size is suitable to accept one molecule of $\gamma$-butyrolactone.

<table>
<thead>
<tr>
<th></th>
<th>A-factor binding</th>
<th>DNA binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>ArpA</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>ArpA (W119A)</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

W127 of CprB $\leftrightarrow$ W119 of ArpA
The A-factor Cascade

A-factor

A-factor receptor (transcriptional repressor)

AdpA

Transcriptional activator

SgiA, SprT, SgmA
SprA, SprB, SprD

Extracellular proteases

Autolysis

Aerial mycelium formation

Morphological differentiation

Sporulation

Secondary metabolism

Streptomycin production
Polyketide production
Grioxazone production
Proposed pathway for A-factor biosynthesis

HO-CH$_2$-CH$_2$-PO$_3$H$_2$ + ACP-S -> 2

Dephosphorylation

nonenzymatically

3

Nonenzymatically

reduction

dephosphorylation

4

reduction

A-factor
## Structural diversity of γ-butyrolactone signals in *Streptomyces*

<table>
<thead>
<tr>
<th>R</th>
<th>Structure</th>
<th>C3</th>
<th>C5</th>
<th>C6</th>
<th>C7</th>
<th>C8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IM-2</td>
<td>VB-C</td>
<td>VB-E</td>
<td>VB-D</td>
<td>VB-A</td>
</tr>
<tr>
<td>C3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Homoserine lactone

γ-butyrolactone

paired

lone

separated

synthase

receptor
LuxR-family ‘solos’: bachelor sensors/regulators of signalling molecules

Sujatha Subramoni and Vittorio Venturi

International Centre for Genetic Engineering and Biotechnology, Trieste, Italy

Microbiology (2009), 155, 1377–1385

Fig. 2. Functional and structural features of LuxR solos in proteobacteria: summary of the features and characteristics of LuxR solos determined so far in AHL-producing and non-AHL-producing bacteria. There are distinct roles in the two scenarios, however; some properties are also shared in both situations.

Fig. 1. Possible roles of LuxR solos in AHL-producing and non-AHL-producing proteobacteria. In AHL-producing bacteria AHLLs can bind either endogenous or exogenous AHLLs; in non-AHL-producing bacteria LuxR solos can bind exogenous AHLLs or other signals also originating in eukaryotes. LuxR solos can then control different sets of target gene expression.
### Table 1. Functionally characterized LuxR solos

<table>
<thead>
<tr>
<th>LuxR solo</th>
<th>Organism</th>
<th>AHL QS systems</th>
<th>Binding molecule(s)</th>
<th>Functions regulated</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>AvhR</td>
<td><em>Agrobacterium vitis</em></td>
<td>AvI/R</td>
<td>Not yet determined</td>
<td>Ability to cause necrosis on grapes and HR* on tobacco plants</td>
<td>Hao <em>et al.</em> (2005)</td>
</tr>
<tr>
<td>AviR</td>
<td><em>Agrobacterium vitis</em></td>
<td>AvI/R</td>
<td>Not yet determined</td>
<td>Ability to cause necrosis on grapes and HR on tobacco plants</td>
<td>Zheng <em>et al.</em> (2003)</td>
</tr>
<tr>
<td>BlxR</td>
<td><em>Brucella melitensis</em></td>
<td>None</td>
<td>Not yet determined</td>
<td>Regulation of virulence factors like type IV secretion system and flagella</td>
<td>Rambow-Larsen <em>et al.</em> (2008)</td>
</tr>
<tr>
<td>CarR</td>
<td><em>Serratia marcescens</em></td>
<td>Smal/R</td>
<td>Ligand independent</td>
<td>Carbapenem antibiotic production</td>
<td>Cox <em>et al.</em> (1998)</td>
</tr>
<tr>
<td>ExpR</td>
<td><em>Sinorhizobium meliloti</em></td>
<td>SinI/SinR</td>
<td>C14-HSL, 3-oxo-C14-HSL, C16:1-HSL, 3-oxo-C16-HSL, C18-HSL</td>
<td>Production of symbiotically active EPSII, succinoglycan production, motility, chemotaxis, nitrogen fixation, metal transport, etc.</td>
<td>Bartels <em>et al.</em> (2007); Hoang <em>et al.</em> (2004); McIntosh <em>et al.</em> (2008); Rambow-Larsen <em>et al.</em> (2008)</td>
</tr>
<tr>
<td>NisR</td>
<td><em>Sinorhizobium meliloti</em></td>
<td>SinI/SinR, Mel</td>
<td>C14-HSL, 3-oxo-C14-HSL, C16:1-HSL, 3-oxo-C16-HSL, C18-HSL</td>
<td>Nutritional and environmental stress response, plant nodulation</td>
<td>Patankar &amp; Gonzalez (2009)</td>
</tr>
<tr>
<td>OryR</td>
<td><em>Xanthomonas oryzae</em> pv. <em>oryzae</em></td>
<td>None</td>
<td>Rice signal molecule</td>
<td>Proline iminopeptidase (pip) gene expression; virulence on rice</td>
<td>Ferluga <em>et al.</em> (2007); Ferluga &amp; Venturi (2009)</td>
</tr>
<tr>
<td>SdiA</td>
<td><em>Escherichia coli</em>, <em>Salmonella enterica serovar Typhimurium</em></td>
<td>None</td>
<td>3-Oxo-C8-HSL, 3-oxo-C6-HSL, 3-oxo-C4-HSL, 3-oxo-C12-HSL, 3-oxo-C10-HSL, C12-HSL</td>
<td>Functions involved in adhesion and resistance to complement killing</td>
<td>Ahmer (2004); Michael <em>et al.</em> (2001); Yao <em>et al.</em> (2006)</td>
</tr>
<tr>
<td>VjbR</td>
<td><em>Brucella melitensis</em></td>
<td>None</td>
<td>C12-HSL</td>
<td>Regulation of virulence factors like type IV secretion system and flagella</td>
<td>Delrue <em>et al.</em> (2005)</td>
</tr>
<tr>
<td>XccR</td>
<td><em>Xanthomonas campestris</em> pv. <em>campestris</em></td>
<td>None</td>
<td>Plant signal molecule</td>
<td>Proline iminopeptidase (pip) gene expression; virulence on cabbage</td>
<td>Zhang <em>et al.</em> (2007)</td>
</tr>
</tbody>
</table>

*HR, hypersensitive response.
Parallel evolution of homoserine lactone system

Non-parallel evolution of \( \gamma \)-lactone system

‘Additional factor’