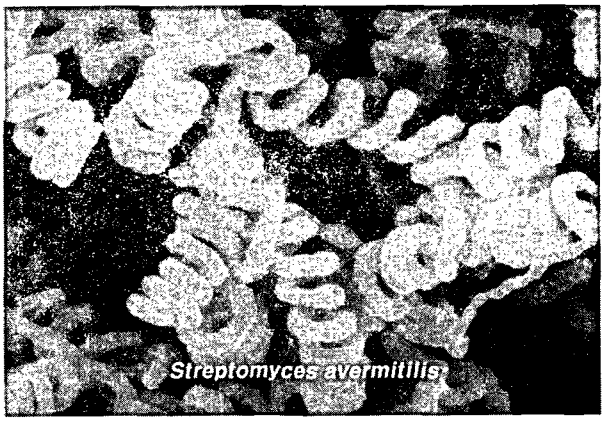
S 1-5

Diversity of Secondary Metabolism in *Streptomyces*

Haruo Ikeda

Kitasato Institute for life Sciences, Kitasato University, 1-15-1 Kitasato, Sagamihara, Kanagawa 228-8555, Japan



Streptomyces is a genus Gram-positive bacterium that grows in soil, marshes, and coastal marine habitats and forms filamentous mycelium-like eukaryote fungi. Morphological differentiation in *Streptomyces* involves the formation of a lawn of aerial hyphae on the colony surface that stands up into the air and differentiate into chains of spores. This process, unique among Gram-positive bacteria, requires the specialized coordination of metabolism and is more complex than other Gram-positive bacteria. The most interesting property of *Streptomyces* is its ability to produce secondary metabolites including antibiotics and bioactive compound value in human and veterinary medicine, agriculture, and unique biochemical tools. Structural diversity is observed in these secondary metabolites that encompass not only antibacterial, antifungal, antiviral, and antitumor compounds, but also metabolites with immunosuppressant, antihypertensive, and antihypercholesterolemic properties. Thus, *Streptomyces* is a rich source of the secondary metabolites in which common intermediates in the cell (amino acids, sugars, fatty acids, terpenes, etc.) are condensed into more complex structures by defined biochemical pathways. Characterization of chromosome ends of eight *Streptomyces* strains has revealed evidence of linear chromosomes, indicating that chromosomal linearity might be common in the streptomycetes. Most *Streptomyces* chromosomal DNA molecules are about 8-Mb long, with terminal-inverted repeats and covalently bound terminal proteins supposedly at the 5' end. This size is unusually large for a bacterium, compared with well known microorganisms such as *Escherichia coli* and *Bacillus subtilis*. Streptomycetes have a higher G+C content (more than 70 mol%) than nearly all other organisms. Thus, the *Streptomyces* chromosome is unique in its structure and size.

S. avermitilis was isolated from a soil sample at Shizuoka, Japan in 1976. The microorganism produces avermectin, a series of eight related pentacyclic lactones that contain a disaccharide of the methylated deoxysugar oleandrose (Fig. 1). Avermectin and the related compounds milbemycin and nemadectin are potent anthelmintic compounds; these compounds are used commercially in animal healthcare and agriculture.

The semisynthetic derivatives of avermectin C22, C23 dihydroavermectin B1, ivermectin, are widely used for the treatment of diseases caused by nematodes and arthropods in veterinary and agricultural fields, respectively. Ivermectin has been used for livestock farming and health care of companion animals. The efficacy of ivermectin in human onchocerciasis has made it a promising candidate for the control of this insidious and intractable tropical disease. Ivermectin also has been found to be effective against human strongyloidiasis in Okinawa, Japan as well as Asian countries.

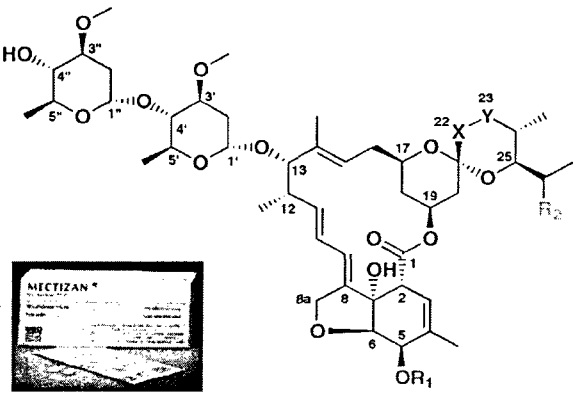


Fig. 1 Structures of avermectins

Table. 1 Features of *S. avermitilis* linear chromosome

Length (bp)	9,025,608
G+C content (mol %)	70.7
Predicted ORFs (>150 bp)	7,577
Assigned function	4,739 (62.5%)
Hypothetical	2,565 (33.8%)
Unknown	273 (3.6%)
Average ORF size (bp)	1,034
Coding region (%)	86.2
Sigma	62 (49 ECF)
rRNA operon(16S-23S-5S)	6
tRNA	68 (43 sp.)
tmRNA	1
scRNA	1

The genome sequence of *S. avermitilis* was obtained by whole genome shotgun sequencing in combination with sequencing of additional cosmid clones. The principle features of the *S. avermitilis* chromosome are summarized in Table 1. The linear chromosome contains 7,577 protein-coding genes (annotation data on 2005), of which we assigned a putative function to 4,739 (62.5%). Out of the remaining 2,838 genes, 2,565 genes (33.8%) showed the conservation to hypothetical proteins of unknown function annotated in other genomes, and 273 genes (3.6%) had no significant similarity to data in the public databases. But 2,291 genes (30.2%) had no significant similarity when excluded *S. coelicolor* A3(2) genes from the databases. The average GC content of the *S. avermitilis* chromosome was 70.7% but some regions were lower than average. For example, the six rRNA operons (16S-23S-5S rRNA) had GC contents ranging from 57.75 to 58.01%. The rRNA operon is known to have similar GC content in all organisms irrespective of the average GC content because of their function as RNA molecules. Transposons including truncated forms, phage and plasmid sequences composed of 1.6% (110 putative transposase and 16 putative phage/plasmid integrase genes) of the *S. avermitilis* chromosome. Most of the mobile sequences (86 transposase and 7 integrase genes) were found to be located in the regions near both chromosomal ends, called sub-telomeric regions.

S. avermitilis has the highest proportion of predicted secondary metabolite gene clusters of all bacterial genomes sequenced. Analysis, using FRAMEPLOT, BLASTP, and HMMERPFAM, showed 32 clusters involving the biosynthesis of pigment, terpene, siderophore, polyketide, and peptide compounds. The total lengths of these gene clusters were estimated to be about 561.3 kb. This analysis predicted that 6.43% of the *S. avermitilis* genome is occupied by genes concerned with the biosyntheses of secondary metabolites, a far higher proportion than has been found in other sequenced genomes. Almost none of these secondary metabolite clusters in *S. avermitilis* were located near the center of the chromosome and more than half

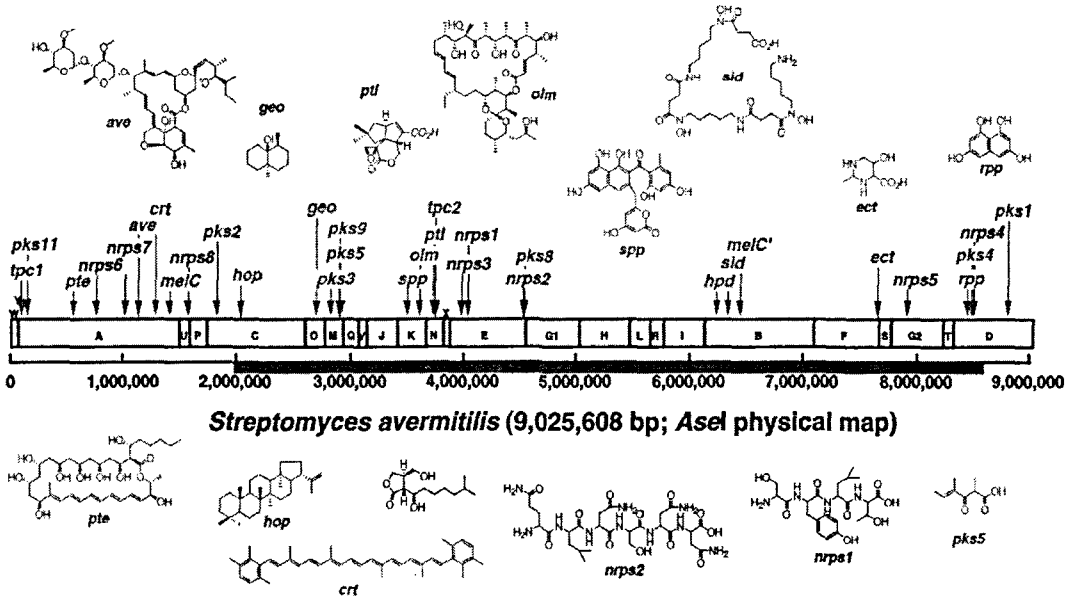


Fig. 2 AseI-Physical map and distribution of genes for secondary metabolism in *S. avermitilis*

were in the left hand from the *oriC*. Furthermore, about half of these clusters were also found near both ends of the chromosome. On the other hand, genes involved with primary metabolism, replication, transcription and translation were located in a region about 6 Mb from *Ase*I-C to -T fragments. These results indicate that some of the secondary metabolite clusters might have been horizontally transferred from donor microorganisms in the past. Furthermore, regions near both ends contain many transposase genes, indicating that transposases played an important evolutionary role in horizontal gene transfer and also in internal genetic rearrangements in the genome. Because some transposase genes were adjacent to secondary metabolite clusters, these transposases might have been involved in the transfer of these clusters.

Cytochrome P450 genes encode a superfamily of heme-thiolate-containing enzymes that are involved in an array of diverse endogenous and exogenous oxidative processes and are found in most organisms. Within streptomycetes (and myxobacteria), cytochrome P450s are often located in macrocyclic lactones biosynthetic gene clusters where they catalyse stereo- and regio-specific oxidation of precursors leading to structural diversity within these molecules. For example, this has been shown for the genes needed for synthesis of the antibacterial agents erythromycin, pikromycin, and tylosin; the antifungal agents amphotericin B and nystatine; the antitumor agent epothiolone; the antiparasitic agent avermectin; and the immunosuppressant rapamycin. The biological importance of the resulting hydroxyl and/or epoxide substituent introduced by the cytochrome P450 to the efficacy of the compound is often represented by a significant increase in antibiotic potency. To date, specific functions of individual cytochrome P450s have been described in 21 different strains of *Streptomyces,* and in the majority of these, the individual cytochrome P450 described is directly involved in the biosynthesis of bioactive secondary metabolites produced by that strain. Thus, a complete understanding of the cytochrome P450 superfamilies of *Streptomyces* is pivotal in generating or improving new and/or novel secondary metabolites from these organisms through protein pathway engineering. *S. coelicolor* A3(2) is a laboratory strain that has been

used extensively for the study of morphological and physiological development. The fully sequenced genome of *S. coelicolor* A3(2) revealed 7,825 open reading frames (ORFs) with 18 putative cytochrome P450 genes in the linear 8.7-Mb chromosome. Furthermore, the genome of *S. avermitilis* revealed 7,577 ORFs with 33 putative cytochrome P450 genes within the 9-Mb chromosome. One-third of cytochrome P450 genes (11 genes) may be involved in the biosynthesis of the secondary metabolites in *S. avermitilis*. The remaining 22 genes may be involved in the defense mechanisms against toxic compounds in the soil environment. It has revealed that some of them catalyze interesting hydroxylation or demethylation reactions

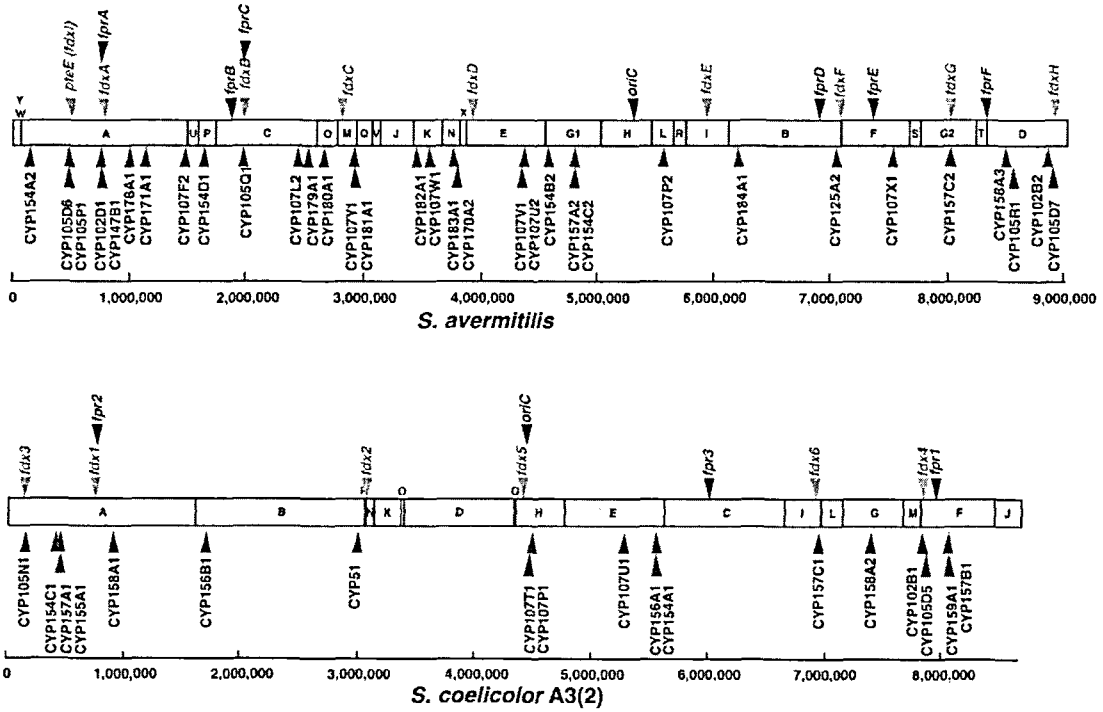


Fig. 3 Distribution of CYPs, ferredoxins and ferredoxin reductases in *S. avermitilis* and *S. coelicolor* A3(2)

References

1. Burg, R. W., Miller, B. M., Baker, E. E., Birnbaum, J., Currie, S. A., Hartman, R., Kong, Y.-L., Monaghan, R. L., Olson, G., Putter, I., Tunac, J. B., Wallick, H., Stapley, E. O., Oiwa, R. & Omura, S.: Avermectins, new family of potent anthelmintic agents: producing organism and fermentation. (1979) *Antimicrob. Agents Chemother.* 15, 361-367.
2. Ikeda, H., Kotaki, H. & Omura, S.: Genetic studies of avermectin biosynthesis in *Streptomyces avermitilis*. (1987) *J. Bacteriol.* 169, 5615-5621.
3. Ikeda, H., Takada, Y., Pang, C.-H., Tanaka, H. & Omura, S. : Transposon mutagenesis by Tn*4560* and its applications in Avermectin-producing *Streptomyces avermitilis*. (1993) *J. Bacteriol.* 175, 2077-2082.
4. Ikeda, H., Nonomiya, T., Usami, M., Ohta, T. & Omura, S.: Organization of the biosynthetic gene

cluster for the polyketide anthelmintic macrolide avermectin in *Streptomyces avermitilis*. (1999) *Proc. Natl. Acad. Sci. USA* 96, 9509-9514.

5. Omura, S., Ikeda, H., Ishikawa, J., Hanamoto, A., Takahashi, C., Shinose, M., Takahashi, Y., Horikawa, H., Nakazawa, H., Osonoe, T., Kikuchi, H., Shiba, T., Sakaki, Y. &, Hattori, M.: Genome sequence of an industrial microorganism *Streptomyces avermitilis*: Deducing the ability of producing secondary metabolites. (2001) *Proc. Natl. Acad. Sci. USA*. 98, 12215-12220.
6. Ikeda, H., Ishikawa, J., Hanamoto, A., Shinose, M., Kikuchi, H., Shiba, T., Sakaki, Y., Hattori, M. & Omura, S.: Complete genome sequence and comparative analysis of the industrial microorganism *Streptomyces avermitilis*. (2003) *Nat. Biotechnol.* 21, 526-531.
7. Lamb, D.C., Ikeda, H., Nelson, D.R., Ishikawa, J., Skaug, T., Jackson, C., Omura, S., Waterman, M.R. & Kelly, S.L.: Cytochrome P450 complement (CYPome) of the avermectin-producer *Streptomyces avermitilis* and comparison to that of *Streptomyces coelicolor* A3(2). (2003) *Biochem. Biophys. Res. Commun.* 307, 610-619.
8. Herai, S., Hashimoto, Y., Higashibata, H., Maseda, H., Ikeda, H., Omura, S. & Kobayashi, M.: Hyper-inducible expression system for streptomycetes. (2004) *Proc. Natl. Acad. Sci. USA*. 102, 14031-14035.