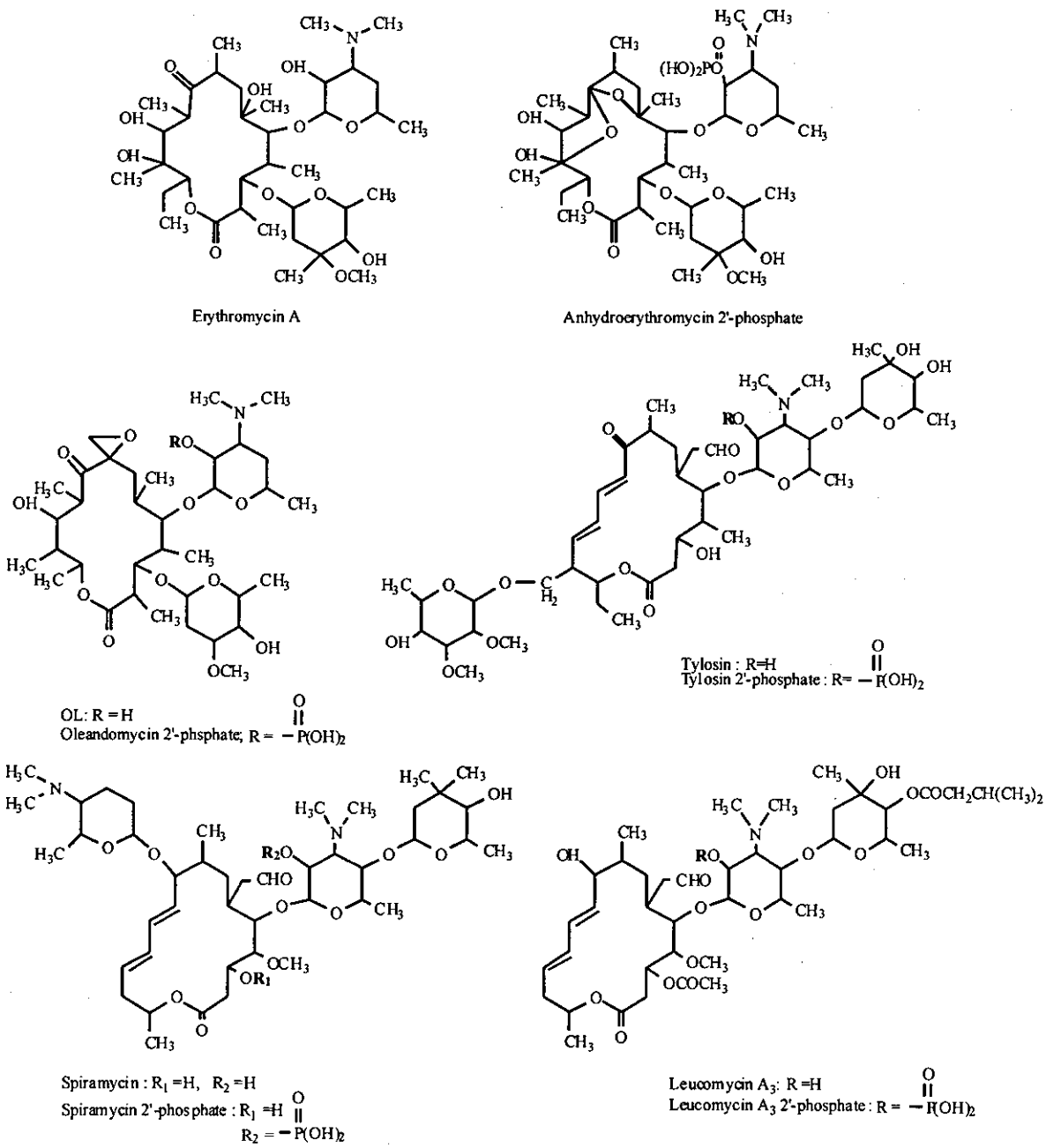


Fig. (1). Structures of substrate and product of erythromycin A, oleandomycin, tylosin, spiramycin I, and leucomycin A_3.

pathway to OL. The culture supernatant of *S. antibioticus* contained another enzyme activity capable of reactivating the glycosylated OL and regenerating the biological activity with the release of a glucose molecule. These two enzyme activities could be an integral part of the OL biosynthetic pathway. OL binds first to the enzyme, followed by UDP-glucose. The ternary complex is thus formed prior to transfer of glucose. UDP is released, followed by the glycosylated OL, and this is the final intracellular product (Fig. **4**). The OleB transporter would be responsible for transmembrane secretion [25-28] of this inactive molecule, which would then be extracellularly reactivated by the product of the *oleR* gene. Sequencing analysis of a 5.2-kb region from the OL gene cluster located between the OL polyketide synthesis gene and sugar biosynthetic gene in *S. antibioticus* revealed the presence of three open reading frames (designated *oleI*,

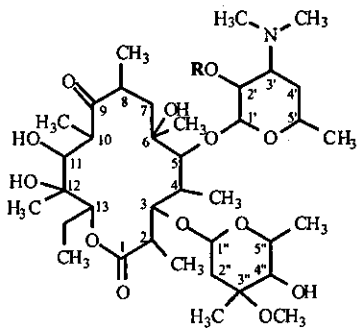


Fig. (2). Structures of erythromycin A (R =H) and 2'-(*O*-[ß-D-glucopyranosyl] erythromycin A (R= glucose).

oleN2 and *oleR*) [29]. The *oleI* gene product resembles other glycosyl-transferases involved in macrolide inactivation, including the *oleD* gene product, a previously described glycosyltransferase from *Streptococcus antibioticus*. The *oleN2* gene product showed similarity with various aminotransferases involved in the biosynthesis of 6-deoxyhexose, and the *oleR* gene product was similar to glucosidases of several origins. The sugar moieties are transferred to a different aglycon by glycosyltransferases such as OleI [30]. The sugar donor was UDP-α-glucose, though the glycosylated product showed a ß-glycoside linkage. The changes in the sugar composition of these molecules may influence the important biological actions of glucosylated OL on ribosomes [29].

Another glycosyltransferase, specific for macrolide monosaccharide, generates a disaccharide linked to the aglycon [31]. Avermectin and ivermectin, a family of oleandrose-containing disaccharide derivatives of 16-membered macrolides produced by *Streptomyces avermitilis*, are glycosylated at C-4" and C-4' by both growing and resting cells of *Saccharopolyspora erythraea* (Fig. **5**). The specificity of the *S. erythraea* enzyme of the glycosyl donor is different from that of the *S. lividans* enzyme. Glycosylated products were formed *in vitro* with UDP-glucose, UDP-galactose, UDP-mannose, and UDP-glucouronic acid, but not with UDP-xylose, UDP-N-acetylglucosamine, UDP-*N*-acetylgalactosamine, or UDP-galactouronic acid. Only the product with UDP-glucose was formed in sufficient quantity to permit its identification. The enzyme in *Streptomyces lividans* utilized only UDP-glucose and UDP-galactose (Table **3**). The *S. lividans* glycosyltransferase, which confers resistance, was induced by substrate macrolides and not by non-substrate macrolides, though that of *S. erythraea* appeared to be constitutive.

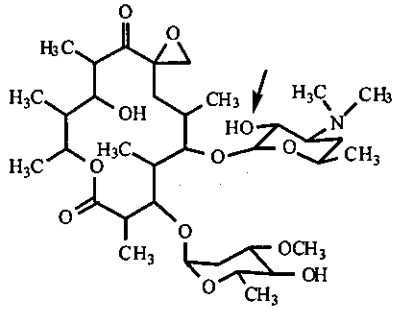


Fig. (3). Chemical structure of oleandomycin. The arrow indicates the hydroxyl group considered to be a potential site for glycosylation.

Streptomyces ambofaciens produces spiramycin, a 16-membered macrolide, and has the *gimA* gene (conferring resistance to spiramycin) downstream of the *srmA* gene, which encodes an rRNA monomethylase [32]. The *gimA* gene product shows a high degree of similarity to the *mgt* gene product in *S. lividans*. In a cloning experiment using a susceptible host mutant of *S. lividans* lacking macrolide-inactivating glycosyltransferase activity, the cloned *gimA* gene was expressed in the presence of UDP-glucose. That is, cell extracts from mutated *S. lividans* could inactivate various macrolides by glycosylation. Spiramycin was not inactivated, but forocidine, a spiramycin precursor, was modified. In *S. ambofaciens*, *gimA* could confer a low level of resistance to some macrolides even under conditions where spiramycin was produced. The putative GTG start codon of the *gimA* gene overlapped with the *srmA* stop codon (GTGA). The ORF of the *gimA* gene consisted of 1254 bp and encoded a 45-kDa protein. The deduced protein showed high homology with the deduced proteins derived from *mgt* of *S. lividans* (82%) [12] and *oleD* of *S. antibioticus* (72%) [24]. Table **4** shows the degree of identity of the DNA and amino acid sequences for four

Table 2. MIC Values of Erythromycin A (EM-A) and 2'-(*O*-[ß-D-glucopyranosyl])EM- A for *Staphylococcus and Streptococcus* Strains [21].

Organism	MIC (μg/ml)	
	EM-A	2'-(*O*-[ß-D-glucopyranosyl]EM-A
Staphylococcus aureus 9218 and 9271	0.5	>32
Streptococcus faecalis 9217	2	>32
Streptococcus pneumoniae 41	0.06	32
Streptococcus pyogenes 152	0.03	16
Streptococcus vendargensis 5315	50	1000

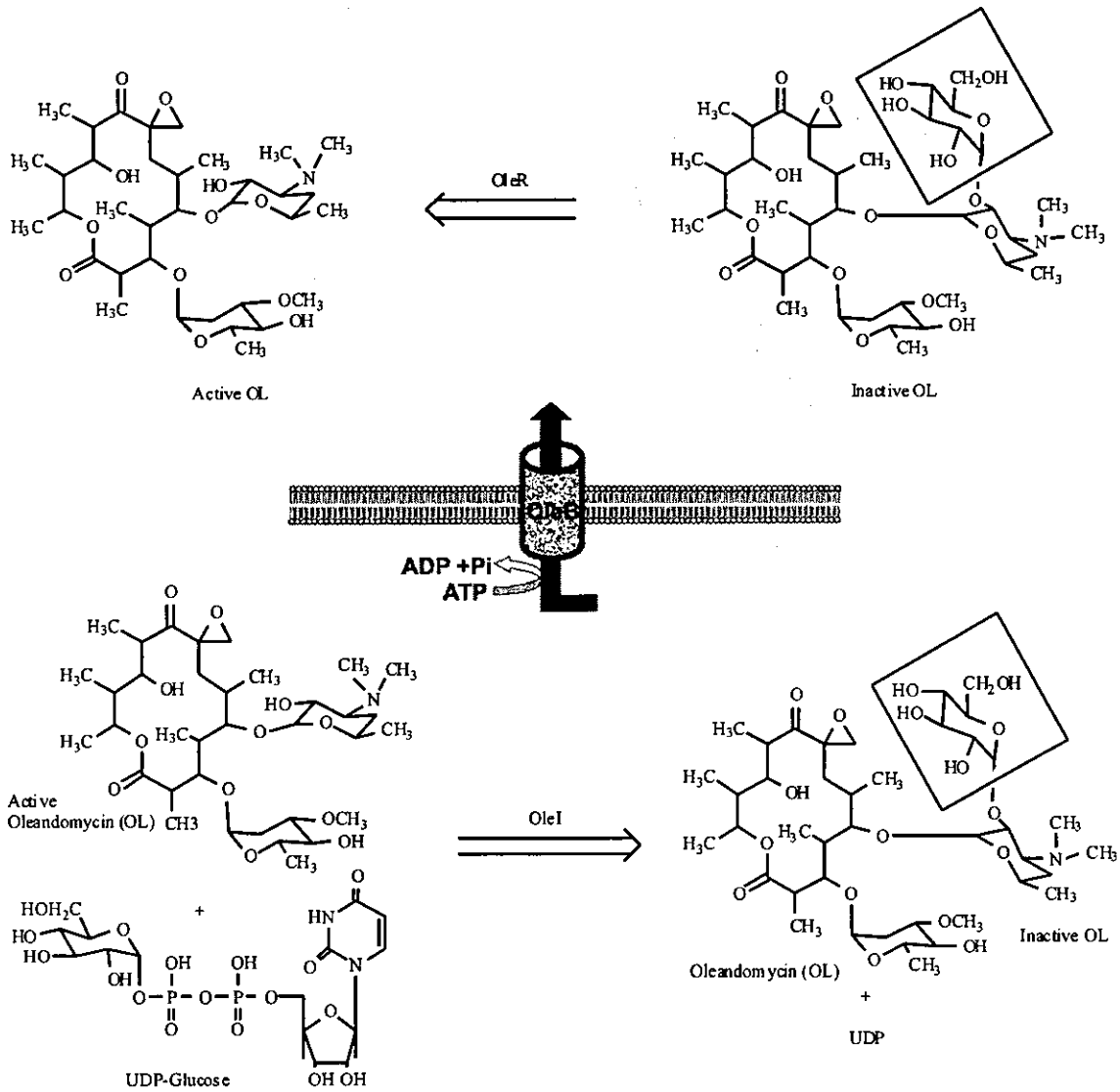


Fig. (4). Strategy for self-protection by oleandomycin producers. Intracellular glucosylation of oleandomycin to an inactive precursor is catalyzed by OleI, then the precursor is pumped out by OleB, and reactivated extracellularly by the glycosidase OleR [28, 29].

glycosyltransferase genes, *mgt, oleI, oleD,* and *gimA*, from different *Streptomyces* spp. In *Streptomyces ambofaciens*, *gimA* was found just downstream of *srmA*, which encodes an rRNA methyltransferase that confers macrolide resistance by target modification [32]. As shown in Fig. (**6**), the glycosyltransferase genes *gimA* and *mgt* are linked with methyltransferase genes, *srmA* in *S. ambofaciens* and *lrm* in *S. lividans*. For *S. antibioticus*, no methyltransferase gene was found upstream of *oleD* or *oleI*. This is in agreement with the observation that ribosomes from *S. antibioticus* are sensitive to OL, even during macrolide production [33]. Upstream of *oleD* are two ORFs, ORF1 and ORF2, which have high degrees of similarity to those of upstream of *srmA*. Thus, some deletion or insertion events may have occurred at the corresponding loci in *S. ambofaciens* or in *S. antibioticus*. The region located upstream of *lrm* in *S. lividans* is completely different from those in the other two strains. This observation suggests that glycosyltransferases like that in *S. vendargensis* are widespread among *Streptomyces* spp [13].

The alignment of the deduced amino acid sequences of three macrolide glycosyltransferases, Mgt from *S. lividans* [12], GimA from *S. ambofaciens* [32] and OleD from *S. antibioticus* [24], is shown in Fig. (**7**). These proteins contain well-conserved amino acid sequences. Analysis of the amino acid sequences of several glycosyltransferases involved in the biosynthesis of polyketides also showed very well conserved regions, including one of two histidine residues [30]. The conserved histidine seems to play an important role in the catalytic activity of the enzyme and in substrate binding

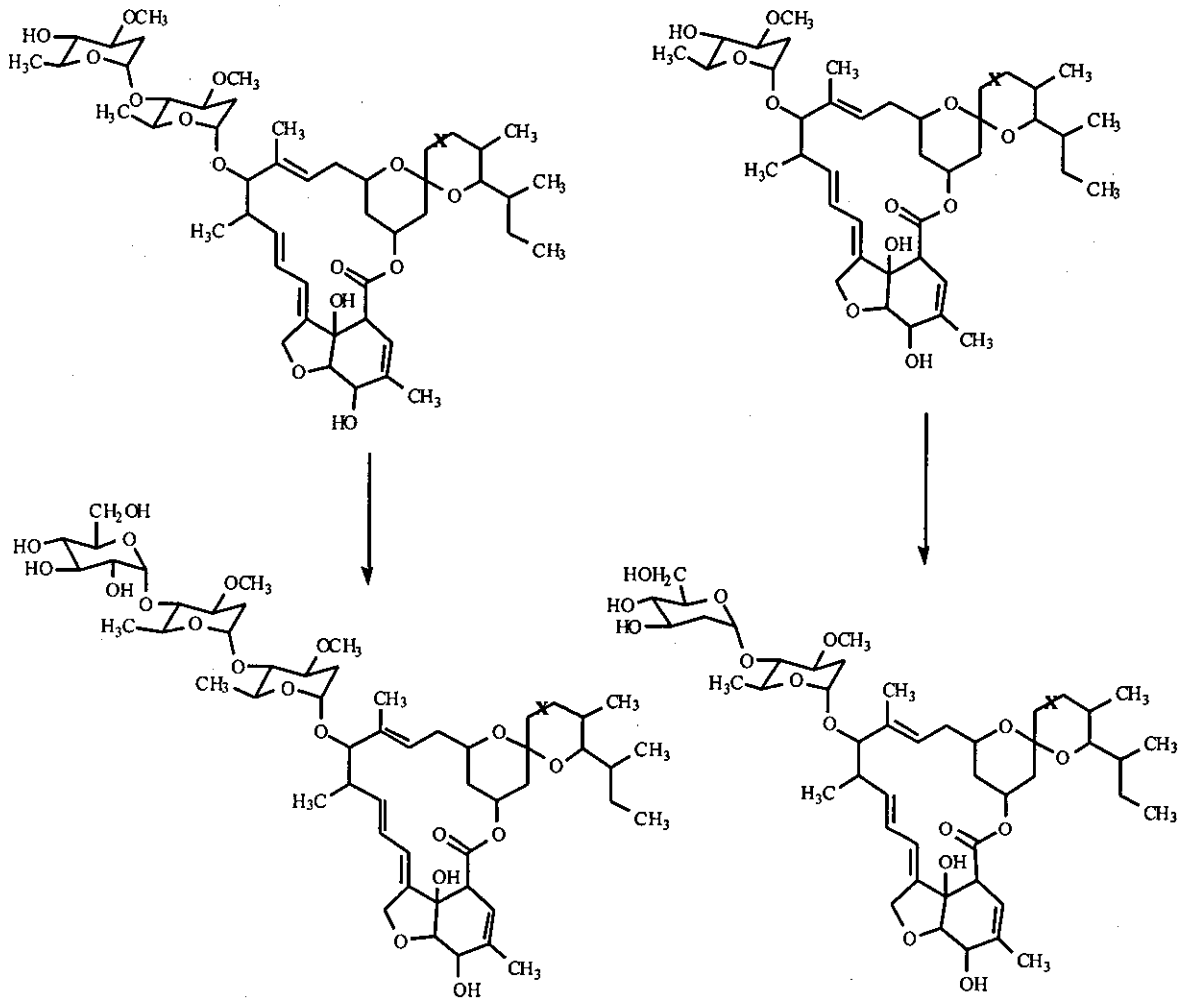


Fig. (5). Glycosylations of evermectin disaccharide and monosaccharide by *Saccharopolyspora*. X, CH_2-CH_2 in invermectin derivatives and CH=CH in avermectin derivatives.

or transition site stabilization in some oligosaccharide-dependent glycosyltransferases.

Table 3. Glycosyl Donors for Glycosyltransferases of *Streptomyces* spp [31].

Glycosyltransferase of	
Streptomyces lividans	*Streptomyces erythraea*
UDP-glucose UDP-galactose	UDP-glucose UDP-galactose UDP-mannose UDP-glucouronic acid

1-3. Deacylation

Deacylation of 16-membered macrolide antibiotics was reported by Nakahama *et al.* [16, 18]. Maridomycin, as well as spiramycin, was deacylated by esterase from *Bacillus megaterium*, *Streptomyces pristinaespiralis* and *Streptomyces olivaceus*. Figure (**8**) shows the deacylation of maridomycin III (MDM III), 9-propionylmaridomycin III (PMDM III) and

Table 4. Homology (%) of DNA and Amino Acid Sequences of Glycosyltransferases from *Streptomyces* spp.

Combination of genes	Homology (%) of	
	DNA	Amino Acid
mgt - gimA	86	82
mgt - oleD	75	72
mgt - oleI	60	40
gimA- oleD	75	72
gimA- oleI	62	41
oleD- oleI	58	38

Accession numbers of genes used for homology analysis were the same as in Table 1.

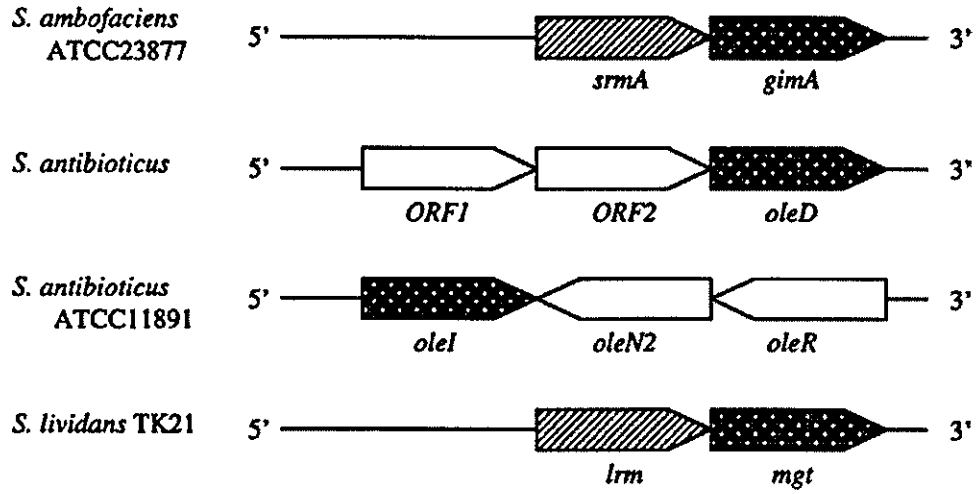


Fig. (6). Comparison of the genetic environments around macrolide resistance genes in various *Streptomyces* spp. *gimA* [32], *oleD* [24], *oleI* [29], and *mgt* [12] encode glycosyltranferase and *srmA* and *lrm* encode methyltransferase.

4"-depropionyl-9-propionylmaridomycin III (PMDM III-M) to 4"-depropionylmaridomycin III (MDM III-M) by an esterase of *Streptomyces* spp. In *B. megaterium*, MDM III and PMDM III are hydrolyzed to MDM III-M and PMDM III-M [16]. *S. olivaceus* also participates in the hydroxylation of maridomycin I (MDM I) to 3"'-hydroxylmaridomycin (HMDM I), as shown in Fig. (**9**) [17]. Josamycin (JM) was also hydroxylated to 3"'-hydroxyjosamycin (HJM) by the same strain, and hydroxylated derivatives of HMD I and HJM exhibited weaker antimicrobial activities [17].

2. Lincosamide Antibiotics

Inactivation studies on lincosamide antibiotics, including lincomycin and clindamycin, have been performed by Argoudelis *et al.* They examined the transformation of lincomycin to lincomycin sulfoxide and 1'-demethylthio-1-hydroxylincomycin by *Streptomyces lincolnensis* [34, 35] and the phosphorylation of lincomycin to lincomycin-3-phosphate by *Streptomyces rochei* [36] (Fig. **10**). These compounds showed loss of the antimicrobial activity during fermentation or growth in a synthetic medium containing *S. lincolnensis* or *S. rochei*, indicating that several *Streptomyces* spp. possess an intrinsic ability to inactivate lincomycin.

Clindamycin is a clinically useful derivative of lincomycin. *Streptomyces coelicolor* completely inactivated clindamycin in less than 48 hr when the antibiotic was added to 24-hr cultures of the organism grown in a complex medium [37]. Clindamycin could be regenerated by treatment of the inactivated fermentation broth with either crude alkaline phosphatase or snake venom phosphodiesterase. This enzymatic behavior suggests that *S. coelicolor* converted clindamycin to compound(s) containing a phosphodiester bond(s). Argoudelis *et al.* have proposed that clindamycin is phosphorylated to clindamycin-3-phosphate [38] and ribonucleotidylated to clindamycin 3-ribonucleotide by *S. coelicolor* [37-39] (Fig. **11**). Enzymic nucleotidylation of lincosamide antibiotics [40] required Mg^{2+} and nucleoside-5'-triphosphates when examined with crude enzyme preparation from *S. coelicolor* in medium maintained at around pH 6.

3. Streptogramin Antibiotics

Streptogramin family antibiotics consist of two groups of components, type A and type B, exemplified by virginiamycin M and virginiamycin S, respectively [41] (Fig. **12**). To date, very few investigations of inactivation in this family have been reported. Hou *et al.* obtained an antibiotic lactonase with a molecular weight of 35,000 from *Actinoplanes missouriensis*, and this enzyme hydrolyzed the lactone bond of dihydrostaphylomycin S (a type B streptogramin antibiotic) [42]. Mikamycin lactonase from *Streptomyces mitakaensis*, a producer of mikamycin A and B, was purified and characterized, and the structure of the reaction product, mikamycin-B acid, was identified [43, 44] (Fig. **13**). This enzyme has a molecular weight of 29,000 and catalyzes the hydrolytic degradation of mikamycin B. The reason for the difference of molecular weight is of interest, since both enzymes appear to hydrolyze the lactone linkage of type B streptogramin acid.

The inactivation of streptogramins in *Streptomyces* spp. other than *S. mitakaensis* was examined by Fernando Fierro *et al.* [45]. *Streptomyces diastaticus*, *Streptomyces loidensis*, and *Streptomyces olivaceus* were quite sensitive to most of the macrolides and lincosamides examined, but resistant to both A (or M) and B (or S) streptogramin components. This phenotypical trait was also observed in inactivation assay using cell-free extracts of the producers and type A and B streptogramin antibiotics. Though the mechanism of this inactivation is not clear, it could be lactone hydrolysis, as mentioned above.

In contrast to the lactone ring hydrolysis by mikamycin B-lactonase, inactivation of virginiamycin M1 from *Streptomyces virginiae* involved reduction of the C-16 carbonyl group [41, 46] (Fig. **14**).

```
Mgt      1 MKRKELHETSRLAYGRRMTTRPAHIAMFSIALHGHVNPSLEVIRELVARGHRVTYAIPRL   60
GimA     1 VRRGDLHETYRLDYAPHMHDPAHIAMFS-IAAHGHVNPSLEVIRELVARGHRVTYAIPPL   59
OleD     1 --------------VTTQTTPAHIAMFSIAAHGHVNPSLEVIRELVARGHRVTYAIPPV   45

Mgt     61 LADKVAEAGAEPKLWNSTLPGPDADPEAWGSTLLDNVEPFLADAIQSLPQLAQAYEGDEP  120
GimA    60 FAEKVAETGAEPKLWNSTLPGPDADPDAWGTTPLDNVEPFLDDAIQALPQLIAAYEGDEP  119
OleD    46 FADKVAATGPRPVLYHSTLPGPDADPEAWGSTLLDNRRTFLNDAIQALPQLADAYADDIP  105

Mgt     121 DLVLHDIASYTARVLGRRWEVPVISLSPCMVAWEGYEQEVGEPMWEEPRKTERGQAYYAR  180
GimA    120 DLVLHDITSYPARVLAHRWGVPAVSLSPNLVAWEGYEEEVGRPTWEEPLKTERGRAYDAR  179
OleD    106 DLVLHDITSYPARVLARRWGVPAVSLSPNLVAWKGYEEEVAEPMWREPRQTERGRAYYAR  165

Mgt     181 FHAWLEENGITDHPDPFIGRPDRSLVLIPKALQPHADRVDETTYTFVGACQGDRTAEGDW  240
GimA    180 FRGWLKENGITEDPDPFVGRPDRSLVLIPKALQPHADRVDEKTHTFVGACQGDRAAEGDW  239
OleD    166 FEAWLKENGITEHPDTFASHPPRSLVLIPKALQPHADRVDEDVYTFVGACQGDRAEEGGW  225

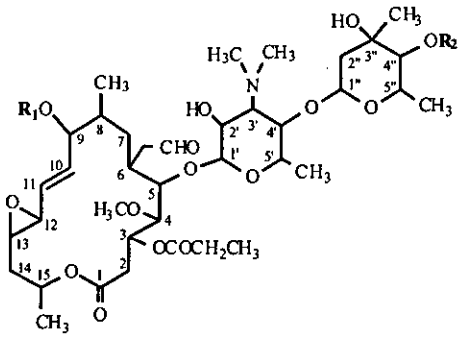
Mgt     241 ARPEGAEKVVLVSLGSAFTKQPAFYRECVRAFGELPGWHTVLQVGRHVDPAELGDVPDNV  300
GimA    240 RRPEGAEKVVLVSLGSSFTKRPAFYRACVEAFGALPGWHVVLQVGRHVDPAELGDVPENV  299
OleD    226 QRPAGAEKVVLVSLGSAFTKQPAFYRECVRAFGNLPGWHLVLQIGRKVTPAELGELPDNV  285

                                 ▼
Mgt     301 EVRTWVPQLAILQQADLFVTHAGAGGSQEGLATATPMIAVPQAADQFGNADMLQGLGVAR  360
GimA    300 EVRSWVPQLAILKQADLFVTHAGAGGSQEGLATATPIVAVPQAVDQFGNADMLQGLGVGR  359
OleD    286 EVHDWVPQLAILRQADLFVTHAGAGGSQEGLATATPMIAVPQAVDQFGNADMLQGLGVAR  345

Mgt     361 TLPTEEATAKALRTAALALVDDPEVAARLKEIQARMAQEAGTRGPADLIEAELAAARG--  418
GimA    360 HLPTEEATAEALRAAGLALVEDPEVARRLKEIQAGMAREGGTRRAADLIEAELAAART--  417
OleD    346 KLATEEATADLLRETALALVDDPEVARRLRRIQAEMAQEGGTRRAADLIEAELPARHERQ  405

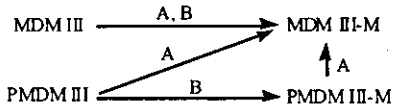
Mgt     419 -------------------------                                     443
GimA    418 -------------------------                                     442
OleD    406 EPVGDRPNVGDRPAGVRSDRQRSAL                                     430
```

Fig. (7). Alignment of the deduced amino acid sequences of three macrolide glycosyltransferases, Mgt [12], GimA [32] and OleD [24]. The histidine residue shown by the arrow seems to play an important role in the catalytic activity of the enzyme and in substrate binding or transition site stabilization in some oligosaccharide-dependent glycosyltransferases.



(Fig. (8). Contd....)

	R_1	R_2
Maridomycin II (MDM III)	H	$COCH_2CH_3$
4"-depropionylmaridomycin III (MDM III-M)	H	H
9-propionylmaridomycin III (PMDM III)	$COCH_2CH_3$	$COCH_2CH_3$
4"-depropionyl-9-propionylmaridomycin III (PMDM III-M)	$COCH_2CH_3$	H



[Deacetylation by *Streptomyces pristinaespiralis* IFO13074 (A) and *Streptomyces olivaceus* 219 (B)]

Fig. (8). Deacylation of maridomycin III (MDM III), 9-propionylmaridomycin III (PMDM III) and 9-propionylmaridomycin III (PMDM III-M) by *Streptomyces pristinaespiralis* IFO13074 and *Streptomyces olivaceus* 219 [18].

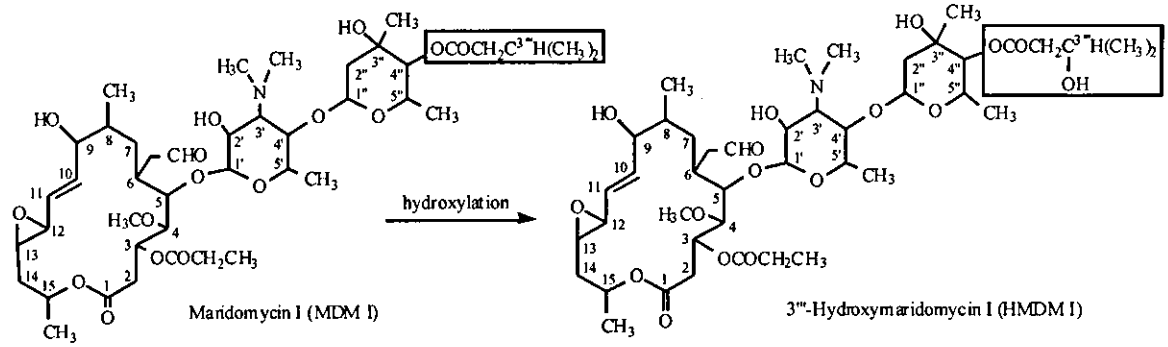


Fig. (9). Hydroxylation of MDM I to HMDM I by *Streptomyces olivaceus* 219 [17].

(B) PATHOGENS

Bacterial inactivation of antibiotics affects only structurally related antibiotics, and is different in this respect from target modification, such as dimethylation of 23S rRNA (the target site of macrolide, lincosamide and streptogramin (MLS) antibiotics). Interestingly, enzymatic inactivation of macrolides appears to be unusual in clinical isolates compared with resistance due to target site modification or efflux [47-52].

Hydrolysis by esterase [53-57] and phosphorylation [58, 59] by phosphotransferase were observed in Gram-negative bacteria such as *E. coli* originally, and in *Providencia stuartii* [56] in recent years. However, the G+C content suggests that the phosphotransferases from Gram-negative bacteria

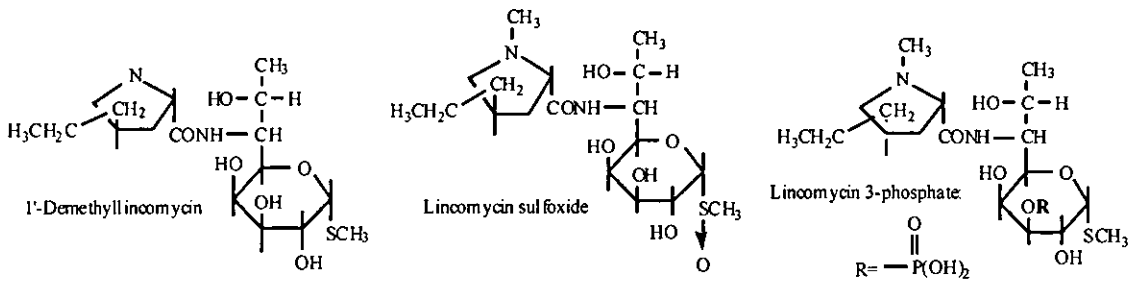


Fig. (10). Structures of 1'-demethylated lincomycin [34], lincomycin sulfoxide [35], and 3-phosphorylated lincomycin [36].

originated from Gram-positive bacteria such as *Staphylococcus aureus* [60]. Resistance owing to inactivation can be divided into two classes, degradation and modification. The former mechanism includes the hydrolysis of the lactone ring in 14- and 16-membered macrolides and type B streptogramin, and the latter includes phosphorylation of 14- and 16-membered macrolides, nucleotidylation of lincosamides, and acetylation of type A streptogramin.

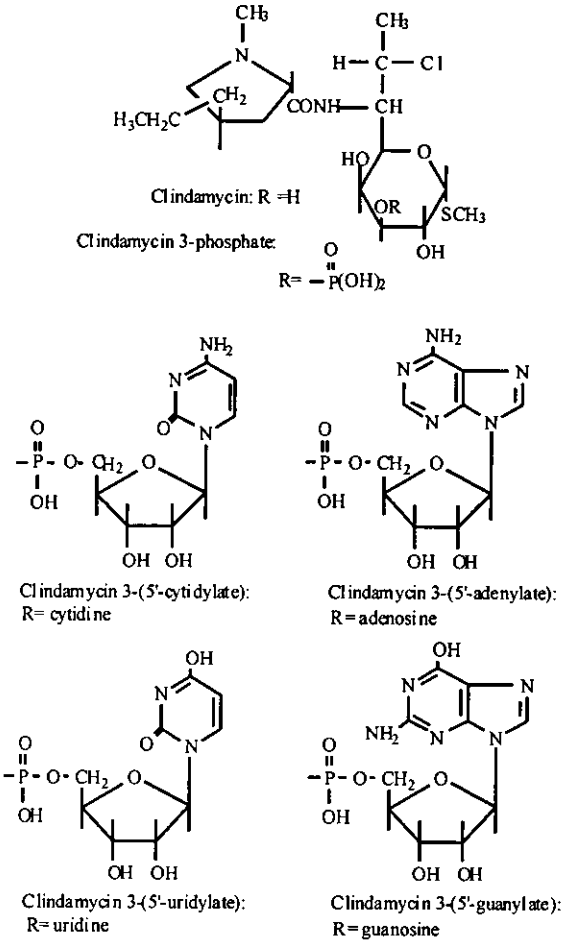


Fig. (11). Structures of clindamycin 3-phosphate and clindamycin 3-ribonucleotides [39].

This brief review outlines the biochemical mechanisms of resistance to MLS antibiotics due to inactivation in human clinical isolates (Table **5**).

1. Macrolide Antibiotics

Bacterial inactivation of macrolides, including 14-, 15-, and 16-membered macrolides, can occur through hydrolysis by erythromycin esterase A [53-55], erythromycin esterase B [57, 61, 62], phosphotransferase A [58, 63, 64], phosphotransferase B [59, 65-72], and phosphotransferase C [60, 73, 74]. Exceptionally, *Nocardia* sp. possesses multiple mechanisms for inactivation of antibiotics, that is, phosphorylation, glycosylation, reduction, and deacylation [75, 76].

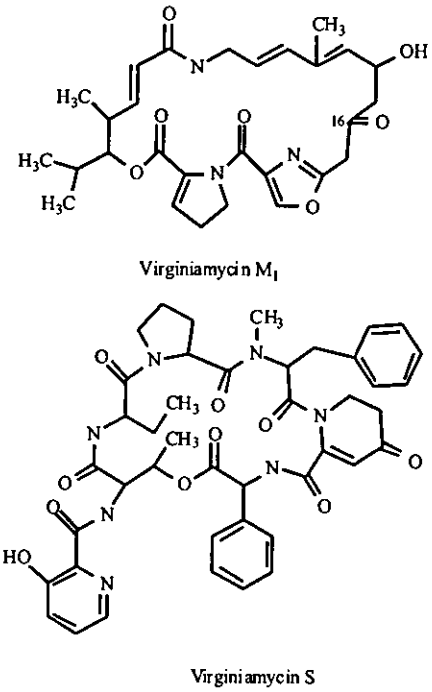


Fig. (12). Structures of virginiamycin M_1 and virginiamycin S [41].

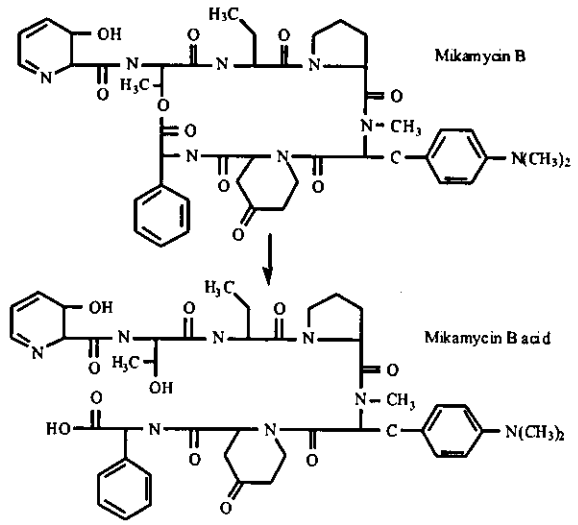
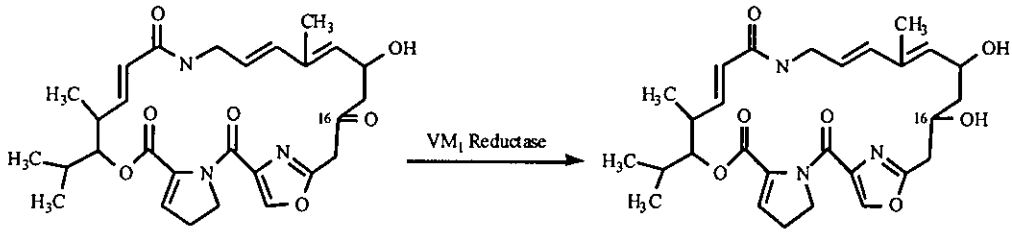


Fig. (13). Reaction of mikamycin B lactonase of *Streptomyces mitakaensis* [43].

1-1. Hydrolysis

Macrolide, lincosamide, and streptogramin (MLS) antibiotics are mainly effective against Gram-positive bacteria. On the other hand, erythromycin (EM) has been used to control the Gram-negative flora of the intestinal tract. Two genes have been identified as being associated with resistance to EM in *E. coli*. One is the *ere*(A) gene, encoding EM esterase A, generated from plasmid pIP1100 in *E. coli* BM2195. The other is the *ere*(B) gene, encoding EM



Virginiamycin M_1 16-Dihydrovirginiamycin M_1

Fig. (14). Structure of 16-dihydrovirginiamycin M_1 [46].

esterase B, generated from plasmid pIP1527 in *E. coli* BM2570. The nucleotide sequence of *ere*(A) on plasmid pI1100, a self-transferable plasmid, revealed that the gene consists of 1032 bp, corresponding to a product with a molecular weight of 37,765, and has a G+C content of 50.5%. Since the G+C content of Gram-positive bacteria is 32-36%, this gene should be indigenous to *E. coli*. *E. coli* BM2570 was isolated from a clinical specimen in France, like *E. coli* BM2195, and was resistant to high levels of EM (MIC >2000 μg/ml) through two different mechanisms. Plasmid pI1527, a 150-kb self-transferable plasmid, in *E. coli* BM2570 carried two genes, *erxA* and *ereB*. The *erxA* gene, which is closely related to the *ermAM* gene encoding the rRNA-methylating enzyme in *Streptococci*, and encodes a single polypeptide with a molecular weight of 27,000, confers high resistance to MLS antibiotics due to dimethylation of 23S rRNA. The *ere*(B) gene product, an enzyme with a molecular weight of 51,000, inactivated EM and oleandomycin (OL) similarly to the enzyme encoded by *ere*(A) and had a G+C content of 35.9%. The low G+C content of *ere*(B) might be due to a specific codon usage in *E. coli*, suggesting an exogenous origin of *ere*(B). Inactivation products of EM generated by enzymic hydrolysis of the lactone ring due to both the *ere*(A) and *ere*(B) gene products are shown in Fig. (**15**). The two genes, *erxA* and *ere*(B), provide different resistance mechanisms to macrolide antibiotics, i.e., dimethylation of 23S rRNA and inactivation. These genes appear similar to *srmA-gimA* in *Streptomyces ambofaciens* [32], and *lrm-mgt* in *Streptomyces lividans* [12].

In recent years, the *ere*(A2) gene, a variant of *ereA* located in an integron cassette has been found in several bacteria [77-80]. Kim *et al.* [81] characterized EM esterase from *Pseudomonas* sp. and the gene encoding this enzyme

Table 5. Inactivation of MLS Antibiotics by Clinically Isolated Microorganisms.

MLS antibiotics /Resistance Profile/Organisms	Plasmid	Gene[1]	Gene[2]	Size (bp)	Protein (size[3])	GenBank	Antibiotics[4]	Reference
Macrolide								
Hydrolysis								
E. coli BM2195	pIP1100	*ereA*	*ere*(A)	1032bp	Ere(A) (344aa)	M11277	EM	[53-55, 82]
E. coli		*ereA2*	*ere*(A2)					[77]
Pseudomonas sp. GD100		*ereA2*	*ere*(A2)					[81]
E. coli BM2570	pIP1527	*ereB*	*ere*(B)	1257bp	Ere(B) (419aa)	X03988	EM, OL	[57, 61, 82]
Phosphorylation								
E. coli Tf481A		*mphA*	*mph*(A)	903bp	Mph(A) (301aa)	D16251	OL, EM, SPCM MDM, JM, LCM	[58, 63, 64]
E. coli 209K	pSK66	*mphK*	*mph*(A)	918bp	Mph(A) (306aa)	U36578	14-, 16-mac	[83]
E. coli BM2506		*mphB*	*mph*(B)	906bp	Mph(B) (202aa)	D85892	14-, 16-mac	[59, 65-72, 84]
S. aureus	pMS97	*mphBM*	*mph*(C)	897bp	MphBM (299aa)	AB013298	14-, 15-, 16-mac EM	[60, 73]
S. aureus 01A1032	pSR1	*mphBM*	*mph*(C)	897bp		AF167161		
Stenotrophomonas maltophilia D457		*mphBM*	*mph*(C)	897bp		AJ251015		[74]

(Table 5. Contd....)

MLS antibiotics /Resistance Profile/Organisms	Plasmid	Gene[1]	Gene[2]	Size (bp)	Protein (size[3])	GenBank	Antibiotics [5]	Reference
Phosphorylation, Glycosylation, Reduction, Deacylation								
Nocardia sp.							EM, RKM, MDM CHA, TL	[75, 76]
Lincosamide								
Nucleotidylation								
S. haemolyticus BM4610	pIP855	*linA*	*lnu*(A)	483bp	LNT(3')(4) (161aa)	M14039	LCM	[85, 86, 88]
S. aureus BM4611		*linA'*	*lnu*(A)	483bp	LNT(3')(4) (161aa)	J03497	LCM, CLDM	[87, 88]
E. faecium HM1025		*linB*	*lnu*(B)	801bp	LinB (267aa)	AF110130	LCM, CLDM	[89]
Streptogramin								
Hydrolysis								
S. aureus STE						PH IA	PRI-1	[90]
S. aureus BM3041	pIP630	*vgb*	*vgb*(A)	897bp	Vgb(A) (298aa)	M20129	VIR-B	[91, 105]
S. cohnii BM1071	pIP1714	*vgbB*	*vgb*(B)	885bp	Vgb(B) (295aa)	AF015628	STG-B	[92]
Acetylation								
S. aureus 71							VIR-M	[95]
S. *aureus* STE					PAC (IIA)		PRI II-A	[96]
S. aureus	pI680	*vat*	*vat*(A)	657bp	VAT (219aa)	L07778	VER-A	[97]
S. aureus BM3385	pIP1633	*vatB*	*vat*(B)	636bp	VatB (212aa)	L38809	STG-A	[98]
S. cohnii BM10711	pIP1714	*vatC*	*vat*(C)	636bp	VatC (212aa)	AF015628	STG-A	[92]
E. faecium BM4145		*satA*	*vat*(D)	627bp	SatA (209aa)	L12033	STG-A	[99, 105]
E. faecium UW1965		*satG*	*vat*(E)	642bp	SatG (214aa)	AF139725	STG-A	[101, 102]
E. faecium		*vat*(E 1-8)[5]		642bp		AF139735	Q-D	[103]

[1] The gene name is based on the original name.
[2] The gene name follows the nomenclature by Roberts M. C. *et al.* [48]
[3] Number of amino acid (aa) is shown in parentheses.
[4] *E. faecium* generates a series of *vat* genes, *vat*(E-1), *vat*(E-2), *vat*(E-3), *vat*(E-4); *vat*(E-5); *vat*(E-6), *vat*(E-7), and *vat*(E-8), and they were deposited as accession numbers AF153312, AY008284, AY043211, AY43209, AY043210, AY043212, and AY043213, respectively.
[5] See Table 1 for abbreviations other than SPCM, spectinomycin; MDM, midecamycin; CHA, charcomycin; STG, streptogramin; Q-D, quinupristin-dalfopristin; mac, macrolide antibiotics.

was similar to the *ere*(A2) gene from *Providencia stuartii* plasmid PLQ1723 [80]. A comparison of EM esterase genes, including *ere*(A), *ere*(A2) and *ere*(B), is shown in Fig. (**16**) and Table **6**. As expected, *ere*(A) and *ere*(A2) showed a high degree of identity in terms of both DNA and amino acid sequences, but *ere*(A) and *ere*(A2) showed relatively low similarity to *ere*(B). EM esterases include *ere*(A) and *ere*(B) appear to be disseminating independently among *Escherichia* sp. *Staphylococcus* sp. and *Pseudomonas* sp. although they are also found together [82].

1-2. Phosphorylation

Inactivation due to phosphorylation of macrolide, lincosamide, and streptogramin (MLS) antibiotics in clinical pathogens was first found by O'Hara *et al.* [63] in *E. coli* with high-level resistance to erythromycin (EM) (MIC >3200 μg/ml), and was similar to the phosphorylation at the 2'-OH group of 14- and 16-membered macrolides in *Streptomyces coelicolor* [19, 20]. Two years later, a similar phosphotransferase gene was discovered in a Gram-positive bacterium, *Staphylococcus aureus*; it was located between two other genes conferring resistance to MLS antibiotics, i.e., *msr*(A), which is related to antibiotic efflux, and *erm*(Y), which is related to methylation of 23S rRNA [73].

To date, three phosphotransferase genes, *mph*(A), *mph*(B), and *mph*(C), have been reported. The *mph*(A) gene, encoding macrolide 2'-OH phosphotransferase I, was found in *E. coli* Tf481. It consists of 903 bp [58] and encodes the inducible enzyme MPH(2')I, which inactivates 14-membered macrolides in the presence of ATP and Mg^{2+} [63, 64] to 2'-OH phosphoryl macrolide antibiotics, as seen in *Streptomyces coelicolor*(Table 1). *E. coli* 209K harboring the plasmid pSK66 was isolated in Korea [83] and was highly

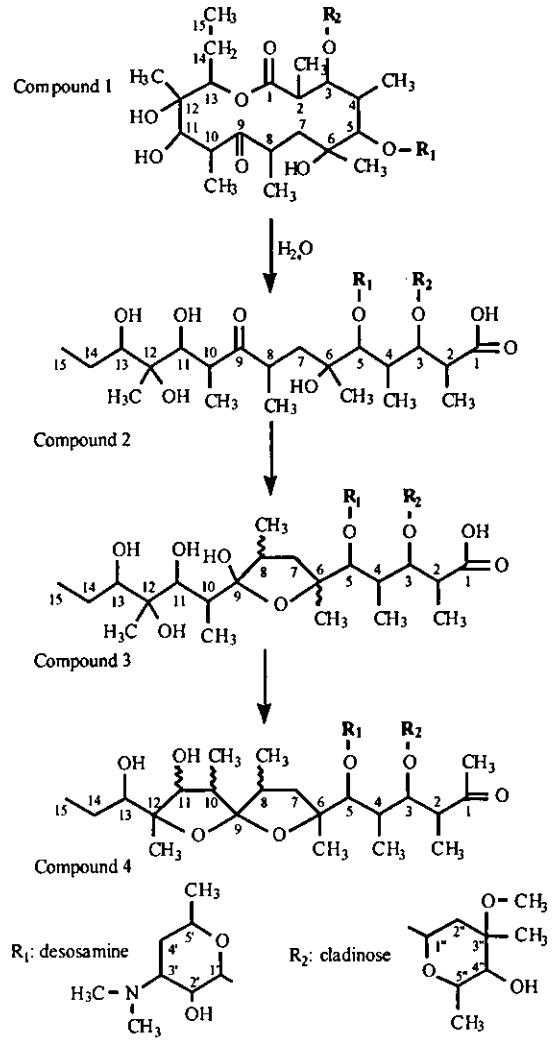


Fig. (15). Reaction catalyzed by erythromycin esterase A and B. Enzymatic hydrolysis of the lactone ring of erythromycin is followed by the formation of an hemiketal by internal condensation and dehydration. Compound 4 is the major end product of detoxification of erythromycin by resting cells at pH 7.0 [57].

resistant to EM (MIC >2000 μg/ml). The *mph*(K) gene on this plasmid is 918 bp long and encodes a protein of 306 amino acids; it is very similar to *mph*(A) (98% identity in DNA and 97% identity in amino acid sequence). The *mph*(B) gene, encoding macrolide 2'-phosphotransferase II (MPH (2')II; 302 amino acids) in *E. coli* BM2506, consists of 906 bp. This enzyme showed potent inactivating activity against 14- and 16-membered macrolides, different from MPH(2')I, which inactivated only 14-membered macrolides [59, 65]. The observation of low similarity between *mph*(A) and *mph*(B) (40% identity in DNA and 37% identity in amino acid sequence) seems to be related to the very different G+C contents of *mph*(A) (65.6%) and *mph*(B) (38.3%). This dissimilarity between the two genes is consistent with the fact that the *mph*(B) gene is only expressed in the Gram-positive bacterium, *S. aureus* [67]. Much work has been done on the *mph*(B) gene [66, 68-72, 84]. The *mph*(C) (formerly *mphBM*) gene, encoding a phosphotransferase, was obtained from *S. aureus* [60] and subsequently from *Stenotrophomonas maltophilia* [74]. The *mph*(C) gene of *S. aureus* is located on plasmid pMS97 in the sequence 5'-*msr*(A)-*mph*(C)-*erm*(Y)-3' and an ATG initiation codon is present 342 bp downstream of the *msr*(A) gene, encoding an ABC-family efflux transporter, while a TAG termination codon is present 414 bp upstream of the *erm*(Y) gene, the product of which dimethylates 23S rRNA [60, 73]. The *mph*(C) gene consists of 897 bp and encodes a deduced protein of 299 amino acids. Furthermore, DNA sequence analysis of *mph*(C) revealed a G+C content of 37.1%, and 62% identity with *mph*(B), despite the 45% identity with *mph*(A). Constructed plasmids pND502, pND5012, pND5013, and pND50A, including the *mph*(C) gene (Fig. **17**), were introduced into the susceptible strain *S. aureus* RN4220, which was then assayed for the inactivation of EM by measurement of the residual activity towards *Micrococcus luteus* ATCC9341. As shown in Fig. (**18**), *S. aureus* RN4220 (pND502) containing only the *mph*(C) gene formed an inhibition zone, although other constructs formed no inhibition zone, suggesting that the expression of *mph*(C) gene is required for the activation of *msr*(A) gene and/or its promoter. Inactivation activity of the *mph*(C) gene was compared with that of the *ere*(A) gene encoding EM-esterase by radioautography and bioautography using [^{14}C]EM and *E. coli* BM694/pAT63 harboring the *ere*(A) gene or *S. aureus* 8325(pMS97) harboring the *mph*(C) gene. Both strains exhibited complete resistance (Fig. **19**). However, the radioautograms showed different peak patterns, indicating that the inactivation mechanism of the *mph*(C) gene product is different from that of the *ere*(A) gene product. The *mph*(C) genes from *Stenotrophomonas maltophilia* D457 [74] and from *S. aureus* harboring plasmid pSR1 showed high similarity to that of pMS97 (more than 98% identity in amino acids) (Fig. **20**). The *mph*(C) gene of *S. maltophilia* D457 was located in a cluster of genes including *mph*(C) and cadmium efflux determinant (*cadA*), together with the gene *cadC* coding for its transcriptional regulator. Nucleotide sequences of plasmid pMS97 (accession number AB179623) resembled those of plasmid pI258, a typical plasmid conferring resistance to macrolides and ß-lactam antibiotics (Fig. **21**). Three plasmids, pI258 containing *erm*(B) (dimethylation of 23S rRNA), pEP2104 containing *msr*(A) (ABC efflux transporter) , and pMS97 containing *msr*(A)-*mph*(C)-*erm*(Y), had the same nucleotide sequences over 20 kb, except for a *SalI*-*PstI* fragment including genes conferring macrolide resistance. These observations suggest that genes such as *msr*(A), *mph*(C), and *erm*(Y) have disseminated among bacterial species by providing a growth advantage in the environment.

The range of MLS antibiotics inactivated by the *mph*(C)-encoded phosphotransferase was compared with that inactivated by *ere*(A)-encoded EM esterase (Table **7**). Both inactivated all 14-membered macrolides other than telithromycin, which is one of the new ketolide antibiotics. The activity of this ketolide was lost upon phosphorylation, but not upon esterase cleavage of the macrolactone ring (Fig. **22**). Inactivation activity of azithromycin mediated by

```
Ere(A)     1 ------------------------------------------------------------  1
Ere(A2)    1 MTWRTTRTLLQPQKLEFNEFEILNPVVEGARIVGIGEGAHFVAEFSLARASLIRYFVERH  60

Ere(A)     2 -MRL-VWKCGAIQASRLSEWLNSTAGAHELERFSDTLTFSVYGSVLIWLKSYLRESGRKL  58
Ere(A2)   61 DFNAIGLECGAIQASRLSEWLNSTAGAHELERFSDTLTFSSYGSVLIWVKSYLRESGRKL 120
                ********************************** ******* ***********

Ere(A)    59 QLVGIALPNTLNPRDDLAQLAEIIQLIDHLMKPHVDMLTHLLASIDGQSAVISSAKWGEL 118
Ere(A2)  121 QLVGIDLPNTLNPRDDLAQLAEIIQVIDHLMKPHVDALTQLLTSIDGQSAVISSAKWGEL 180
             ***** ******************** ********** ** ** ******************

Ere(A)    119 ETARQEKAISGVTRLKLRLASLAPVLKKHVNSDLFRKASDRIESIEYTLETLRIMKTFFD 178
Ere(A2)   181 ETAQQEKAISGVTRLKLRLASLAPVLKNHVNSDFFRKASDRIESIEYTLETLRVMKAFFD 240
              *** ************************ ***** ******************** ** ***

Ere(A)    179 GTSLEGDTSVRDSYMAGVVDGMVRANPDVKIILLAHNNHLQKTPVSFSGELTAVPMGQHL 238
Ere(A2)   241 GTSLEGDTSVRDSYMAGVVDGMVRANPDVRIILLAHNNHLQKTPVSFSGELTAVPMGQHL 300
              ****************************** ********************************

Ere(A)    239 AERVNYRAIAFT--HLGPTVPEMHFPSPKSPLGFSVVTTPADAIREDSMEQYVIDACGTE 296
Ere(A2)   301 AEREEGDYRAIAFTHLGLTVPEMHFPSPDSPLGFSVVTTPADAIREDSVEQYVIDACGKE 360
              ***      *    *** ********** ******************** ********* *

Ere(A)    297 NSCLTLTDAPMEAKRMRSQSASVETKLSEAFDAIVCVTSAGKDSLVAL              344
Ere(A2)   361 DSCLTLTDDPMEAKRMRSQSASVETNLSEAFDAIVCVPSAGKDSLVAL              408
              ******* ***************** *********** **********
```

Fig. (16). Comparison of the deduced amino acid sequences of erythromycin esterase Ere(A) from *E. coli* (accession number M11277) and Ere(A2) from *Providensia stuartii* (accession number AF099140). Identical residues are indicated by asterisks.

mph(C) was lower that by *ere*(A), and 16-membered macrolide was more strongly inactivated by *mph*(C) than by *ere*(A).

Table 6. Homology (%) of DNA and Amino Acid Sequences for Erythromycin Esterase.

Combination of genes	Homology (%) of	
	DNA	Amino Acid
ere(A) - *ere*(A2)	80	78
ere(A2) - *ere*(B)	49	26
ere(A) - *ere*(B)	47	24

Accession numbers of genes used for homology analysis were the same as in Table 5.

2. Lincosamide Antibiotics

Phosphorylation and nucleotidylation of the hydroxyl group at position 3 of lincosamide have been observed in several species of *Streptomyces* [19, 39]. Among clinical isolates, *Staphylococcus aureus* BM4611 and *Staphylococcus haemolyticus* BM4610 were highly resistant to lincomycin (MIC 64 μg/ml), but susceptible to clindamycin (MIC 0.12 μg/ml). Lincosamide *O*-nucleotidyltransferases coded by two closely related genes, *linA* (lincosamide inactivation nucleotidylation) from *S. haemolyticus* and *linA'* from *S. aureus*, provide resistance to lincosamide [85-88] by inactivation to afford lincomycin 3-(5'-adenylate) and clindamycin 4-(5'-adenylate) (Fig. **23**). Both *linA* and *linA'* encode 3-lincomycin, 4-clindamycin *O*-nucleotidyltransferase, and the two 161-amino acids isozymes differ by only 14 amino acids (Fig. **24**) [88]. A nucleotidyl donor and Mg^{2+} as a cofactor are required for inactivation of lincomycin, as in the case of *Streptomyces coelicolor* [37-40]. Recently, a new resistance gene, *linB*, was identified in *Enterococcus faecium* 1025 [89]. This gene encodes a lincosamide nucleotidyltransferase (267 amino acids) that catalyzes 3-(5'-adenylation) of lincomycin and clindamycin, different from *linA* and *linA'* that catalyze 4-(5'-adenylation) of clindamycin (Fig. **25**). Comparison of nucleotide sequences and deduced amino acid sequences revealed no significant homology with *linA* and *linA'* (Table **8**).

3. Streptogramin Antibiotics

3-1. Hydrolysis

Plasmid-mediated pristinamycin IA (PH IA, belonging to the streptogramin type B category) resistance involving

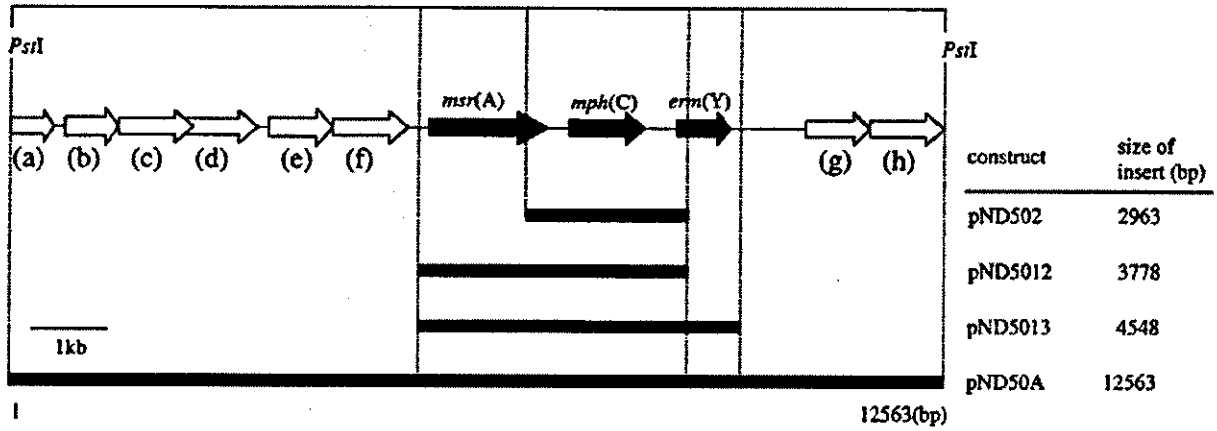


Fig. (17). Schematic representation of constructs containing fragments of genes coding for resistance to macrolides. The constructs pND502, pND5012, pND5013 and pND50A contain *mph*(C), *msr*(A)-*mph*(C), *msr*(A)-*mph*(C)-*erm*(Y), and *msr*(A)-*mph*(C)-*erm*(Y) including the extra region, respectively. The amino acid sequence deduced from the DNA sequence of fragment A (12563 bp) gives eight putative proteins ((a) to (h)) in addition to three macrolide resitance gene products. The constructed fragment is shown by black bars. Arrows show orientation of cloning (5' to 3') and approximate size of the genes of the constructs. Black arrows show the macrolide resistance genes: *msr*(A), *mph*(C), and *erm*(Y). ORFs of (a), (b), (c), (d), (e), (f), (g), and (h) were homologious with reblication-associated protein of *Staphylococcus aureus* plasmid pI9789 with 100%, two-component response regulator of *Clostridium acetoutylicum* with 55%, ABC-type multidrug transport system of *Clostridium acetobutylicum* with 46%, transmembrane protein of *Clostridium acetobutylicum* with 30%, sensory transduction histidine kinase of *Clostridium acetobutylicum* with 36%, hypothetical protein of *Enterococcus faecium* with 61%, transcriptional regulator, AcrR family, of *Clostridium acetobutylicum* with 42%, and ABC transporter ATP-binding protein of *Oceanobacillus iheyensis* with 77% homology, respectively [73].

PH1A hydrolase was first reported in *Staphylococcus aureus* [90]. Thereafter, two genes encoding hydrolases of streptogramin type B antibiotics, *vgb*(A) (formerly *vgb*) from *S. aureus* BM3041 [91] and *vgb*(B) (formerly *vgb*B) from plasmid pI1714 in *Staphylococcus cohnii* subsp. *cohnii* strain were reported [92]. The *vgb*(A) gene consists of 894 bp with a G+C content of 37.5%, and the enzyme (33 kDa) encoded by this gene is similar in molecular weight to an enzyme from *Actinoplanes missouriensis* (35 kDa) inactivating the B component of virginiamycin antibiotics by cleavage of the lactone ring [42]. The *vgb*(B) is linked with *vat*(C), encoding

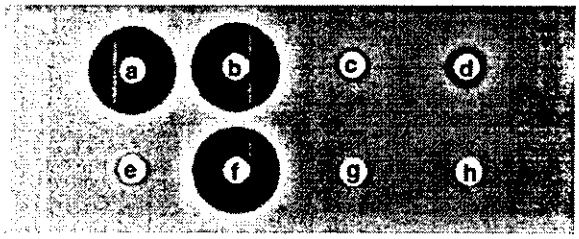


Fig. (18). Inactivation assay of erythromycin (EM) with several *Staphylococcus aureus* strains. EM activity after 24 h incubation with *S. aureus* in the presence of the drug was assayed by the disk plate method using *Micrococcus luteus* ATCC9341 as an indicator microorganism. The residual EM activity was determined in the supernatant (80 µl) of the mixtures incubated with the following bacteria. The supernatants applied to the paper disks were follows: a, 800 ng of EM as a blank; b, *S. aureus* RN4220; c, *E. coli* BM694(pAT63) encoding *ere*(A) gene; d, *S. aureus* 4220(pMS97); e, *S. aureus* 4220(pND50A); f, *S. aureus* 4220(pND502); g, *S aureus* 4220(pND5012); h, *S. aureus* 4220(pND5013).

an acetyltransferase that inactivates streptogramin B on plasmid pIP1714 from *S. cohnii*, and consists of 855 nucleotides encoding a 295-amino acid lactonase. These enzymes, Vgb(A) and Vgv(B), exhibits 67% amino acid identity as shown in Fig. (**26**). A recent study showed that *vgb* from *S. aureus* inactivated streptogramin B antibiotics by elimination, not by hydrolysis of the ester bond [93, 94]. Because the streptogramin B of hexadepsipeptide is linearized through an elimination reaction across the ester bond with generation of an N-terminal dehydrobutyrine group (Fig. **27**), it was suggested that *vgb* is not a hydrolase, but a lyase.

3-2. Acetylation

Acetylation of streptogramin antibiotics is a specific reaction for type A component, whereas hydrolysis reaction is specific for type B *Staphylococcus aureus*, isolated in the virginiamycin production plant from a workman in continuous contact with this antibiotic, inactivated virginiamycin M to the *O*-acetyl derivative [95]. Other strains of *S. aureus* isolated from skin burns inactivated pristinamycin IIA to the *O*-acetylate, as shown in Fig. (**28**) [96].

To date, five different *vat* genes encoding acetyltransferase and eight variations of *vat*(E) from *Enterococcus faecium* have been reported (Table **5**). The genes *vat*(A) [97], *vat*(B) [98], and *vat*(C) [92] (formerly *vat*, *vatB*, and *vatC*, respectively) were isolated from pI680 and pIP1633 on *S. aureus* and from pIP1714 on *Staphylococcus cohnii*. The *vat*(A) gene on plasmid pI680 is linked with *vgb*(A) immediately upstream and with *vga*(A) (formerly *vga*; an ABC–transporter gene) more distantly downstream, while the *vat*(C) gene on plasmid pIP1714 is linked closely downstream of *vgb*(B). The *vat*(D) [99, 100] and *vat*(E)

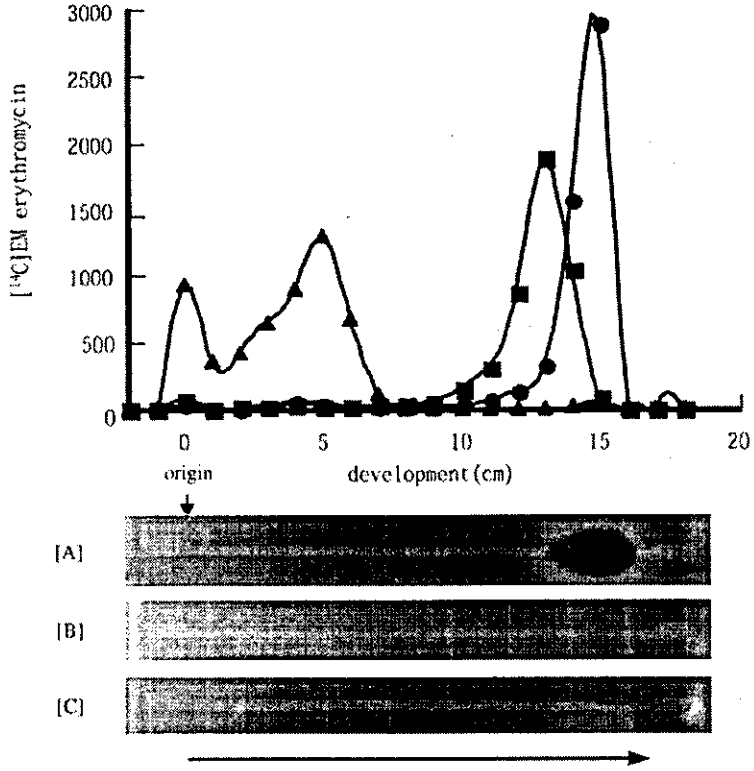


Fig. (19). Radioautogram and bioautogram of [^{14}C]erythromycin (EM). [^{14}C] EM was incubated for 63 h at 37°C with *E. coli* BM694(pAT63) (closed square and [B]) or *Staphylococcus aureus* 8325(pMS97) (closed triangle and [C]). The arrow indicates the direction of development with a solvent system of chloroform-methyanol-34.2% aqueous ammonia (9:1:0.1) [73].

```
pMS97     1 MTRHNEIIKCAEKYQLHIQPQTISLNESGLDFQVAFGKDKHGVEWVLRLPRRPDVYKRTK   60
pSR1      1 MTRHNEIIKCAEKYQLHIQPQTISLNESGLDFQVAFGKDKHGVEWVLRLPRRPDVYKRTK   60
D457      1 MTRHNEIIKCAEKYQLHIQPQTISLNESGLDFQVAFGKDKHGVEWVLRLPRRPDVYKRTK   60
            ************************************************************

pMS97    61 PEKQTVDFLQKNVSFEVPKWKVHERDLIAYPKLTGKPAATIDPEIQNYVWEIEHKPLPEN   120
pSR1     61 PEKQTVDFLQKNVSFEIPKWKVHAKDLIAYPKLTGKPAATIDPEIQNYVWEIEHKPLPEN   120
D457     61 PEKQTVDFLQKNVSFEIPKWKVHAKDLIAYPKLTGKPAATIDPEIQNYVWEIEHKPLPEN   120
            ***************.******..************************************

pMS97   121 FINTLAETLVDLHNIPEENINVQHINIKTIQEIKNDFQRRMNKVKETYGVSDELWNRWKQ   180
pSR1    121 FINTLAETLVDLHNIPEENINVQHINIKTIQEIKNDFQRRMNKVKETYGVSDELWNRWKQ   180
D457    121 FINTLAETLVDLHNIPEENINVQHINIKTIQEIKNDFQRRMNKVKETYGVSDELWNRWKQ   180
            ************************************************************

pMS97   181 WLENDELWPRHATMIHGDLHPGHIMVDNQANVTGLIDWTEATHSDPSMDFMGHHRVFDDE   240
pSR1    181 WLENDELWPRHATMIHGDLHPGHIMVDNQANVTGLIDWTEATHSDPSMDFIGHHRVFDDE   240
D457    181 WLENDELWPRHATMIHGDLHPGHIMVDNQANVTGLIDWTEATHSEPSMDFIGHHRVFDDE   240
            ***********************************************.*****.*********

pMS97   241 GLEQLITAYGKAGGEIWPRMKEHIIELNAVFPMFIAEFAMESGESAYETMALKELGMKE   299
pSR1    241 GLEQLITAYGKAGGEIWPRMKEHIIELNAVFPMFIAEFAMESGESAYETMALKELGMKE   299
D457    241 GLEQLITAYGKAGGEIWPRMKEHIIELNAVFPMFIAEFAMESGESAYETMALKELGMKE   299
            ************************************************************
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Fig. (20). Comparision of the deduced amino acid sequences of Mph(C) conferring erythromycin resistance by plasmid pMS97 (accession number AB013298) and pSR1 (accession number AF167161) from *Staphylococcus aureus* and by *Stenotrophomonas maltophillia* D457 (accession number AJ251015).

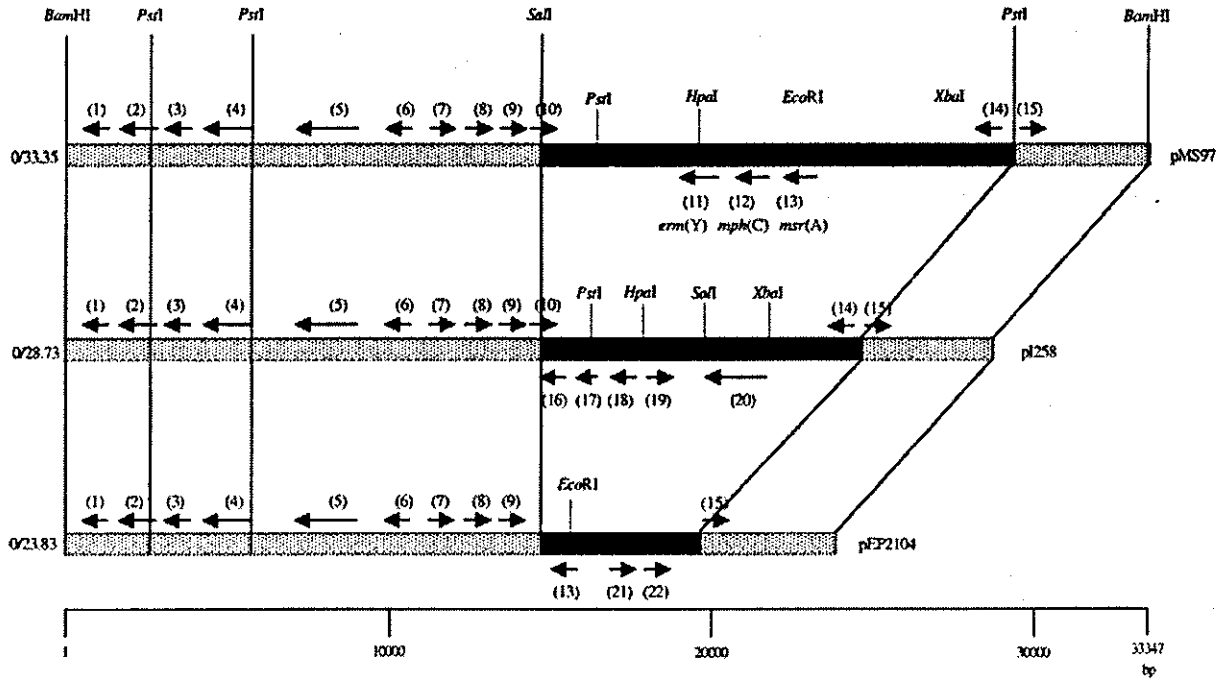


Fig. (21). Genetic maps of macrolide resistance plasmids, pMS97 (accession number AB179623), pI258 [112] and pEP2104 (accession numberAB016613) from *Staphylococcus aureus*. Genes are indicated by the following numbers: (1), *merB*; (2), *merA*; (3), *merT*; (4), *merR*; (5), *cadA*; (6), *blaZ*; (7) *blaRI*; (8), *blaI*; (9), *binR*; (10), *bin3*, (11), *erm*(Y); (12), *mph*(C); (13), *msr*(A), replication associated protein; (15), replication initiation protein; (16), *arsC*; (17), *arsB*: (18), *arsR*; (19), *erm*(B); (0), transposase; (21), *stp*(A); (22) *smp*(A).

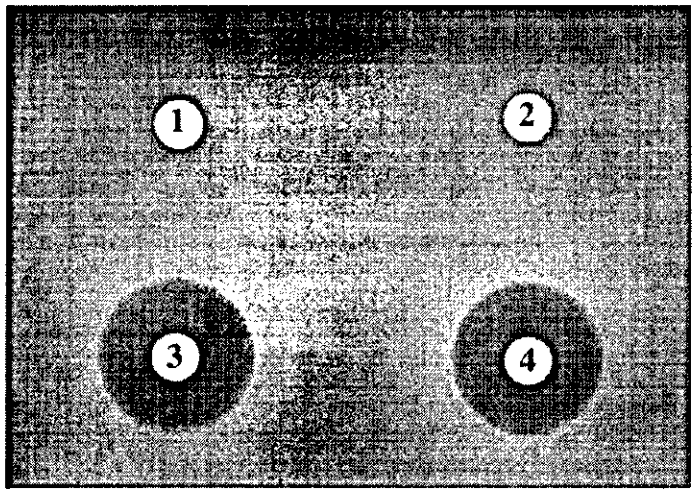


Fig. (22). Inactivation assay for telithromycin. Disks contained incubation supernatant of bacteria and telithromycin as follows: *Staphylococcus aureus* 8325(pMS97) encoding *mph*(C) (disk 1), *S. aureus* 8325(pMS97) induced by EM (disk 2), *E. coli* BM694/pAT63 encoding *ere*(A) (disk 3), and 800 ng of telithromycin as a blank (disk 4).

[101, 102] genes (former *satA* and *satG*, respectively) were isolated from *E. faecium*. The *vat*(D) is homologous with a family of chloramphenicol acetyltransferases present in *Agrobacterium tumefaciens*, *E. coli*, *Pseudomonas aerginosa*, and *S. aureus*. Another Enterococcal gene, *vat*(E) were isolated from quinupristin-dalfopristin-resistant *E. faecium* UW1965 at a sewage treatment plant in Germany [101]. The DNA and amino acid sequences of five *vat* genes were compared (Table **9**, Fig. **29**), and the amino acid sequences of eight variants of the *vat*(E) gene product of *E. faecium* isolated from retail poultry samples [103, 104], *vat*(E-1) to *vat*(E-8), were also compared (Fig. **30**). The prevalence of streptogramin resistance gene in *Enterococci* found in retail poultry [105] might be due to the use of virginiamycin in

Table 7. Comparison of the Inactivation Spectra of Macrolide, Lincosamide, and Streptogramin B Antibiotics for *S. aureus* 8325(pMS97) Containing *mph*(C) and *E. coli* BM694(pAT63) Containing *ere* (A) Gene [73].

Antibiotics	Group	Inactivation (%)*	
		S. aureus 8325(pMS97)	*E. coli* BM694(pAT63)
Erythromycin	14-membered	100	100
Oleandomycin	14-membered	100	100
Roxithromycin	14-membered	100	100
Clarithromycin	14-membered	100	100
Telithromycin	14-membered	100	4
Azithromycin	15-membered	54	100
Rokitamycin	16-membered	6	13
Mycinamicin	16-membered	53	6
Spiramycin	16-membered	8	17
Tylosin	16-membered	12	4
Rosamicin	16-membered	100	0
YM133	16-membered	63	8
Clindamycin	lincosamide	6	3
Lincomycin	lincosamide	5	4
Mikamycin-B	streptograminB	0	0

* Extent of inactivation (%) is expressed as the ratio of decrease in amount of drug activity (i.e. difference between initial drug activity and residual drug activity after incubation) to the initial amount of drug before incubation with bacteria.

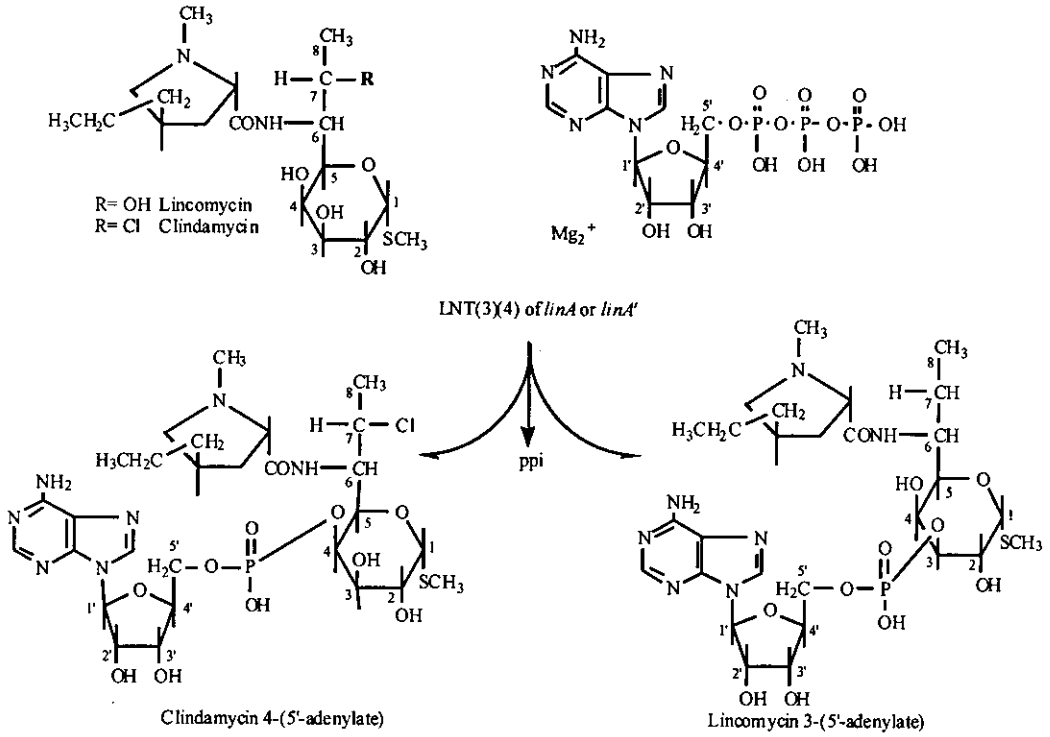


Fig. (23). Adenylation of lincosamides by *Staphylococcus haemolyticus* BM4610 and *Staphylococcus aureus* BM4611. LNT (3)(4); 3-lincomycin, 4-clindamycin nucleotidyl transferase [88].

```
LNT(3)(4)/linA      1 MKNNNVTEKELFYILDLFEHMKVTYWLDGGWGVDVLTGKQQREHRDIDIDFDAQHTQKVI   60
LNT(3)(4)/linA'     1 MKIDNVTEKDLFYILDLFEKMEVTHWLDGGWGVDVLTGKQQREHRDIDIDFDAQHTQKVI   60
                      **  ***** ********* * ** ************************************

LNT(3)(4)/linA     61 QKLEDIGYKIEVHWMPSRMELKHEEYGYLDIHPINLNDDGSITQANPEGGNYVFQNDWFS  120
LNT(3)(4)/linA'    61 KKLEDIGYKIEVDWMPSRMELKHKEYGYLDIHPINLNDDGSITQANPEGGNYIFQNEWFS  120
                       *********** ********** ***************************** *** ***

LNT(3)(4)/linA     121 ETNYKDRKIPCISKEAQLLFHSGYDLTETDHFDIKNLKSIT                    161
LNT(3)(4)/linA'    121 ETNYKGRKIPCISKEAQLLFHSGYELTEKDHFDIKNLKSIT                    161
                       ***** ****************** *** ************
```

Fig. (24). Comparision of amino acid sequences of LNT(3)(4), 3-lincomycin, 4-clindamycin O-nucleotidyl transferase encoded by *linA* (accession number M14039) or *linA'* (accession number J03497).

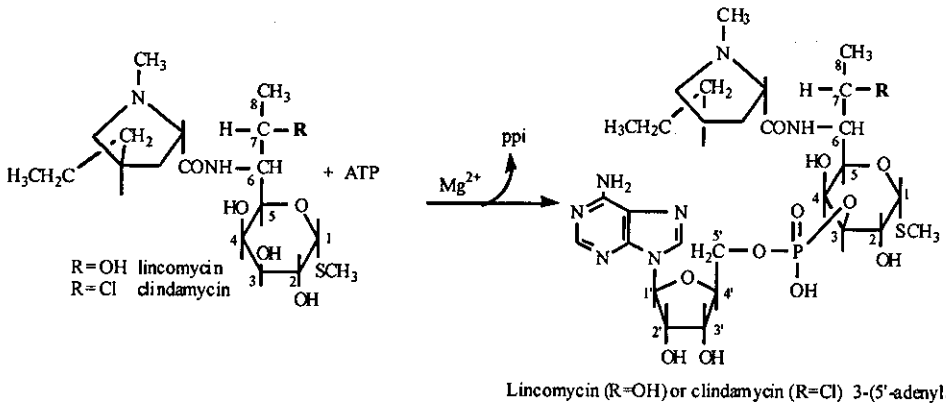


Fig. (25). Adenylation of lincomycin and clindamycin by *linB* gene product of *Enterococcus faecium* HM1025 [89].

Table 8. Homology (%) of DNA and Amino Acid Sequences for the Lincosamide Nucleotidyl Transferase.

Combination of genes	Homology (%) of	
	DNA	Amino Acid
linA - linA'	93	91
linA' - linB	43	19
linA - linB	42	17

Accession numbers of genes used for homology analysis were the same as in Table 5.

many countries for many years as a feed additive to enhance growth of food animals. Consequently, large numbers of virginiamycin-resistant *E. faecium* have been isolated from the feces of food animals, and these were also resistant to quinupristin-dalfopristin, indicating the presence of cross-resistance between virginiamycin and quinupristin-dalfopristin. Many instances of resistance to MLS antibiotics originating from animals have been reported, as shown in *Lactobacillus* [106-108], *Staphylococci* [109], and *Clostridium perfringens* [110].

As MLS antibiotics are widely used as human and veterinary medicines [111], human and veterinary bacteria may acquire resistance genes against MLS antibiotics. In considering the resistance mechanisms of human pathogens, it is always important to ask "Where did the resistance gene come from?" Clinical isolates resistant to MLS antibiotics possess similar resistance mechanisms to those of antibiotic-producing bacteria, except for the hydrolytic inactivation of 14-, 15- and 16-membered macrolides and acetylation of streptogramin A. Resistance genes of antibiotic-producing bacteria might have been transferred into human bacteria through any of the pathways shown in Fig. (31). Pathway [I] shows resistance gene transfer to human bacteria mediated by soil bacteria, animal bacteria, and so on [106, 107, 109, 111]. Pathway [II] shows gene transfer involving human bacteria found in hospitals where antibiotics are used as chemotherapeutic agents [92]. Pathway [III] shows transfer

```
Vgb(A)      1 MEFKLQELNLTNQDTGPYGITVSDKGKVWITQHKANMISCINLDGKITEYPLPTPDAKVM   60
Vgb(B)      1 MNFYLEEFNLSIPDSGPYGITSSEDGKVWFTQHKANKISSLDQSGRIKEFEVPTPDAKVM   60
                * * * * **   * ****** *  **** ****** **     * * *   ********

Vgb(A)     61 CLTISSDGEVWFTENAANKIGRITKKGIIKEYTLPNPDSAPYGITEGPNGDIWFTEMNGN  120
Vgb(B)     61 CLIVSSLGDIWFTENGANKIGKLSKKGGPTEYPLPQPDSGPYGITEGLNGDIWFTQLNGD  120
                **  ** *  ***** *****   ***   ** ** *** ******* *******  **

Vgb(A)    121 RIGRITDDGKIREYELPNKGSYPSFITLGSDNALWFTENQNNAIGRITESGDITEFKIPT  180
Vgb(B)    121 RIGKLTADGTIYEYDLPNKGSYPAFITLGSDNALWFTENQNNSIGRITNTGKLEEYPLPT  180
                ***  * ** * ** ******** ******************** *****  *   *  **

Vgb(A)    181 PASGPVGITKGNDDALWFVEIIGNKIGRITPLGEITEFKIPTPNARPHAITAGAGIDLWF  240
Vgb(B)    181 NAAAPVGITSGNDGALWFVEIMGNKIGRITTTGEISEYDIPTPNARPHAITAGKNSEIWF  240
                 *  ***** *** ******* ********  *** *  **************     **

Vgb(A)    241 TEWGANKIGRLTSNNIIEEYPIQIKSAEPHGICFDGETIWFA-MECDKIGKLT-LIKDNM  298
Vgb(B)    241 TEWGANQIGRITNDKTIQEYQLQTENAEPHGITFGKDGSVWFALKCKIG-KLNLNE----  295
                ****** *** *    * **  *   ****** *          *    **

Vgb(A)    299 E
Vgb(B)    296 -
```

Fig. (26). Comparision of amino acid sequences of Vgb(A) [91] and Vgb(B) [92], which hydrolyze mikamycin B antibiotics.

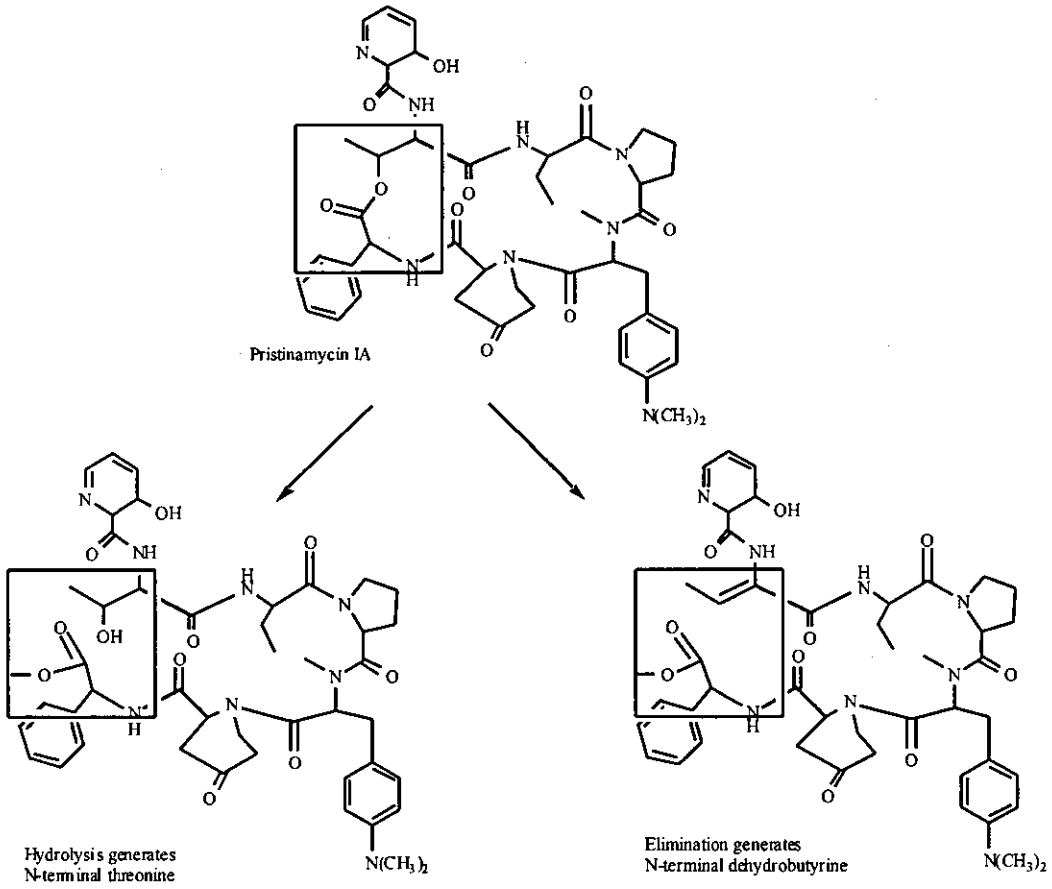
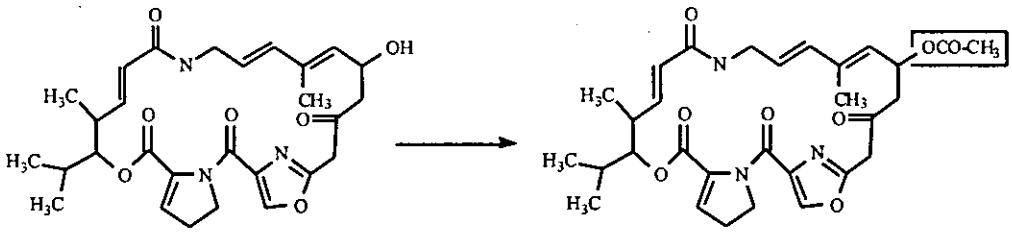


Fig. (27). Structures of possible products of lactone cleavage of pristinamycin IA [93].



Pristinamycin IIA Acetylpristinamycin IIA

Fig. (28). Acetylation of pristinamycin IIA [96].

```
Vat(A)    1 MNLNNDHGP--DPENILPIKGNRNLQFIKPTITNENILVGEYSY-YDSKRGESFEDQVLY  57
Vat(B)    1 ------MKYGPDPNSIYPHEEIKSVCFIKNTITNPNIIVGDYTYYSDVNGAEKFEEHVTH  54
Vat(C)    1 MKWQNQQGP--NPEEIYPIEGNKHVQFIKPSITKPNILVGEYSY-YDSKDGESFESQVLY  57
Vat(D)    1 ------MGP--NPMKMYPIEGNKSVQFIKPILEKLENVEVGEYSYYDSKNGETFDKQILY  52
Vat(E)    1 ------MTIP-DANAIYPNSAIKEVVFIKNVIKSPNIEIGDYTYYDDPVNPTDFEKHVTH  53
                     ... .. ..*..... ..***. .. ... .. . ...*.. .. *. ....

Vat(A)   58 HYEVIGDKLIIGRFCSIGPGTTFIMNGANHRMDGSTYPF-HLFRNGWEKYMPSLKDLPLK  116
Vat(B)   55 HYEFRGDKLVIGKFCAIAEGIEFIMNGANHRMNSITTYPFNIMGNGWEKATPSLEDLPFK  114
Vat(C)   58 HYELIGDKLILGKFCSIGPGTTFIMNGANHRMDGSTFPF-NLFGNGWEKHTPTLEDLPYK  116
Vat(D)   53 HYPILNDKLKIGKFCSIGPGVTIIMNGANHRMDGSTYPF-NLFGNGWEKHMPKLDQLPIK  111
Vat(E)   54 HYEFLGDKLIIGKFCSIASGIEFIMNGANHVMKGISTYPFNILGGDWQQYTPELTDLPLK  113
              **. .***..*.**.*..* ..*******.*.... .. ......*.. .* * .** *

Vat(A)  117 GDIEIGNDVWIGRDVTIMPGVKIGDGAIIAAEAVVTKNVAPYSIVGGNPLKFIRKRFSDG  176
Vat(B)  115 GDTVVGNDVWIGQNVTVMPGIQIGDGAIVAANSVVTKDVPPYRIIGGNPSRIIKKRFEDE  174
Vat(C)  117 GNTEIGNDVWIGRDVTIMPGVKIGNGAIIAAKSVVTKNVDPYSVVGGNPSRLIKIRFSKE  176
Vat(D)  112 GDTIIGNDVWIGKDVVIMPGVKIGDGAIVAANSVVVKDIAPYMLAGGNPANEIKQRFDQD  171
Vat(E)  114 GDTVVGNDVWFGQNVTVLPGVKIGDGAIIGANSVVTKDVAPYTIVGGNPIQLIGPRFEPE  173
              *.. .*****.* .*...**..**.***..*..**.*...** ..****  *. **  .

Vat(A)  177 VIEEWLALQWWNLDMKIINENLPFIING----------------------DIEMLKRKR  213
Vat(B)  175 LIDYLLQIKWWDWSAQKIFSNLET--------------LCSSDLEKIKSIRD-------  212
Vat(C)  177 KIAALLKVRWWDLEIETINENIDCILNG----------------------DIKKVKRS-  212
Vat(D)  172 TINQLLDIKWWNWPIDIINENIDKILDN----------------------SIIREVIWK  208
Vat(E)  174 VIQALENLAWWNKDIEWITANVPKLMQTTPTLELINSLMEK------------------  214
              *  ..  **. . *..*  .                  .

Vat(A)  214 KLLDDT  219
Vat(B)  213 ------  218
Vat(C)  213 ------  218
Vat(D)  209 K-----  214
Vat(E)  215 ------  220
```

Fig. (29). Alignment of amino acid sequences of acetyltransferases Vat(A) [97], Vat(B) [98], Vat(C) [92], Vat(D) [99], and Vat(E) [101] from *Staphylococci* and *Enterococci*, conferring resistance to streptogramin A antibiotics. Identical residues are indicated by asterisks. Highly conserved regions are blodfaced.

```
Vat(E-1)   1 MTIPDANAIYPNSAIKEVVFIKNVIKSPNIEIGDYTYYDDPVNPTDFEKHVTHHYEFLGD  60
Vat(E-2)   1 ..........H.....................................................  60
Vat(E-3)   1 ............P.....................................................  60
Vat(E-4)   1 ..............MI...................................................  60
Vat(E-5)   1 ............L.....................................................  60
Vat(E-6)   1 ............L.....................................................  60
Vat(E-7)   1 ..............I...................................................  60
Vat(E-8)   1 ........V...LILI.LD..............................................  60
             ********.*.*  ...*..*************************************
```

(Fig. (30). Contd....)

```
Vat(E-1)   61 KLIIGKPCSIASGIEFIMNGANHVMKGISTYPFNILGGDWQQYTPELTDLPLKGDTVVGN 120
Vat(E-2)   61 .........L.................................................... 120
Vat(E-3)   61 ..........................................K..................... 120
Vat(E-4)   61 ............................................................... 120
Vat(E-5)   61 ............................................................... 120
Vat(E-6)   61 ............................................................... 120
Vat(E-7)   61 ............................................................... 120
Vat(E-8)   61 ............................................................... 120
              ********* ******************************** ******************

Vat(E-1)  121 DVWFGQNVTVLPGVKIGDGAIIGANSVVTKDVAPYTIVGGNPIQLIGPRFEPEVIQALEN 180
Vat(E-2)  121 ............................................................... 180
Vat(E-3)  121 ............................................................... 180
Vat(E-4)  121 ............................................................... 180
Vat(E-5)  121 ............................................................... 180
Vat(E-6)  121 ..............................................S.................. 180
Vat(E-7)  121 ............................................................... 180
Vat(E-8)  121 ............................................................... 180
              ********************************************** ************

Vat(E-1)  181 LAWWNKDIEWITANVPKLMQTTPTLELINSLMEK                        214
Vat(E-2)  181 ..............................                               214
Vat(E-3)  181 .......V....V...........V.....                               214
Vat(E-4)  181 ..............................                               214
Vat(E-5)  181 ..............................                               214
Vat(E-6)  181 ..............................                               214
Vat(E-7)  181 ..............................                               214
Vat(E-8)  181 ..............................                               214
              ******** **** *********** *********
```

Fig. (30). Amino acid sequences variations of Vat(E) alleles encoded by streptogramin A acetyltransferase gene found in Enterococcus faecium from retail samples of poultry. GenBank accession numbers of *vat*(E-1), *vat* (E-2), *vat* (E-3), *vat*(E-4), *vat*(E-5), *vat*(E-6), *vat*(E-7), and *vat*(E-8) are AF242872, AF153312, AY008284, AY043211, AY0430209, AY043210, AY043212, and AY043213, respectively.

Table 9. Homology (%) of DNA and Amino Acid Sequences for Virginiamycin Acetyltransferase.

Combination of genes	Homology (%) of	
	DNA	Amino Acid
vat(A) - *vat*(B)	63	52
vat(A) - *vat*(C)	70	69
vat(A) - *vat*(D)	64	59
vat(A) - *vat*(E)	59	49
vat(B) - *vat*(C)	63	57
vat(B) - *vat*(D)	63	51
vat(B) - *vat*(E)	62	61
vat(C) - *vat*(D)	64	64
vat(C) - *vat*(E)	60	50
vat(D) - *vat*(E)	59	49

Accession numbers of genes used for homology analysis were the same as in Table 5.