

A NOVEL PRODUCER OF THE  
ANTIBIOTIC KIRROMYCIN  
BELONGING TO THE  
GENUS *Actinoplanes*

Sir:

In the course of a screening program for actinomycetes which produce antibacterial antibiotics, we repeatedly isolated aurodox-type antibiotics which were active against *Neisseria caviae* ATCC 14659 and inactive against *Staphylococcus aureus* ATCC 6538. These antibiotics, which act upon elongation factor Tu, have a characteristic spectrum of antibacterial activity: they inhibit anaerobes, neisseriae and streptococci<sup>1~4)</sup>, but are ineffective against *S. aureus*<sup>5)</sup>.

The producing strains we isolated were mainly *Streptomyces* spp. occurring with a frequency of about one producer found for every three thousand strains screened. Among the sixteen antibiotics belonging to the aurodox-type class (Table 1), aurodox<sup>5)</sup>, kirromycin<sup>6)</sup>, A73A<sup>7)</sup>, factumycin<sup>8)</sup> and kirrothricin<sup>9)</sup> occurred more frequently. We also observed that the streptomycetes co-produced humidin-type antibiotics. These macrolides, which often contaminate preparations of the aurodox-type antibiotics, exhibit high toxicity on both prokaryotic and eukaryotic cells<sup>10~14)</sup>. Humidin-type antibiotics were found mainly adsorbed onto the mycelium of the producing strains and were determined in crude extracts from their UV spectrum and their toxic effect on yeast cells.

From a soil sample collected in India, we isolated an *Actinoplanes* strain, A8924, which produced kirromycin and did not produce any humidin-type antibiotic. Identification of the antibiotic was made using <sup>1</sup>H NMR and LC-MS<sup>15)</sup>.

Strain A8924 typically had a bright orange<sup>16)</sup> vegetative mycelium composed of twisted hyphae with a diameter of 1~1.5 μm, bearing on the surface multispored spherical sporangia with a diameter of 9~13 μm (Fig. 1). The sporophores are straight to slightly curved about 8~15 μm long and 2 μm in diameter. The oval spores, 1.5~2 μm in diameter, are highly motile by means of several flagella arranged in polar tufts.

Chemotaxonomic assessment of strain A8924 revealed *meso*-diaminopimelic acid and glycine as distinguishing components of the cell wall<sup>17)</sup>. Xylose and arabinose were the major sugars in the whole cell hydrolysate<sup>18)</sup>. According to the classification of LECHEVALIER and LECHEVALIER<sup>19)</sup>, this is a cell

wall-type IID.

On the basis of both its morphological and chemotaxonomic characteristics, strain A8924 was assigned to the genus *Actinoplanes*. There are no other examples reported in the literature (Table 1) of a member of genus *Actinoplanes* producing an antibiotic of the kirromycin-like family.

Fig. 1. *Actinoplanes* sp. A8924: spherical and sub-spherical sporangia forming on the vegetative mycelium.

Stalks supporting the sporangia are visible. Light microscope (×1,000).

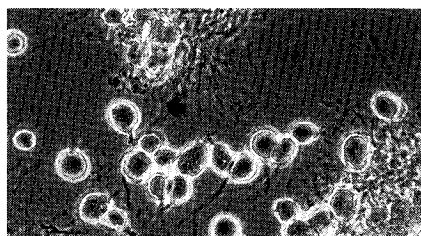


Table 1. Aurodox-type antibiotics.

Antibiotic	Producer
Kirromycin <sup>6)</sup> (Mocimycin <sup>20)*</sup> )	<i>Streptomyces ramocissumus</i> CBS 190-69 <i>S. collinus</i> Tu 365
Aurodox <sup>5)</sup>	<i>S. goldiniensis</i> ATCC 21386
Heneicomycin <sup>21)</sup> SB22484 <sup>22)</sup> )	<i>S. filipinensis</i> NRRL 11044 <i>Streptomyces</i> sp. NRRL 15496
Efrotomycin <sup>23,24)</sup>	" <i>Nocardia lactamdurans</i> " MA5887 <i>Nocardia</i> sp. ATCC 53758
Azdimycin <sup>25)</sup>	<i>S. diastatochromogenes</i> ATCC 31013
MSD A63A <sup>26)</sup>	<i>S. hirosimense</i> ATCC 31586
Factumycin <sup>8)</sup> A73A <sup>7)</sup> UK69753 <sup>27)</sup> )	<i>S. lavendulae</i> ATCC 31312 <i>S. viridifaciens</i> ATCC 31495 <i>Amycolatopsis orientalis</i> ATCC 53550
L-681,217 <sup>28)</sup>	<i>S. cattleya</i> ATCC 39203
Phenelfamycin <sup>29)</sup>	<i>S. violaceoniger</i> NRRL 18084 <i>S. violaceoniger</i> NRRL 18920
LL-E19020 <sup>30)</sup>	<i>S. lydicus</i> NRRL 18036
Dihydromocimycin <sup>31)</sup>	<i>S. ramocissumus</i> CBS 190-69
Kirrothricin <sup>9)</sup> New elfamycin <sup>32)</sup> (A83010F <sup>33)*</sup> )	<i>S. cinnamoneus</i> Tu 89 <i>Streptomyces</i> sp. CBS 473.89 unidentified Actinomycetes

\* Identical aurodox-type antibiotics described by different authors.

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### References

- 1) FROST, B. M.; M. E. VALIANT, B. WEISSBERGER & E. L. DULANEY: Antibacterial activity of efrotomycin. *J. Antibiotics* 29: 1083~1091, 1976
- 2) FROST, B. M.; M. E. VALIANT & E. L. DULANEY: Antibacterial activity of heneicomycin. *J. Antibiotics* 32: 626~629, 1979
- 3) SWANSON, R. N.; D. J. HARDY, N. L. SHIPKOWITZ, C. W. HANSON, N. R. RAMER, L. J. COEN & P. B. FERNANDES: Phenelfamycins, a novel complex of elfamycin-type antibiotics. III. Activity *in vitro* and in a hamster colitis model. *J. Antibiotics* 42: 94~101, 1989
- 4) BERGER, J.; H. H. LEHR, S. TEITEL, H. MAEHR & E. GRUNBERG: A new antibiotic X-5108 of *Streptomyces* origin. I. Production, isolation and properties. *J. Antibiotics* 26: 15~22, 1973
- 5) HALL, C. C.; J. D. WATKINS & N. H. GEORGOPAPADAKOU: Effects of elfamycins on elongation factor Tu from *Escherichia coli* and *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* 33: 322~325, 1989
- 6) WOLF, H. & H. ZÄHNER: Stoffwechselprodukte von mikroorganismen. 99. Mitteilung. Kirromycin. *Arch. Mikrobiol.* 83: 147~154, 1972
- 7) DEWEY, R. S.; J. E. FLOR, P. J. CASSIDY, S. OMURA & R. OIWA (Merck & Co.): Discovery of A73A, a new efrotomycin-like antibiotic in fermentation broth. U.S. 4,262,002, Apr. 14, 1981
- 8) GULLO, V. P.; S. B. ZIMMERMAN, R. S. DEWEY, O. HENSENS, P. J. CASSIDY, R. OIWA & S. OMURA: Factumycin, a new antibiotic (A40A): Fermentation, isolation and antibacterial spectrum. *J. Antibiotics* 35: 1705~1707, 1982
- 9) THEIN-SCHRANNER, I.; H. ZÄHNER, H.-U. HOPPE, I. HUMMEL & A. ZEECK: Metabolic products of microorganisms. 209. Kirrothricin, a new member of the kirromycin group. *J. Antibiotics* 35: 948~956, 1982
- 10) BÉRDY, J.: Humidin-Type. *In Handbook of Antibiotic Compounds*. Vol. II. *Ed.*, J. BÉRDY *et al.*, pp. 347~354, CRC Press Inc., 1980
- 11) CHACKO, C. I. & D. GOTTLIEB: The isolation of flavensomycin and humidin from the same strain of *Streptomyces griseus* and their antimicrobial properties. *Phytopathology* 55: 587~591, 1965
- 12) OTOGURO, K.; A. NAKAGAWA & S. OMURA: Setamycin, a 16-membered macrolide antibiotic identification and nematocidal activity. *J. Antibiotics* 41: 250~252, 1988
- 13) OMURA, S.; K. OTOGURO & H. TANAKA: The mode of action of a novel 18-membered macrolide, virustomycin A (AM-2604 A), on *Trichomonas foetus*. *J. Antibiotics* 36: 1755~1761, 1983
- 14) HUANG, L.; G. ALBERS-SCHONBERG, R. L. MONAGHAN, K. JAKUBAS, S. S. PONG, O. D. HENSENS, R. W. BURG, D. A. OSTLIND, J. CONROY & E. O. STAPLEY: Discovery, production and purification of the Na<sup>+</sup>, K<sup>+</sup>, activated ATPase inhibitor, L-681,110 from the fermentation broth of *Streptomyces* sp. MA-5038. *J. Antibiotics* 37: 970~975, 1984
- 15) EDWARDS, D. M. F.; E. SELVA, S. STELLA, L. F. ZERILLI & G. G. GALLO: Mass spectrometric techniques for structure and novelty determination of kirromycin-like antibiotics. *Biol. Mass Spectrometry* 21: 51~59, 1992
- 16) MAERZ, A. & M. R. PAUL: A dictionary of color. 2nd Edition, McGraw Hill Book Company Inc., New York, 1950
- 17) STANECK, J. L. & G. D. ROBERTS: Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Appl. Microbiol.* 28: 226~231, 1974
- 18) SADDLER, G. S.; P. TAVECCHIA, S. LOCIURO, M. ZANOL, L. COLOMBO & E. SELVA: Analysis of madurose and other actinomycete whole cell sugars by gas chromatography. *J. Microbiol. Methods* 14: 185~191, 1991
- 19) LECHEVALIER, M. P. & H. A. LECHEVALIER: Chemical composition as a criterion in the classification of aerobic actinomycetes. *Int. J. Syst. Bacteriol.* 20: 435~443, 1970
- 20) VOS, C. & J. DEN ADMIRAL (Koninklijke Nederlandsche): Antibiotic MYC 8003 and process for producing same. U.S. 3,927,211, Dec. 16, 1975
- 21) ZIMMERMAN, S. B.; J. H. CHALMERS, Jr., R. S. DEWEY, E. O. STAPLEY & S. HERNANDEZ: Heneicomycin, a new antibiotic (A21A): Fermentation, isolation, and antibacterial spectrum. *J. Antibiotics* 32: 665~666, 1979
- 22) SELVA, E.; G. BERETTA, R. PALLANZA, B. P. GOLDSTEIN, M. BERTI, D. M. F. EDWARDS & M. DENARO: Antibiotic SB22484: A novel complex of the aurodox group. I. Taxonomy of the producing organism, isolation of the antibiotics and chemical and biological characterization. *J. Antibiotics* 43: 1349~1358, 1990
- 23) WAX, R.; W. MAIESE, R. WESTON & J. BIRNBAUM: Efrotomycin, a new antibiotic from *Streptomyces lactamdurans*. *J. Antibiotics* 29: 670~673, 1976
- 24) JEFSON, M. R., J. KANEDA, S. NISHIYAMA & J. TONE (Pfizer): Production of *N*-demethyl-efrotomycin by microbial glycosylation of mocimycin, using new efrotomycin-producing *Nocardia* species. U.S. 4,937,184-A, June 26, 1990

- 25) NIMECK, M. W.; E. MEYERS & W. LIU (Squibb & Sons Inc.): Antibiotic azdimycin. U.S. 3,898,327, Aug. 5, 1975
- 26) HERNANDEZ, S.; S. B. ZIMMERMAN, V. P. GULLO & R. S. DEWEY (Merck & Co.): Discovery of MSD A63A, a new efrotomycin-like antibiotic in fermentation broth. U.S. 4,311,693, Jan. 19, 1982
- 27) PACEY, M. S.; M. R. JEFSON, L. H. HUANG, W. P. CULLEN, H. MAEDA, J. TONE, S. NISHIYAMA, K. KANEDA & M. ISHIGURO: UK-69,753, a novel member of the efrotomycin family of antibiotics. I. Taxonomy of the producing organism, fermentation and isolation. *J. Antibiotics* 42: 1453~1459, 1989
- 28) KEMPF, A. J.; K. E. WILSON, O. D. HENSENS, R. L. MONAGHAN, S. B. ZIMMERMAN & E. L. DULANEY: L-681,217, a new novel member of the efrotomycin family of antibiotics. *J. Antibiotics* 39: 1361~1367, 1986
- 29) HOCHLOWSKI, J. E.; M. H. BUYTENDORP, D. N. WHITTERN, A. M. BUKO, R. H. CHEN & J. B. McALPINE: Phenelfamycins, a novel complex of elfamycin-type antibiotics. II. Isolation and structure determination. *J. Antibiotics* 41: 1300~1315, 1988
- 30) CARTER, G. T.; D. W. PHILLIPSON, J. J. GOODMAN, T. S. DUNNE & D. B. BORDERS: LL-E19020 $\alpha$  and  $\beta$ , novel growth promoting agents: Isolation, characterization and structures. *J. Antibiotics* 41: 1511~1514, 1988
- 31) JONGSMA, H. M.; H. J. KOOREMAN & J. L. Os (Gist-Brocades, N.V., Delft (Netherland)): Dihydro-mocimycin, dessen Sälze mit Säuren, Verfahren zu seiner Herstellung und Futtermittel. *Offenlegungsschrift* 2,621,615, Nov. 25, 1976
- 32) MARGRAFF, R.; T. KIENER & A. KIES (Rhône-Poulenc): Nouvelle elfamycine, son procédé de préparation et son utilisation. *Eur. Pat. Appl.* 442,783 Al., Feb. 7, 1991
- 33) SMITKA, T. A.; R. BONJOUKLIAN, T. J. PERUN, Jr., A. H. HUNT, L. D. BOECK & R. C. YAO: A83016F, a new member of the aurodox family. *J. Antibiotics* 45: 433~443, 1992