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MINI-REVIEW

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# Lincomycin, cultivation of producing strains and biosynthesis

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**Abstract** Lincomycin and its derivatives are antibiotics exhibiting biological activity against Gram-positive bacteria. The semi-synthetic chlorinated lincomycin derivative is used in clinical practice. The chemical structure of lincosamide antibiotics, cultivation of producing strains and analytical procedures used for separation and isolation of these compounds are described in this review. Biosynthesis of lincomycin and related compounds and its genetic control are briefly discussed.

## Introduction

Lincomycin, the first of two lincosamide antimicrobial agents, was isolated in 1962 from a soil actinomycete found near Lincoln, Nebraska, which gave origin to its name (Stratton 1998). The actinomycete was classified as *Streptomyces lincolnensis* var. *lincolnensis*, a new streptomycete species. The announcement by Mason et al. (1962) of the discovery of lincomycin coincided with chemical and physical characterisation of the crystalline hydrochloride (Herr and Bergy 1962). Initial reports indicated that lincomycin possessed good in vitro and in vivo potency against a variety of Gram-positive microorganisms (Lewis et al. 1963). It did not exhibit cross resistance with known antibiotics and possessed a low degree of toxicity (Mason and Lewis 1964). Lincomycin has gained clinical acceptance as a major antibiotic for the treatment of diseases caused by Gram-positive microbes. The reports of extensive experimental and clinical studies with lincomycin will be discussed in the second part of our review.

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## Chemical structure

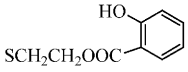
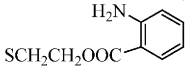
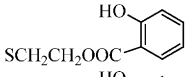
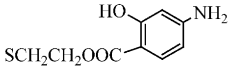
A study of the structure of lincomycin by Hoeksema et al. (1964), employing both classical chemical degradation and nuclear magnetic resonance (NMR) data, led to the announcement of the structure of lincomycin (**1**; see Table 1). It consists of an unusual amino acid, viz. trans-*N*-methyl-4-*n*-L-proline (propylhygric acid), linked via a peptide bond with the sugar methylthiolincosamide. Details of the chemical characterisation and NMR studies of lincomycin and its degradation products subsequently appeared (Herr and Slomp 1967; Schroeder et al. 1967; Slomp and MacKellar 1967; Magerlein and Kagan 1969). The electron-impact mass spectrum and the chemical-ionisation mass spectrum of lincomycin were reported somewhat later (Kagan and Trostic 1972; Horton et al. 1974).

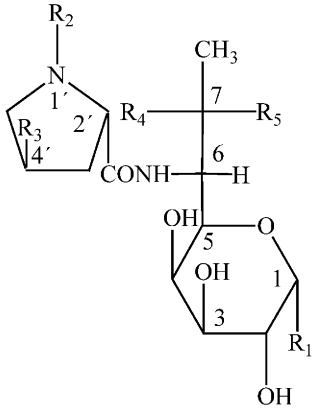
Lincomycin proved to be a member of a new class of antibiotics (Table 2) characterised by an alkyl 6-amino-6,8-dideoxy-1-thio-D-erythro-α-D-galacto-octopyranoside joined with a proline moiety by an amide linkage. The availability of this interesting antibiotic presented an excellent opportunity for chemical and microbiological modification of lincomycin.

## Cultivation

A typical cultivation is performed in a 1-l fermentor inoculated with a spore suspension or with frozen vegetative cultures. In practice, cells from shake flasks are transferred to an 8-l fermentor and finally to a 250-l fermentor. The growth of the culture is followed by determining the concentration of diaminopimelic acid. A sufficient oxygen supply, as well as the temperature and pH of the cultivation medium, play a significant role in determining production levels. Under optimum conditions, the wild-type *S. lincolnensis* strain produced 25 mg/l lincomycin A and negligible amounts of lincomycin B. The strain *S. lincolnensis* 78–11 used by Zhang (1993) is a phage-resistant, lincomycin-overproducing mutant of *S.*

**Table 1** Structure of lincomycin and related antibiotics

| | Name | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ |
|---|---|---|---|---|---|---|
| **1** | Lincomycin A | $SCH_3$ | $CH_3$ | $CH_2CH_2CH_3$ | OH | H |
| **4** | Lincomycin B | $SCH_3$ | $CH_3$ | $CH_2CH_3$ | OH | H |
| **5** | Lincomycin C | $SCH_2CH_3$ | $CH_3$ | $CH_2CH_2CH_3$ | OH | H |
| **7** | Lincomycin D | $SCH_3$ | H | $CH_2CH_2CH_3$ | OH | H |
| **6** | Lincomycin S | $SCH_2CH_3$ | $CH_2CH_3$ | $CH_2CH_2CH_3$ | OH | H |
| **8** | Lincomycin K | $SCH_2CH_3$ | H | $CH_2CH_2CH_3$ | OH | H |
| **10** | Lincomycin sulfoxide | $S(=O)-CH_3$ | $CH_3$ | $CH_2CH_2CH_3$ | OH | H |
| **11** | 1-Demethylthio-1-hydroxylincomycin | OH | $CH_3$ | $CH_2CH_2CH_3$ | OH | H |
| **2** | Celesticetin A |  | $CH_3$ | H | $OCH_3$ | H |
| **14** | Celesticetin B | $SCH_2CH_2OOCCH_2CH(CH_3)_2$ | $CH_3$ | H | $OCH_3$ | H |
| **15** | Celesticetin C |  | $CH_3$ | H | $OCH_3$ | H |
| **16** | Celesticetin D | $SCH_2CH_2OOCCH_3$ | $CH_3$ | H | $OCH_3$ | H |
| **3** | Desalicetin | $SCH_2CH_2OH$ | $CH_3$ | H | $OCH_3$ | H |
| **17** | N-Demethylcelesticetin |  | H | H | $OCH_3$ | H |
| **18** | Desalicetinsalicylate |  | $CH_3$ | H | $OCH_3$ | H |
| **9** | Clindamycin | $SCH_3$ | $CH_3$ | $CH_2CH_2CH_3$ | H | Cl |
| **12** | Clindamycin sulfoxide | $S(=O)-CH_3$ | $CH_3$ | $CH_2CH_2CH_3$ | H | Cl |
| **13** | 1′-demethylclindamycin | $SCH_3$ | H | $CH_2CH_2CH_3$ | H | Cl |



*lincolnensis* NRRL 2936, synthesising about 2.5 g/l lincomycin A.

Before reviewing the other naturally occurring members of the lincomycin family of antibiotics, processes used for the cultivation of producing strains and methods for separation of lincomycin A will be described.

The first patent describing lincomycin production was registered by Upjohn (Bergy et al. 1963). The producing *S. lincolnensis* var. *lincolnensis* has a compact cream-pink aerial growth, yellow-tan vegetative growth, and is melanin positive. Spore chains are flexuous and smooth. Its growth characteristics on standard biological media and its carbon assimilation were also described in this patent. Cultivation in a 380-l fermentor containing 250 l sterile medium proceeded at 28°C for 5 days while agitating at 6.5 Hz and aerating at a rate of 100 l air per minute. At the end of the fermentation (114 h), 210 l cultivation broth were harvested from the fermentor (pH 7.9; assay, 43 biounits/ml).

The entire cultivation broth from the fermentor was adjusted from a harvest pH of 7.9 to pH 6.7 with 70 ml concentrated sulfuric acid and filtered. The filter cake was washed with one-tenth volume of water, based on the whole broth, and added to the clear liquid. The clear liquid (250 l; assay, 20 biounits/ml) was adjusted to pH 10 with 300 ml 59% aqueous sodium hydroxide solution and extracted twice with one-third volume of 1-butanol. The washed butanol extract was freeze-dried to give about 105 g dried preparation assaying 125 biounits/mg. After further purification, the yield was 32 g lincomycin A at a purity of 232 biounits/mg.

The novel actinomycete *S. vellosus* var. *vellosus* NRRL 8037 isolated from Arizona soil was used according to the invention patent (Bergy et al. 1981) for the production of lincomycin. The culture can be readily differentiated from other lincomycin producers according to its characteristics such as colour, overall appearance, and cultivation and biochemical properties. Most importantly, the strain produces lincomycin A without concomitant production

**Table 2** Strains producing the lincomycin group of antibiotics

| Organisms | Literature |
|---|---|
| *Streptomyces lincolnensis* 78–11 | Zhang et al. (1992), Peschke et al. (1995) |
| *S. lincolnensis* NRRL 2936 | Bergy et al. (1963), Brahme et al. (1984a), Peschke et al. (1995) |
| *S. lincolnensis* RIA 1246 | Neusser et al. (1998) |
| *S. lincolnensis* UC 5124 | Chung und Crose (1990) |
| *S. espinosus* NRRL 5729 | Peschke et al. (1995) |
| *S. espinosus* NRRL 3890 | Peschke et al. (1995) |
| *S. espinosus* | Argoudelis et al. (1972) |
| *S. espinosus* | Reusser and Argoudelis (1974) |
| *S. pseudogriseolus* NRRL 3985 | Peschke et al. (1995) |
| *S. variabilis* var. *liniabilis* | Argoudelis and Coats (1974) |
| *S. vellosus*NRRL 8037 | Bergy et al. (1963), Peschke et al. (1995) |
| *Micromonospora halophytica* | Bibikova et al. (1989) |
| *Actinomyces roseolus* | Kuznetsov et al. (1974) |

of lincomycin B. Another strain characteristic is the production of comparable titres of lincomycin at 28°C and 45°C.

Several strains were obtained by extensive screening of different wild-type strains and appropriate patents were published. According to the patent of Argoudelis et al. (1972), the novel actinomycete produced only lincomycin A. *S. espinosus* Dietz sp. Nov. NRRL 3890 produced 50 μg/ml of the desirable lincomycin A during a 4-h cultivation at 28°C in shake flasks with classical medium. According to the US Patent (Argoudelis and Coats 1974) *S. pseudogriseolus* var. *linmyceticus* Dietz var. nova NRRL 3985 cultivated for 2 days at 28°C produces ~22 μg/ml lincomycin. Another patent describing production of lincomycin A only, without contamination with lincomycin B, was registered by Upjohn. *S. variabilis* var. *liniabilis* NRRL 5618 produces 26 μg/ml lincomycin A after a 120-h cultivation at 28°C in shaken 500-ml Erlenmeyer flasks.

In the last 5 years, Chinese investigators have become interested in the cultivation of lincomycin-producing strains; mainly in the isolation of lincomycin A with a simultaneous decrease or complete removal of the undesirable lincomycin B (Wang and Qi 1999, 2000, 2001; Dong and Dong 2001; Li et al. 2001).

Although lincomycin was the first member of its family to have its structure completely elucidated, a related antibiotic, celesticetin, was reported some years earlier in the culture broth of a new actinomycete species *Streptomyces caelestis*. This antibiotic was isolated by Hoeksema et al. (1955). Employing some of the techniques used in structural studies on lincomycin, Hoeksema (1968) assigned structure **2** (Table 1) to celesticetin. Desalicetin, the alkaline hydrolysis product of celesticetin, was shown to possess structure **3**. Both of these antibiotics proved to be less effective than lincomycin when assayed in vitro and in vivo against a spectrum of microorganisms (Mason and Lewis 1964).

In addition to lincomycin, a very closely related antibiotic was isolated from fermentations of *S. lincolnensis* var. *lincolnensis*. Structural studies indicated this compound to be lincomycin B, i.e. 4′-depropyl-4-ethyllincomycin (**4**). Lincomycin B, described as a minor component in *S. lincolnensis* cultures as early as 1965, exhibits only 25% of the antibiotic activity shown by lincomycin A. As shown in Table 1, lincomycin B contains one less methylene group in the side chain of propylhygric acid. Several patents (Visser 1972; Witz 1972; Reusser and Argoudelis 1974) describing ways to reduce the amount of lincomycin B during fermentation have been published. The addition of propylproline to the cultivation medium was found to decrease the amount of lincomycin B produced. It has been assumed that propylproline is a likely precursor of propylhygric acid in lincomycin A, whereas ethylproline is a tentative precursor of the ethylhygric acid in lincomycin B. Aromatic amino acids, primarily L-tyrosine, L-dihydroxyphenylalanine and other compounds similar to tyrosine, are incorporated into lincomycin A during cultivation of *S. lincolnensis*. Addition of L-tyrosine or L-dihydroxyphenylalanine to the culture medium was found to induce accumulation of propylproline; however, although to a lesser extent, accumulation of ethylproline was also induced. Despite the fact that the mechanism by which the addition of propylproline or L-tyrosine and similar compounds influences the biosynthesis of lincomycin has not yet been clarified, the results obtained so far indicate that *N*-demethyllincomycin synthetase, the enzyme catalysing the linkage between the amino acid and sugar moiety of lincomycin, preferentially utilises propylproline. Effects of different salts, nitrogen and carbon sources on specific ratios of lincomycin production in *S. lincolnensis* in a chemically defined medium have also been investigated. Effects of biomass concentration, pH and utilisation of carbon sources such as glucose and citrate in lincomycin production are shown in Fig. 1. Maximum lincomycin production is detected during the late stages of growth. When carbon sources are exhausted, growth rapidly decreases, whereas lincomycin production remains constant for some time. The addition of phosphates as physiological regulators of lincomycin production has also been described. Maximal lincomycin production was

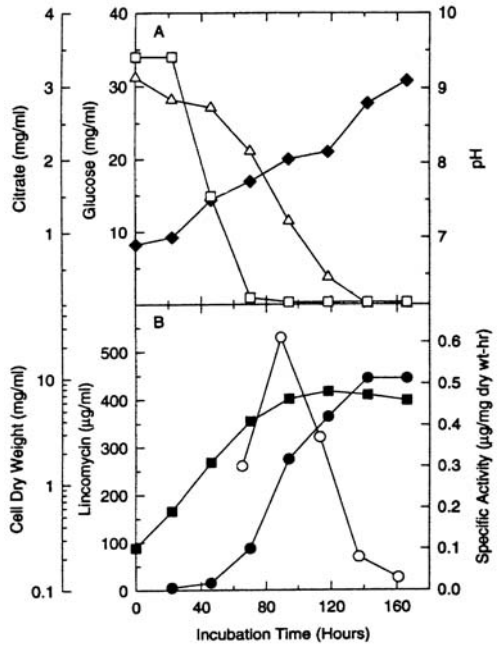



**Fig. 1** Profiles of glucose utilisation (△), citrate utilisation (□) and pH (◆) (**A**), and cell dry weight accumulation (■), lincomycin titers (●) and specific rate of lincomycin productivity (○) (**B**) by *Streptomyces lincolnensis* inoculated into a chemically defined medium. The specific rate of lincomycin production was determined in washed-cell reactions. Redrawn from Grady 1968

detected at 3 mM phosphate; at higher phosphate concentrations production decreases sharply whereas the amount of biomass significantly increases. Another biogenic element, nitrogen (in the form of ammonium salts), influences not only lincomycin production but also growth. The optimum concentration for lincomycin production varies between 40 and 200 mM. Glucose at 20–30 g/l seems to be the best carbon source in chemically defined media.

The introduction of various chemicals to the fermentation of *S. lincolnensis* var. *lincolnensis* was found to induce the formation of lincomycin-related antibiotics. For example, introduction of the biosynthetic precursor *n*-propyl-L-proline increases production of the desirable lincomycin A, as described in the patent by Bruce et al. (1973). Thus, the addition of DL-ethionine led to the isolation of 1-demethylthio-l-ethylthiolincomycin (**5**) and 1-demethyl-l-demethylthio-1′-ethyl-l-ethylthiolincomycin (**6**); methylthiolincosamide induced formation of 1′-demethyllincomycin (**7**); and ethyl thiolinconsamide yielded 1′-demethyl-l-demethylthio-l-ethylthiolincomycin (**8**). Sulfonamides, and particularly sulfanilamide, inhibit N-methylation rather selectively, resulting in the production of 1′-demethyllincomycin (**7**). In each case, lincomycin was also noted in the fermentation extracts.

The production and isolation of a new antibiotic, celesticetin, in *S. caelestis* NRRL 2418 was first described in 1960. The strain was obtained from a soil sample from Utah. Celesticetin exhibits a broad antibacterial spectrum, particularly against Gram-positive bacteria. For cultivation, a 380 l tank was filled with 240 l fermentation medium. After 67 h, cultivation was terminated and celesticetin at 26 µg/ml was obtained by means of commonly used methods. New antibiotics, *N*-demethyl-7-*O*-demethylcelesticetin and *N*-demethylcelesticetin were found to be produced by cultivation of the new strain *S. caelestis* NRRL 5481. The cultivation proceeded in a 7,000 l fermentor containing the following cultivation medium: glucose (15 g/l), enzymatically hydrolysed proteins of animal origin (15 g/l), yeast extract (1 g/l). The culture was grown for 4 days at 28°C. From 4,400 l whole cultivation broth, 1.6 kg crude mixture was isolated that was further purified by means of counter current distribution. Both metabolites were obtained as pure crystalline compounds. *S. caelestis* NRRL 5320 cultivated for 4 days at 28°C produced 7-*O*-demethylcelesticetin. Total production was 38 µg/ml (after extraction and silica gel chromatography).

Chemical modification (partial synthesis) of lincomycin derivatives consisted mainly of substitution of hydrogen atoms bound to a heteroatom (Birkenmeyer 1981, 1982a, 1982b; Argoudelis and Stroman 1984; Patt et al. 1984). Tens of lincomycin esters, either with organic (from acetate to stearate) or inorganic (phosphoric, carbonic) acids or of lincomycin alkyl derivatives (ethers) have been prepared. Substitutions were performed primarily in positions 2, 3, 4 and 7. Salts of lincomycin with inorganic acids, i.e. hydrochlorides and derivatives of sulfamic acid, were also synthesised. Replacement of hydroxyl in the side chain (i.e. C-7 of octose) is another modification of lincomycin. By introduction of chlorine, the commercially used derivative with the generic name clindamycin was produced (**9**). Additional thio-analogs, of which, e.g., the C-1 β-anomer exhibits only one-tenth of the activity of the natural α-anomer, were synthesised. The changed configuration of 1-methyl-4-proline is also very important. It was demonstrated that the derivative with the D amino acid exhibits only 50% of the expected antibacterial activity. It can be concluded that although hundreds of lincomycin derivatives have been prepared, including derivatives produced totally by chemical synthesis, clindamycin is the only drug that has been successfully used in clinical practice. Detailed synthetic procedures leading to hundreds of lincomycin derivatives together with their biological activity were described by Magerlein (1971).

A new and stereoselective route to the aminoglycoside components of the antibiotics lincomycin and clindamycin was presented. The key step involves diastereoselective introduction of the amino group at C-6 of D-galactose by (3,3)-sigmatropic rearrangements of the corresponding allylic (Z)-trifluoroacetimidate and (Z)- and (E)-allylic thiocyanates. Epoxidation of the resulting trifluoroacetamide with m-CPBA led to an epoxide with high threo-selectivity (Gonda et al. 2000). A further paper (Bowden and Stevens 2000) reported the novel synthesis of clindamycin from lincomycin using *N*-chlorosuccinimide and triphenylphosphine.

Microbial modification of lincomycin and clindamycin does not lend itself to the formation of the number and diversity of transformation products encountered in chem-

ical modification. However, several types of interesting microbial transformations have been reported.

The addition of lincomycin to a fermentation of *S. lincolnensis* var. *lincolnensis* resulted in the formation of lincomycin sulfoxide (**10**). This sulfoxide was also prepared by oxidation of lincomycin hydrochloride with hydrogen peroxide (Pospíšil et al. 2001). As compared with lincomycin it exhibits only 1% activity against *Sarcina lutea*. A lower yield of 1-demethylthio-l-hydroxy-lincomycin (**11**) was also isolated from the fermentation broth.

Similarly, clindamycin sulfoxide (**12**) was the major transformation product when clindamycin (**9**) was added to fermentations of *S. armentorus*. Trace amounts of sulfoxide were detected in similar fermentations of other *Streptomyces* species, particularly *S. punipalus*. Clindamycin sulfoxide is about equal in potency to lincomycin when assayed with the test organism *Sarcina lutea*.

In addition to sulfoxide formation, several species of streptomycetes, including those mentioned above, perform a partial 1-demethylation. *S. punipalus* was particularly successful in demethylating clindamycin (**9**) to 1′-demethylclindamycin (**13**), which was previously prepared by chemical modification.

Argoudelis and Coats (1969) observed that *S. rochei* grown in synthetic medium converted lincomycin and lincomycin-related antibiotics to the 3-phosphate esters. Lincomycin 3-phosphate was inactive in vitro against several organisms. It did protect *Staphylococcus aureus* infected mice with $CD_{50}$ of about 30 mg/kg when administered subcutaneously.

## Analysis and chromatographic separations

For the success of physiological experiments it was necessary to develop methods to determine the amount of lincomycin by either biological or chemical means. In addition to classical methods of biological assay, e.g. the diffusion disc plate assay, physico-chemical methods were also used. In the mid-1960s gas chromatography was first used followed later by HPLC methods. An advantage of chromatographic methods over biological procedures is that they make it possible to determine not only the overall titre of the antibiotic but also the lincomycin A:lincomycin B ratio.

A recovery process using reverse-phase (RP) preparative HPLC yielding a highly purified preparation of lincomycin hydrochloride was patented (Hofstetter 1982). As well as giving a highly purified preparation of lincomycin hydrochloride with less than only about 0.5% lincomycin B, the process also yields analytically pure lincomycin B hydrochloride that can be used as a laboratory standard.

Comparison of RP-HPLC and HPLC on normal phase revealed that the latter method yielded a product of insufficient purity (40–50%), required high volumes of solvent (more than three-fold with respect to RP-HPLC), and was also demanding in terms of materials (cartridge with silica gel). Therefore, the results presented below were obtained mainly with RP-HPLC.

Lincomycin salvage mother liquor, containing about 30% lincomycin B was used as the starting material. Lincomycin B eluted at between about 5 and 6 void volumes; lincomycin A eluted next between 6 and 8.5 column void volumes. Yields were about 64 g crystalline lincomycin B, and ca. 40 g high purity lincomycin A.

A crude crystalline lincomycin A preparation contained 20% of lincomycin B; the remaining solid material was primarily lincomycin A. This starting material was used for 20 18 g runs over two cartridges eluted isocratically with (2:1) water:methanol. The lincomycin B-rich pool was concentrated to an aqueous phase to which enough methanol was added to make a mobile phase, which was then reinjected. Rich eluent from the second HPLC was concentrated as before and rechromatographed a third time. Yields were 24 g 99% pure lincomycin B and ca. 125 g lincomycin A of high purity.

Procedures for the determination of clindamycin in human plasma using HPLC with UV detection have been described in the literature (Liu et al. 1997; La Follette et al. 1988; Fieger-Büschges et al. 1999). These methods are either not very sensitive, or are time-consuming and require laborious sample preparation. Recently, two methods using HPLC coupled with MS detection have been published for the quantification of clindamycin in human plasma. Yu et al. (1999) used an LC/ESI-MS/MS method, with acceptable linearity in the 0.05–20 μl/ml range and a quantification limit of 0.050 μg/ml. Martens-Lobenhoffer and Banditt (2001) used an LC/APCI-MS method for both human plasma and bone. The method showed good linearity in the 0.1–4 μg/ml range for plasma with a limit of quantification of 0.1 μg/ml.

A method for the quantification of clindamycin in animal plasma using LC/ESI-MS/MS has also been published (Cherlet et al. 2002). Lincomycin was used as the internal standard. The sample preparation includes a simple deproteinisation step with trichloroacetic acid. Chromatographic separation is achieved on an RP-18 Hypersil column using gradient elution with 0.01 M ammonium acetate and acetonitrile as mobile phase. Good linearity was observed in the range 0–10 μg/ml. The limit of quantification of the method is 50 μg/ml and the detection limit is 1.3 ng/ml. The method was used for pharmacokinetic studies of clindamycin formulations in dogs.

For further analysis of lincomycin and spectinomycin an RP ion-pair LC method with a base-deactivated column and pulsed electrochemical detection by means of a gold electrode was used (Szunyog et al. 2002). This method allows the separation of a mixture of spectinomycin sulfate, lincomycin hydrochloride and related substances. A step gradient was necessary to obtain good separation together with a reasonable analysis time of 40 min. The mobile phases consisted of an aqueous solution of 3.3 or 0.55 g/l pentanesulfonic acid, 10 mM acetic acid and 20 ml/l tetrahydrofuran. Both mobile phases were adjusted to pH 4.0 with diluted sodium hydroxide.

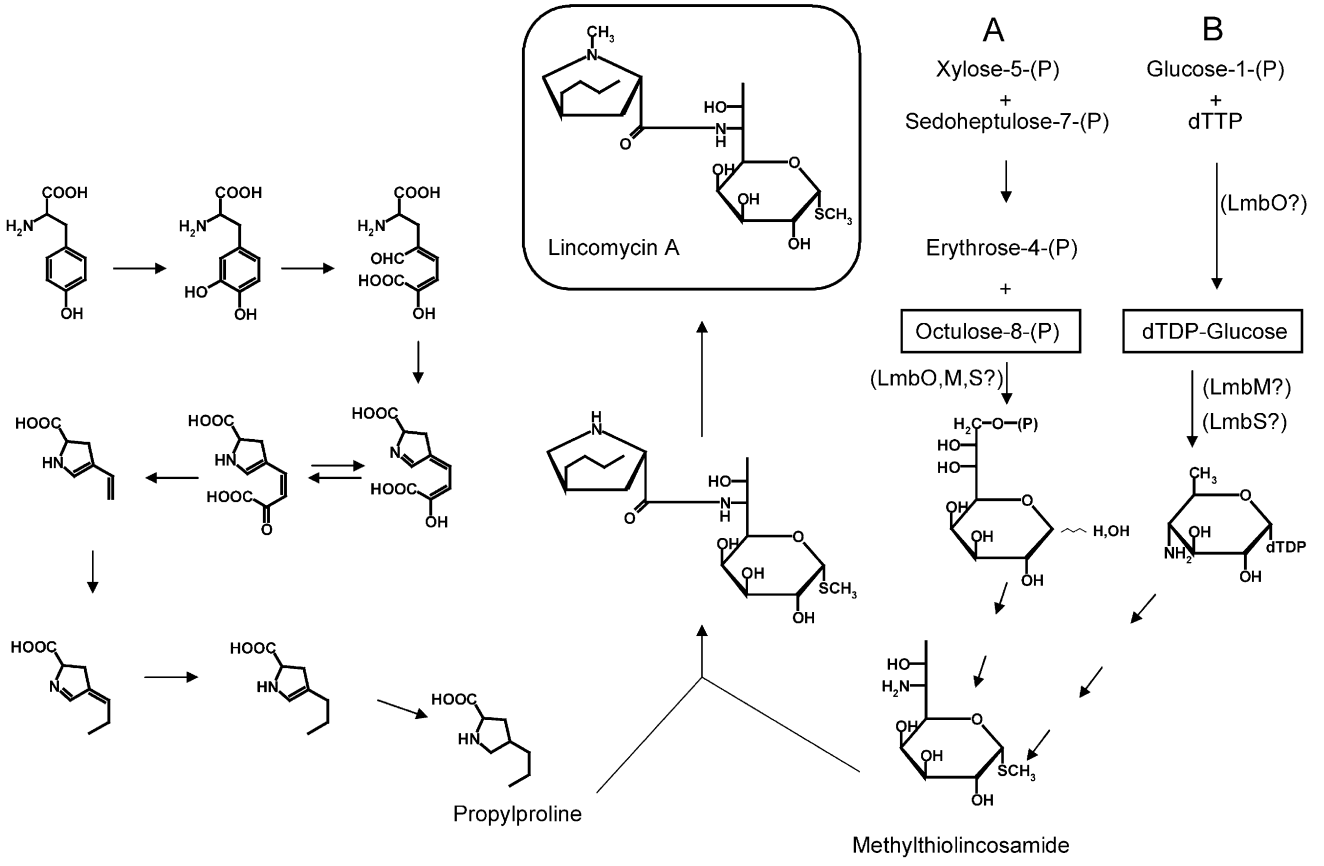



**Fig. 2** Hypothetical biosynthetic pathway for lincomycin A (based on Brahme et al, 1984a, 1984b; Kuo et al. 1992 and Peschke et al. 1995). Two alternative pathways are proposed for methylthiolincosamide biosynthesis: *A* from intermediates of the pentose phosphate cycle via formation of a $C_8$ sugar precursor; *B* from dTDP-glucose via a 6-deoxyhexose pathway and a later extension of the carbon chain (Thorson et al. 1993). The possible involvement of three of the gene products of the putative methylthiolincosamide genes is indicated

Capillary electrophoresis was used for simultaneous determination of the five aminoglycoside antibiotics (netilmicin, tobramycin, lincomycin, kanamycin and amikacin). Under the optimum separation conditions (separation voltage of 6.2 kV, electrophoretic medium of 125 mM NaOH), the aminoglycoside antibiotics were baseline separated within 20 min. At a working electrode potential of 0.7 V (versus saturated calomel electrode), the calibration curves were linear over two orders of magnitude of concentration, and the detection limits (S/N=3) were below 6.7 μM for lincomycin (Yang et al. 2001).

HPLC-integrated pulsed amperometric detection is a good primary detection method to complement or replace absorbance detection for nonchromophoric sulfur-containing antibiotics and their sulfur-containing impurities and decomposition products (Hanko et al. 2001).

A thin layer chromatography (TLC)/densitometric method was developed with mobile phases e.g. 10% citric acid:*n*-*n*-hexane:ethanol (80:1:1, v/v) and/or *n*-butanol:ethanol:chloroform:25% ammonia (4:5:2:5, v/v) for the identification and quantitation of oxytetracycline, tiamulin, lincomycin, and spectinomycin in veterinary preparations (Krzek et al. 2000).

Lincomycin and related antibiotics were analysed by a tandem mass spectral technique with in-source collision-induced dissociation in bovine milk extract. Lincomycin gave a limit of detection of 0.83 pg on-column, despite the fact that trifluoroacetic acid (TFA) was required to give adequate chromatography (Crellin et al. 2003).

## Genetics of lincomycin biosynthesis

Similar to other antibiotics (Martin and Liras 1989) produced by actinomycetes, genes coding for lincomycin biosynthesis are clustered together in a single genomic region and are closely linked to the corresponding resistance determinants (Wovcha et al. 1986; Chung and Crose 1990; Zhang et al. 1992).

The 35 kb-long chromosomal region bearing the lincomycin production gene cluster from the overproducing industrial strain *S. lincolnensis* 78–11 was cloned and sequenced by Peschke et al. (1995). There are 27 open reading frames (ORFs) with putative biosynthetic or regulatory functions (*lmb* genes) and three resistance (*lmr*) genes in the region (Fig. 3). Genes designated *lmr*A and *lmr*C flank the cluster and appear to code for proteins

involved in lincomycin export (Zhang et al. 1992; Peschke et al. 1995). The *lmr*B gene codes for a protein very similar to several 23S RNA methyltransferases (Zhang et al. 1992). Four other lincomycin producers probably share the overall cluster organisation; however, the clusters are embedded in non-homologous chromosomal surroundings. DNA sequence analysis suggests a complicated transcription pattern with at least 12 possible transcription units.

By virtue of experiments using transposon-4560-mediated mutagenesis of the lincomycin production cluster of a strain derived from *S. lincolnensis* UC 5124, it is assumed that genes coding for enzymes involved in propylproline synthesis are located close to the *lmr*A gene whereas those controlling methythiolincosamide synthesis are located further from it (Chung and Crose 1990). Thus, biosynthetic genes may be organised according to functional pathways within the cluster region; however, the same experiments suggest that genes coding for subunits of the NDL synthetase, the key enzyme catalysing propylproline and methylthiolincosamide condensation, are located at three widely separated loci (Chung et al. 1997). The functions coded for by just a few of the above mentioned ORFs have been demonstrated experimentally. In addition to resistance genes, only the functions of the genes *lmbB1* and *lmbB2*, coding for enzymes converting L-tyrosine and L-dihydroxyphenylalanine, have been clarified (Neusser et al. 1998). Gene *lmbJ* apparently codes for a specific N-demethyllincomycin methyltransferase (Janata et al. 1999). Functions of other proteins encoded by lincomycin cluster genes can only be deduced.

The molecular mechanism by which clindamycin inhibits ribosomal protein biosynthesis in prokaryotic microorganisms has hitherto been unclear. However, recently it has been shown that the three-dimensional structure of clindamycin closely resembles the biomolecules L-Pro-Met and the D-ribosyl ring of adenosine (Fitzhugh 1998), which occur proximal to one another at the 3′-ends of L-Pro-Met-tRNA and deacylated-tRNA for a brief interval following the formation of a peptide bond between L-Pro-tRNA and L-Met-tRNA. This finding strongly suggests that clindamycin and other lincosamides act as mimics of the 3′-ends of L-Pro-Met-tRNA and deacylated-tRNA as they are positioned during an initial phase of pretranslocation in the peptide elongation cycle. As such, clindamycin constitutes a structural analogue of an intermediate state in protein biosynthesis.

## Biosynthesis of lincomycin

Lincomycin is chemically classified as a member of the lincosamide group of antibiotics. A typical feature of these antibiotic molecules is the presence of 6,8-dideoxy-6-aminooctose lincosamine. In lincomycin A, the unusual sugar moiety α-amino-6,8-dideoxy-1-thio-D-*erythro*-α-D-galactooctopyranoside (commonly referred to as methylthiolincosamide) is linked by an amide bond to the amino acid derivative trans-*N*-methyl-4-*n*-propyl-L-proline (commonly referred to as propylhygric acid) (Piepersberg 1994).

The lincomycin biosynthetic pathway (Retzlaff et al. 1993) proceeds via a heterogeneously rooting biphasic pathway giving rise to propylproline (Fig. 2) and methylthiolincosamide (Fig. 2). These subunits then become condensed to *N*-demethyllincomycin, which is finally methylated to yield lincomycin.

### Biosynthesis of the amino acid moiety

Tyrosine seems to be the principal precursor of the amino acid moiety. Feeding studies using L-[1-$^{14}$C]tyrosine and L-[U-$^{14}$C]tyrosine by Witz et al. (1971) suggested that seven of the nine carbons in the propylhygric acid come from tyrosine. The other two carbons in the propylhygric acid, namely, the *N*-methyl and terminal aliphatic side chain methyl groups, are added in reactions involving transfer from *S*-adenosylmethionine (Argoudelis et al. 1969; Witz et al. 1971; Brahme et al. 1984a, 1984b).

Biosynthetic experiments with D-($^{13}C_6$)glucose suggest that glucose is converted via glycolysis and the hexose monophosphate shunt to phosphoenolpyruvate and erythrose-4-phosphate, respectively, which are in turn converted via the shikimic acid pathway to tyrosine and then to dihydroxyphenylalanine. The pathway seems to continue through the 2,3-extradiol cleavage of the aromatic ring of dihydroxyphenylalanine, followed by condensation to form a pyrrole ring (Brahme et al. 1984a). Lincomycin probably shares a similar biosynthetic route—leading from tyrosine through dihydroxyphenylalanine and pyrrole ring formation—with the pyrrolo[1,4]benzodiazepine antibiotics anthramycin, sibiromycin and tomaymycin (Hurley et al. 1979; Hurley 1980). The multistep conversion of dihydroxyphenylalanine to propylproline remains unknown, but it seems to lead through 1,2,3,6-tetradehydro-propylproline, which was shown to accumulate in mutants lacking a reductase that requires lincomycin cosynthetic factor (Kuo et al. 1992) identified as 7,8-didemethyl-8-hydroxy-5-deazariboflavin (Kuo et al. 1989). Based on this finding, Kuo and coworkers modified the scheme of Brahme et al. (1984a, 1984b) and designed the remaining steps leading to propylproline as shown in (Fig. 2).

### Biosynthesis of the sugar moiety

To date, no intermediates of the aminooctose moiety methylthiolincosamide biosynthesis have been identified. A likely pathway leading from glucose to methylthiolincosamide was predicted by Brahme et al. (1984b), who studied the metabolic origin of methythiolincosamide by means of analysis of $^{13}C$–$^{13}C$ spin coupling patterns in methythiolincosamide and lincomycin derived from a biosynthetic experiment with D-($^{13}C_6$)glucose, and the specific $^{13}C$ enrichments in methylthiolincosamide synthesised from specifically labeled substrates. The data

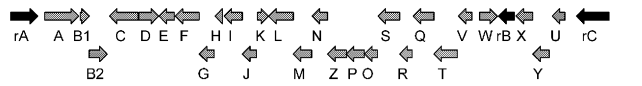



**Fig. 3** The lincomycin gene cluster (according to Peschke et al. 1995). *Grey arrows* designated *A–Z* indicate putative biosynthetic genes and *black arrows* designated *rA–rC* indicate genes coding for functions that impart a lincomycin resistance phenotype

obtained from both these approaches suggested that the C8 carbon skeleton of methythiolincosamide arises from condensation of a pentose unit (C5) and a C3 unit. The pentose unit could be derived either from glucose through the hexose monophosphate shunt as an intact unit or result from condensation of glyceraldehyde-3-phosphate with a C2 donor such as sedoheptulose-7-phosphate via a transketolase reaction. The C3 unit probably comes from a suitable donor molecule, such as sedoheptulose-7-phosphate, and is added via a transaldolase reaction. According to the origin of the C3 unit donor, the unit could consist either of an intact C3 unit or a C2 unit combined with a C1 unit. The C3 and C5 units then condense giving rise to octose, which is then converted to methylthiolincosamide. The final conversion of the C8-skeleton to methylthiolincosamide was postulated to involve isomerisation of the octulose to octose, dephosphorylation and reduction of the C8 carbon, transamination of the precursor 6-ketooctose, and final thiomethylation of the C1 carbon. First attempts to determine the metabolic origin of the sugar moiety were provided by means of a combination of radioactive labeling and mass spectroscopy. The S-methyl group of the methylthiolincosamide subunit was found to be derived from C1 fragments at the oxidation state of methyl groups.

Analysis of the lincomycin gene cluster sequence lead Peschke et al. (1995) to design an alternative route (Fig. 2, route B) to the above scheme.

The eight genes, *lmb-LMNZPOSQ*, form a "sugar subcluster" which probably codes for a set of enzymes involved in sugar metabolism (Fig. 3) (Pissowotzki et al. 1991). Putative proteins LmbO, LmbM and LmbS show similarity to enzymes involved in the central steps of many NDP-6-deoxyhexose pathways (Stockmann and Piepersberg 1992), including NDP hexose synthases (LmbO), NDP-hexose dehydratases (LmbM), and (NDP-) ketosugar (or cyclitol) aminotransferases (LmbS). The genetic record led Peschke et al. (1995) to the conclusion that methylthiolincosamide biosynthesis presumably involves a nucleotide (probably dTDP) activation step and a series of modification steps on dNTP-activated sugar intermediates. Thus, two totally different pathways, starting from either D-glucose or NDP-activation and modification of a C8 sugar intermediate, both represent routes leading to the production of methylthiolincosamide.

The authors assume that the $^{13}C$-labelling pattern in methylthiolincosamide published by Brahme et al (1984b), suggesting that an octulose-phosphate intermediate is formed first, could support glucose as a starter unit if there is rapid and efficient equilibrium in the hexose phosphate pool mediated by a hexose monophosphate shunt during the production phase. This type of rearrangement of labelling has been described for other streptomycete products directly derived from glucose (Rinehart et al. 1992).

The detection of a gene (*lmbR*) encoding a transaldolase-like enzyme in the "sugar subcluster" could support an alternative route via a pyranosidic octose intermediate that is NDP-activated and further modified in this form. Finally, a thiomethyl unit at the C1 position of the postulated (NDP-)6-amino-6,8-deoxyoctose intermediate would be added, which could be transferred from 5′-thiomethyladenosine, a side product of polyamine biosynthesis from *S*-adenosyl-methionine (Piepersberg and Distler 1997).

## Condensation and final modification

Formation of the amide bond between the carboxyl group of the aglycone propylproline and the amino group of the methylthiolincosamide yielding *N*-demethyllincomycin seems to be the penultimate step in the lincomycin biosynthesis. *N*-Demethyllincomycin-synthetase, which catalyses this reaction, is probably a complex of readily dissociable subunits with non-identical molecular weights (Hausknecht and Wolf 1986).

The final step in the pathway is *N*-methylation of *N*-demethyllincomycin, catalysed by *S*-adenosylmethionine: *N*-demethyllincomycin methyl transferase (Patt and Horvath 1985).

## Conclusion

Chemical and microbial modifications of lincomycin have succeeded in producing derived antibiotics possessing greater potency, extended spectrum (including antiparasitic activity), improved taste, and varied absorption characteristics. Several total syntheses of lincomycin have been achieved. These syntheses offer opportunities for further modification of the lincomycin molecule.

The direction and extent to which these new opportunities, as well as the older synthetic patterns, are developed and extended, make modification of lincomycin an exciting area of future research.

## References

Argoudelis AD, Coats JH (1969). Microbial transformation of antibiotics. II. Phosphorylation of lincomycin by *Streptomyces* species. J Antibiot 22:341–343

Argoudelis AD, Coats JH (1974) Process for preparing lincomycin. US Patent 3,812,014

Argoudelis AD, Stroman DW (1984) Process of producing lincomycin nucleotides. US Patent 4,464,466

Argoudelis AD, Eble TE, Fox JA, Mason DJ (1969) Studies on the biosynthesis of lincomycin. IV. The origin of methyl groups. Biochemistry 8:3408–3411

Argoudelis AD, Coats JH, Pyke TR (1972) Lincomycin production. US Patent 3,697,380

Bergy ME, Herr RR, Mason DJ (1963) Antibiotic lincolnensin and method of production. US Patent 3,086,912

Bergy ME, Coats JH, Malik VS (1981) Process for preparing lincomycin. US Patent 4,271,266

Bibikova MV, Singal EM, Ivanitskaia LP, Zhdanovich IV (1989) Production of lincomycin by *Micromonospora halophytica* culture. Antibiot Khimioter 34:723–726

Birkenmeyer RD (1981) Lincomycin compounds. Novel useful analogs of the well known antibiotics lincomycin and clindamycin. US Patent 4,278,789

Birkenmeyer RD (1982a) Lincomycin compounds. Novel useful analogs of the well known antibiotics lincomycin and clindamycin. US Patent 4,309,533

Birkenmeyer RD (1982b) Lincomycin compounds. Novel useful analogs of the well known antibiotics lincomycin and clindamycin. US Patent 4,310,660

Bowden K, Stevens GP (2000) An alternative synthesis of clindamycin. J Serbian Chem Soc 65:691–693

Brahme NM, Gonzalez JE, Mizsak S, Rolls JR, Hessler EJ, Hurley LH (1984a) Biosynthesis of the lincomycins. 2. Studies using stable isotopes on the biosynthesis of methylthiolincosaminide moiety of lincomycin A. J Am Chem Soc 106:7878–7883

Brahme NM, Gonzalez JE, Rolls JR, Hessler EJ, Mizsak S, Hurley LH (1984b) Biosynthesis of the lincomycins. 1. Studies using stable isotopes on the biosynthesis of the propyl- and ethyl-L-hygric acid moieties of lincomycin A and B. J Am Chem Soc 106:7873–7878

Bruce W, Churchill BJ, Rakow ME (1973) The amino acids trans-4-*n*-propyl-L-proline and trans-4-ethyl-L-proline. US Patent 3,753,859

Cherlet M, Croubels S, De Backer P (2002) Determination of clindamycin in animal plasma by high-performance liquid chromatography combined with electrospray ionization mass spectrometry. J Mass Spectrom 37:848–853

Chung ST, Crose LL (1990) Transposon Tn4556-mediated DNA insertion and site-directed mutagenesis. In: Heslot H, Davies J, Florent J, Bobichon L, Durand G, Peneasse L (eds) Proceedings of the 6th International Symposium on Genetics of Industrial Microorganisms. Societe Francaise de Microbiologie, Strasbourg, pp 207–218

Chung ST, Manis JJ, McWethy SJ, Patt TE, Witz DF, Wolf HJ, Wovcha MG (1997) Fermentation, biosynthesis, and molecular genetics of lincomycin. In: Strohl WR (ed) Biotechnology of industrial antibiotics, 2nd edn. Dekker, New York, pp 165–186

Crellin KC, Sible E, Van Antwerp J (2003) Quantification and confirmation of identity of analytes in various matrices with in-source collision-induced dissociation on a single quadrupole mass spectrometer. Int J Mass Spectrom 222:281–311

Dong B, Dong M (2001) Extracting technology for lincomycin. Chinese Patent 1,301,703

Fieger-Büschges H, Schussler G, Larsimont V, Blume H (1999) Determination of clindamycin in human plasma by high-performance liquid chromatography using coupled columns. J Chromatogr B 724:281–286

Fitzhugh AL (1998) Antibiotic inhibitors of the peptidyl transferase center. 1. Clindamycin as a composite analogue of the transfer RNA fragments L-Pro-Met and the D-ribosyl ring of adenosine. Bioorg Med Chem Lett 8:87–92

Gonda J, Zavacka E, Budesinsky M, Cisarova I, Podlaha J, (2000) Stereocontrolled introduction of an amino group at C-6 of D-galactose via (3,3)-sigmatropic rearrangements—novel synthesis of lincosamine and 7-epi-lincosamine precursors. Tetrahedron Lett 41:525–529

Grady JE (1968) Recent developments in lincomycin research. Int J Pharmacol 16:533–538

Hanko VP, LaCourse WR, Dasenbrock CO, Rohrer JS (2001) Determination of sulfur-containing antibiotics using high-performance liquid chromatography with integrated pulsed amperometric detection. Drug Dev Res 53:268–280

Hausknecht EC, Wolf HJ (1986) Radiometric assay for N-demethyllincomycin synthetase. In: Proceedings of the American Society of Microbiology Annual Meeting, 1986. ASM, Washington, D.C.

Herr RR, Bergy ME (1962) Lincomycin, a new antibiotic. II. Isolation and characterization. Antimicrob Agents Chemother pp 560–564

Herr RR, Slomp G (1967) Lincomycin. II. Characterization and gross structure. J Am Chem Soc 89:2444–2447

Hoeksema H (1968) Celesticetin. V. Structure of celesticetin. J Am Chem Soc 90:755–757

Hoeksema H, Crum GF, DeVries WH (1955) Isolation and purification of celesticetin. Antibiot Ann 2:837–841

Hoeksema H, Bannister B, Birkenmeyer RD, Kagan F, Magerlein BJ, MacKellar FA, Schroeder W, Slomp G, Herr RR (1964) Chemical studies on lincomycin. 1. The structure of lincomycin. J Am Chem Soc 86:4223–4224

Hofstetter JR (1982) Process for the purification of lincomycin. US Patent 4,317,903

Horton D, Wander JD, Foltz RL (1974) Chemical-ionization mass spectrometry of Lincomycin and Clindamycin. Anal Biochem 59:452–461

Hurley LH (1980) Elucidation and formulation of novel biosynthetic pathways leading to the pyrrolo-benzodiazepine antibiotics anthramycin, tomaymycin, and sibiromycin. Acc Chem Res 13:263–269

Hurley LH, Laswell WL, Ostrander JM, Parry R (1979) Pyrrolo [1,4]benzodiazepine antibiotics. Biosynthetic conversion of tyrosine to the C2- and C3-proline moieties of anthramycin, tomaymycin and sibiromycin. Biochemistry 18:4230–4237

Janata J, Kadlec J, Gakh O, Lichá I, Kopecký J, Tichý P, Spížek J (1999) LmbJ protein alone catalyzes conversion of N-demethyllincomycin to lincomycin. In: Abstracts, 11th International Symposium on the Biology of Actinomycetes. Sissi-Heraklion, Crete, Greece, 24–28 October 1999, p 188

Kagan F, Trostic MF (1972) Mass spectra of lincomycin, lincomycin analogs, and degradation products. Org Mass Spectrom 6:1217–1223

Krzek J, Kwiecien A, Starek M, Kierszniewska A, Rzeszutko W (2000) Identification and determination of oxytetracycline, tiamulin, lincomycin, and spectinomycin in veterinary preparations by thin-layer chromatography/densitometry. J Assoc Off Anal Chem Int 83:1502–1506

Kuo MS, Yurek DA, Coats JH, Li GP (1989) Isolation and identification of 7,8-didemethyl-8-hydroxy-5-deazariboflavin, an unusual cosynthetic factor in streptomycetes, from *Streptomyces lincolnensis*. J Antibiot 42:475–478

Kuo MS, Yurek DA, Coats JH, Chung ST, Li GP (1992) Isolation and identification of 3-propylidiene acid, a biosynthetic precursor of lincomycin. J Antibiot 45:1773–1777

Kuznetsov VD, Bushueva OA, Bryzgalova LS (1974) Variation of *Actinomyces roseolus*. A lincomycin-producing organism. Antibiotiki 19:690–693

La Follette G, Gambertoglio J, White JA, Knuth DW, Lin ET (1988) Determination of clindamycin in plasma or serum by high-performance liquid chromatography with ultraviolet detection. J Chromatogr 431:379–388

Lewis C, Clapp HW, Grady JE (1963) In vitro and in vivo evaluation of lincomycin, a new antibiotic. Antimicrob Agents Chemother pp 570–582

Li C, Yu W, Li B (2001) Producing technology for lowering lincomycin B content in lincomycin by vibrative sieve plate extraction tower. Chinese Patent 1,280,133

Liu CM, Chen YK, Yang TH, Hsieh SY, Hung MH, Lin ET (1997) High-performance liquid chromatographic determination of clindamycin in human plasma or serum: application to the bioequivalency study of clindamycin phosphate injections J Chromatogr B 696:298–302

Magerlein BJ (1971) Lincomycin. XII. Preparation of methyl N-methyl-α-thiolincosaminide. J Org Chem 36:596–598

Magerlein BJ, Kagan F (1969) Lincomycin. IX. 7-Thiol and thioamido analogs of lincomycin. J Med Chem 12:974–977

Martens-Lobenhoffer J, Banditt P (2001) Sensitive and specific determination of clindamycin in human serum and bone tissue applying liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry. J Chromatogr B 755:143–149

Martin JF, Liras P (1989) Organization and expression of genes involved in the biosynthesis of antibiotics and other secondary metabolites. Annu Rev Microbiol 43:173–206

Mason DJ, Lewis C (1964) Biological activity of the lincomycin related antibiotics. Antimicrob Agents Chemother pp 7–12

Mason DJ, Dietz A, DeBoer C (1962) Lincomycin, a new antibiotic. I. Discovery and biological properties. Antimicrob Agents Chemother pp 554–559

Neusser D, Schmidt H, Spížek J, Novotná J, Peschke U, Kaschabeck S, Tichý P, Piepersberg W (1998) Arch Microbiol 169:322–332

Patt TE, Horvath BA (1985) Isolation and characterization of N-demethyllincomycin methyltransferase. In: Abstracts, 13th International Congress of Biochemistry, Amsterdam, The Netherlands, August 25–30 1985

Patt TE, Argoudells AD, Marshall VP (1984) Process for preparing lincomycin and clindamycin ribonucleotides. US Patent 4,430,495

Peschke U, Schmidt H, Zhang HZ, Piepersberg W (1995) Molecular characterization of the lincomycin production gene cluster of *Streptomyces lincolnensis* 78–11. Mol Microbiol 16:1137–1156

Piepersberg W (1994) Streptomycin and related aminoglycoside antibiotics. In: Vining L, Stuttard C (eds) Biochemistry and genetics of antibiotic biosynthesis. Butterworth-Heinemann, Newton, pp 531–570

Piepersberg W, Distler J (1997) Aminoglycosides and sugar components in other secondary metabolites in biotechnology. In: Kleinkauf H, von Dören H (eds) Biotechnology, 2nd edn, vol 7. Products of secondary metabolism. Wiley-VCH, Weinheim, pp 397–488

Pissowotzki K, Mansouri K, Piepersberg W (1991) Genetics of streptomycin production in *Streptomyces griseus*: molecular structure and putative function of genes strELMB2N. Mol Gen Genet 231:113–123

Pospíšil S, Sedmera P, Halada P, Spížek J (2001) Oxidation of lincomycin by hydrogen peroxide restricts its potential biotransformation with haloperoxidases. Folia Microbiol 46:376–378

Retzlaff L, Mayer G, Beyer G, Ahlert J, Verseck S, Distler J, Piepersberg W (1993) Streptomycin production in streptomycetes: a progress report. In: Hegeman GD, Baltz RH, Skatrud PL (eds) Industrial microorganisms: basic and applied molecular genetics. American Society for Microbiology, Washington, D.C., pp 183–194

Reusser F, Argoudelis AD (1974) Process for preparing lincomycin. US Patent 3,833,475

Rinehart KL, Snyder WC, Staley AL, Lau RCM (1992) Biosynthetic studies on antibiotics. In: Petroski RJ, McCormick SP (eds) Secondary metabolite biosynthesis and metabolism. Plenum Press, New York, pp 41–60

Schroeder W, Bannister B, Hoeksema H (1967) Lincomycin. The structure and stereochemistry of the carbohydrate moiety. J Am Chem Soc 89:2448–2453

Slomp G, MacKellar FA (1967) Lincomycin. IV. Nuclear magnetic resonance studies on the structure of lincomycin, its degradation products, and some analogs. J Am Chem Soc 89:2454–2459

Stockmann M, Piepersberg W (1992) Gene probes for the detection of 6-deoxyhexose metabolism in secondary metabolite-producing streptomycetes. FEMS Microbiol Lett 90:185–190

Stratton CW (1998) Macrolides, lincosamides, and streptogramins: new agents and new roles. Antimicrobics Infect Dis Newslett 17:89–92

Szunyog J, Adams E, Liekens K, Roets E, Hoogmartens J (2002) Analysis of a formulation containing lincomycin and spectinomycin by liquid chromatography with pulsed electrochemical detection. J Pharm Biomed Anal 29:213–220

Thorson JS, Lo SF, Liu H-W, Hutchinson CR (1993) Biosynthesis of 3,6-dideoxyhexoses: new mechanistic reflections upon 2,6-dideoxy, 4,6-dideoxy, and amino sugar construction. J Am Chem Soc 115:6963–6994

Visser J (1972) Lincomycin production. US Patent 3,676,302

Wang B, Qi P (1999) Process for recovering lincomycin from filtered dregs of its fermented liquid. Chinese Patent 1,239,100

Wang B, Qi P (2000) Process for extracting lincomycin by using organic acid. Chinese Patent 1,266,063

Wang B, Qi P (2001) Lincomycin extracting process. Chinese Patent 1,288,895

Witz DF (1972) Lincomycin production. US Patent 3,687,814

Witz DF, Hessler EJ, Miller TL (1971) Bioconversion of tyrosine into the propylhygric acid moiety of lincomycin. Biochemistry 10:1128–1133

Wovcha MG, Steuerwald DC, Manis JJ (1986) Cloning and characterization of a lincomycin resistance gene from *Streptomyces lincolnensis*. In: Abstracts of the 5th International Symposium on the Genetics of Industrial Microorganisms. Split, Yugoslavia, 14–20 September 1986. Abstract W4-P4

Yang WC, Yu AM, Chen HY (2001) Applications of a copper microparticle-modified carbon fiber microdisk array electrode for the simultaneous determination of aminoglycoside antibiotics by capillary electrophoresis. J Chromatogr A 905:309–318

Yu LL, Chao CK, Liao WJ, Twu TY, Liu CM, Yang TH, Lin ET (1999) Determination of clindamycin in human plasma by liquid chromatography electrospray tandem mass spectrometry: application to the bioequivalence study of clindamycin. J Chromatogr B 724:287–294

Zhang HZ (1993) Genetik und Biochemie der Lincomycin-Biosynthese in *Streptomyces lincolnensis* 78–11. Klonierung und Analyse der Produktions und Resistenzgene. Dissertation, Bugh, Wuppertal

Zhang HZ, Schmidt H, Piepersberg W (1992) Molecular cloning and characterization of two lincomycin-resistance genes, lmrA and lmrB, from *Streptomyces lincolnensis* 78–11. Mol Microbiol 6:2147–2157