

**Table 1-1. A list of enzymes involved in the biosynthesis of 4,5-disubstituted aminoglycosides**

| 4,5-Disubstituted aminoglycosides                                   | Neomycin                    |            |            |                       | Butirosin                 |            |            | Ribostamycin            |                       | Lividomycin       | Paromomycin       |
|---|-----------------------------|------------|------------|-----------------------|---------------------------|------------|------------|-------------------------|-----------------------|-------------------|-------------------|
| GenBank Accession no.   | AJ843080                    | AB211959   | AJ786317   | AJ629247 <sup>a</sup> | AB097196 <sup>a</sup>     | AJ494863   | AJ781030   | AJ748131                | AJ744850 <sup>a</sup> | AJ748832          | AJ628955          |
| Producing Organism  | <i>Streptomyces fradiae</i> |            |            |                       | <i>Bacillus circulans</i> |            |            | <i>S. ribosidificus</i> |                       | <i>S. lividus</i> | <i>S. rimosus</i> |
| Proposed or Characterized Function                                  | MCIMB 8233                  | NBRC 12773 | ATCC 10745 | DSM 40063             | SANK 72073                | NRRL B3312 | ATCC 21557 | ATCC 21294              | NRRL B-11466          | CBS 844.73        | NRRL 2455         |
| 2DOI-synthase   | Neo7                        | NeoC       | NemA       | NeoC                  | BtrC                      |            | BtrC       | RbmA                    | RibC                  | LivC              | ParC              |
| 2DOI-aminotransferase   | Neo6                        | NeoB       | NemB       | NeoS                  | BtrS                      | BtrR       |            | RbmB                    | RibS                  | LivS              | ParS              |
| 2DOI-synthase stabilizer  |                             |            |            |                       | BtrC2 <sup>b</sup>        |            |            |                         |                       |                   |                   |
| 2DOIA-dehydrogenase (NAD <sup>+</sup> dependent)                    | Neo5                        | NeoA       | NemC       | NeoE                  | BtrE                      |            | BtrE       | RbmC                    | RibE                  | LivE              | ParE              |
| 2DOIA-dehydrogenase (radical SAM protein)                           |                             |            |            |                       | BtrN                      |            |            |                         |                       |                   |                   |
| 1 <sup>st</sup> Glycosyltransferase (GlcNAc and/or Glc transferase) | Neo8                        | NeoD       | NemD       | NeoM                  | BtrM                      |            | BtrM       | RbmD                    | RibM                  | LivM              | ParM              |
| 2'-N-acetylparomamine deacetylase <sup>c</sup>                      | Neo16                       | NeoL       | Nac25      | NeoD                  | BtrD                      |            | BtrD       | RacJ                    | RibD                  | LivD              | ParD              |
| 6'-Paromamine dehydrogenase <sup>d</sup>                            | Neo11                       | NeoG       | NemG       | NeoQ                  | BtrQ                      |            | BtrQ       | RbmG                    | RibQ                  | LivQ              | ParQ              |
| 6'-Oxoparomamine aminotransferase <sup>d</sup>                      | Neo18                       | NeoN       |            | NeoB                  | BtrB                      |            | BtrB       | RbmH                    | RibB                  | LivB              | ParB              |



| Proposed or Characterized Function        | Neomycin |          |          |                       | Butirosin             |          |          | Ribostamycin |                       | Lividomycin | Paromomycin        |
|---|----------|----------|----------|-----------------------|-----------------------|----------|----------|--------------|-----------------------|-------------|--------------------|
| GenBank Accession no.                     | AJ843080 | AB211959 | AJ786317 | AJ629247 <sup>a</sup> | AB097196 <sup>a</sup> | AJ494863 | AJ781030 | AJ748131     | AJ744850 <sup>a</sup> | AJ748832    | AJ628955           |
| Hypothetical protein                      | Neo14    | NeoJ     |          | NeoX                  | BtrV                  |          |          | RbmX         | RibX                  | LivX        | ParX               |
| Hypothetical protein                      |          |          |          |                       | BtrT                  |          |          |              |                       |             |                    |
| Hypothetical protein                      |          |          |          |                       |                       | BtrN     |          |              |                       |             |                    |
| Hypothetical protein                      |          |          |          |                       |                       |          |          |              |                       | LivV        |                    |
| ABC transporter                           | Neo9     | NeoE     | NemE     | NeoT                  |                       |          |          | RbmE         | RibT                  | LivT        | ParT               |
| ABC transporter                           | Neo10    | NeoF     | NemF     | NeoU                  |                       |          |          | RbmF         | RibU                  | LivU        | ParU               |
| ABC transporter                           |          |          |          |                       | BtrW                  |          |          |              |                       |             |                    |
| ABC transporter                           |          |          |          |                       | BtrX                  |          |          |              |                       |             |                    |
| <i>N</i> -Acetyltransferase (resistance)  | Neo20    | NeoP     |          | AacC8                 |                       |          |          | RbmI         | AacC8                 |             | AacC7 <sup>g</sup> |
| <i>O</i> -phosphotransferase (resistance) | Neo1     | NeoU     | Aph      | AphA                  |                       |          |          | Rph          | Rph                   |             | ParR               |
| Transcriptional regulator                 |          |          |          |                       | BtrR1                 | BtrW     |          |              |                       |             |                    |
| Transcriptional regulator                 |          |          |          |                       | BtrR2                 | BtrU     |          |              |                       |             |                    |
| Putative regulator                        | Neo4     | NeoR     | Nac1     | NeoI                  |                       |          |          | RacA         | RibI                  | LivI        | ParI               |
| Putative regulator                        | Neo3     | NeoS     | Nac3     | NeoH                  |                       |          |          | RacB         | RibH                  | LivH        | ParH               |
| Putative regulator                        | Neo2     | NeoT     | Nac4     | NeoG                  |                       |          |          | RacC         | RibG                  | LivG        | ParG               |
| Putative regulator                        |          | Orf6     | NemH     | Orf SfrA10.3          |                       |          |          |              |                       |             |                    |
| Putative LuxR regulator                   | Neo21    | NeoQ     |          | NeoR                  |                       |          |          |              |                       |             |                    |
| Extracellular aminoglycoside phosphatase  |          |          |          |                       |                       |          |          |              |                       | LivK        | ParK               |

<sup>a</sup> Representative annotations described in the main text are mostly taken from the selected GenBank databases, which have been deposited by Piepersberg and co-worker at Bergische Universität Wuppertal in Germany, whereas those for butirosin biosynthesis are solely taken from Eguchi and co-worker at Tokyo Institute of Technology in Japan; <sup>b</sup> Located outside of of the gene cluster, and deposited as GenBank accession no. AB066209; <sup>c</sup> A dual function enzyme as 2'-*N*-acetylparomamine deacetylase and *N*-acetylglucosaminyribostamycin deacetylase; <sup>d</sup> NeoQ/NeoB catalyzes amination of paromamine and 6'''-hydroxyneomycin to generate neamine and neomycin C, respectively. Meanwhile, LivQ (NeoQ homolog) is a monofunctional 6'''-hydroxyneomycin oxidoreductase and LivB (NeoB homolog) is a bifunctional aminotransferase like NeoB; <sup>e</sup> LivY and LivW are homologous to AprD3 and AprD4 (GenBank accession no. AJ875019), respectively, which are responsible for C3' deoxygenation in the apramycin biosynthesis; <sup>f</sup> BtrL/BtrP catalyzes ribosylation of neamine and paromamine to generate ribostamycin and pseudoribostamycin, respectively; <sup>g</sup> Located outside of of the gene cluster, and deposited as GenBank accession no. AJ749845.

**Table 1-2. A list of enzymes involved in the biosynthesis of 4,6-disubstituted aminoglycosides**

| 4,6-Disubstituted aminoglycosides                                   | Kanamycin               |                         |                         | Tobramycin            |                       | Gentamicin                        |                       |                       | Sisomicin           |
|---|-------------------------|-------------------------|-------------------------|-----------------------|-----------------------|-----------------------------------|-----------------------|-----------------------|---------------------|
| GenBank Accession no.   | AB164642                | AJ628422 <sup>a</sup>   | AJ582817                | AJ579650              | AJ810851 <sup>a</sup> | AJ575934                          | AY524043              | AJ628149 <sup>a</sup> | FJ160413            |
| Producing Organism  | <i>S. kanamyceticus</i> | <i>S. kanamyceticus</i> | <i>S. kanamyceticus</i> | <i>S. tenebrarius</i> | <i>S. tenebrarius</i> | <i>Micromonospora echinospora</i> | <i>M. echinospora</i> | <i>M. echinospora</i> | <i>M. inyoensis</i> |
| Proposed or Characterized Function                                  | M1164                   | DSM 40500               | ATCC 12853              | ATCC 17920            | DSM 40477             | ATCC 15835                        | ATCC 15835            | DSM 43036             | TS388               |
| 2DOI-synthase   | Orf11                   | KanC                    | KanA                    | TbmA                  | TobC                  | GtmA                              | GntB                  | GenC                  | Sis11               |
| 2DOI-aminotransferase   | Orf10                   | KanS1                   | KanB                    | TbmB                  | TobS1                 | GtmB                              | GntA                  | GenS1                 | Sis10               |
| 2DOIA-dehydrogenase (NAD <sup>+</sup> dependent)                    | Orf6                    | KanE                    | KanK                    | TacD                  | TobE                  | GacH                              | GntP                  | GenE                  | Sis22               |
| 1 <sup>st</sup> Glycosyltransferase (GlcNAc and/or Glc transferase) | Orf20                   | KanM1                   | KanF                    |                       | TobM1                 | GtmG                              | GntZ                  | GenM1                 | Sis8                |
| 2'-N-acetylparomamine deacetylase                                   | Orf15                   | KanN                    | KacA                    |                       | TobN                  | GtmM <sup>b</sup>                 |                       | GenD                  | Sis29               |
| 6'-Paromamine dehydrogenase <sup>c</sup>                            | Orf9                    | KanQ                    | KanI                    | TacB                  | TobQ                  | GacJ                              | GntX                  | GenQ                  | Sis6                |
| 6'-Oxoparomamine aminotransferase <sup>c</sup>                      | Orf8                    | KanB                    | KacL                    | TacC                  | TobB                  | GacK                              | GntJ                  | GenB1                 | Sis5                |
| Putative dehydrogenase  |                         |                         |                         |                       |                       | GtmH                              | GntY                  | GenD3                 | Sis7                |
| 2 <sup>nd</sup> Glycosyltransferase (Kns and/or Glc transferase)    | Orf14                   | KanM2                   | KanE                    | TbmD                  | TobM2                 |                                   |                       |                       |                     |

| Proposed or Characterized Function                          | Kanamycin |                       |          | Tobramycin |                       | Gentamicin |          |                       | Sisomicin |
|---|-----------|-----------------------|----------|------------|-----------------------|------------|----------|-----------------------|-----------|
| GenBank Accession no.                                       | AB164642  | AJ628422 <sup>a</sup> | AJ582817 | AJ579650   | AJ810851 <sup>a</sup> | AJ575934   | AY524043 | AJ628149 <sup>a</sup> | FJ160413  |
| 2 <sup>nd</sup> Glycosyltransferase<br>(Xylose transferase) |           |                       |          |            |                       | GtmE       | GntD     | GenM2                 | Sis13     |
| Carbamoyltransferase  |           |                       |          | TacA       | TobZ                  |            |          |                       |           |
| Carbamoyl-phosphate synthase                                |           |                       |          |            | TobL                  |            |          |                       |           |
| UDP-Glc 3-dehydrogenase                                     | Orf12     | KanD2                 | KanC     | TbmC       | TobD2                 | GtmC       | GntC     | GenD2                 | Sis12     |
| UDP-3-keto Glc aminotransferase                             | Orf16     | KanS2                 | KanD     |            | TobS2                 | GtmD       | GntF     | GenS2                 | Sis15     |
| Dioxygenase<br>(Ketoglutarate dependent)                    | Orf17     | KanJ                  | KacB     |            |                       |            |          |                       |           |
| Reductase<br>(NADPH dependent)                              | Orf18     | KanK                  | KacC     |            |                       |            |          |                       |           |
| Putative methyltransferase                                  |           |                       |          |            |                       |            |          | GenT                  | Sis28     |
| Putative methyltransferase                                  |           |                       |          |            |                       |            |          | GenN                  | Sis30     |
| Putative radical SAM methyltransferase                      |           |                       |          |            |                       | GacD       | GntK     | GenK                  |           |
| Putative radical SAM protein                                |           |                       |          |            |                       | GtmI       | GntE     | GenD1                 | Sis14     |
| Putative radical SAM protein                                |           |                       |          |            |                       |            | GntS     | GenF                  | Sis25     |
| Putative aminotransferase                                   |           |                       |          |            |                       | GacE       | GntH     | GenB2                 |           |
| Putative aminotransferase                                   |           |                       |          |            |                       | GacC       | GntW     | GenB3                 | Sis18     |
| Putative aminotransferase                                   |           |                       |          |            |                       | GacB       | GntL     | GenB4                 |           |
| Hypothetical protein  | Orf22     | KanO                  | KacE     |            |                       |            |          |                       |           |

| Proposed or Characterized Function           | Kanamycin |                       |          | Tobramycin |                       | Gentamicin |          |                       | Sisomicin |
|--|-----------|-----------------------|----------|------------|-----------------------|------------|----------|-----------------------|-----------|
| GenBank Accession no.                        | AB164642  | AJ628422 <sup>a</sup> | AJ582817 | AJ579650   | AJ810851 <sup>a</sup> | AJ575934   | AY524043 | AJ628149 <sup>a</sup> | FJ160413  |
| Hypothetical protein                         | Orf23     | KanP                  | KacF     |            |                       |            |          |                       |           |
| Hypothetical protein                         |           |                       |          |            | TobX                  |            |          |                       |           |
| Hypothetical protein                         |           |                       |          |            |                       | GacF       | GntM     | GenX                  | Sis19     |
| Hypothetical protein                         |           |                       |          |            |                       | GacG       | GntN     | GenU                  | Sis20     |
| Hypothetical protein                         |           |                       |          |            |                       |            |          | GenG                  |           |
| Hypothetical protein                         |           |                       |          |            |                       | GacN       | GntT     | Orf MecE04.1          | Sis1      |
| Transport protein                            | Orf7      | KanT                  | KanJ     | TbmE       | TobT                  |            |          |                       |           |
| Transmembrane efflux protein                 |           | KanX                  | KanN     |            |                       |            |          |                       |           |
| Transmembrane efflux protein                 |           |                       |          |            |                       | GtmK       | GntO     | GenV                  | Sis21     |
| Efflux protein                               |           | KanZ                  |          |            |                       |            |          |                       |           |
| Membrane antiporter                          |           |                       |          |            |                       | GacI       | GntQ     | GenY                  | Sis23     |
| Putative exporter                            |           |                       |          |            |                       |            |          | GenH                  | Sis26     |
| Putative exporter                            |           |                       |          |            |                       |            |          | GenI                  | Sis27     |
| <i>O</i> -phosphoryltransferase (resistance) |           |                       |          |            |                       | GtmJ       | GntI     | GenP                  | Sis17     |
| <i>N</i> -acetyltransferase (resistance)     | Kac       | KanA                  | KanM     |            |                       |            |          |                       |           |
| 16S rRNA methylase (resistance)              | Kmr       | Kmr                   | Kmr      |            |                       | GtmF       | GrmA     | GmrA                  | Sis9      |
| Transcriptional regulator                    | Orf5      | KanL                  | KanR     |            |                       | GtmL       | GrmO     | GmrB                  | Sis4      |

| Proposed or Characterized Function               | Kanamycin |                       |          | Tobramycin |                       | Gentamicin |          |                       | Sisomicin |
|--|-----------|-----------------------|----------|------------|-----------------------|------------|----------|-----------------------|-----------|
| GenBank Accession no.                            | AB164642  | AJ628422 <sup>a</sup> | AJ582817 | AJ579650   | AJ810851 <sup>a</sup> | AJ575934   | AY524043 | AJ628149 <sup>a</sup> | FJ160413  |
| Transcriptional regulator                        |           |                       |          |            |                       |            |          |                       | Sis32     |
| Putative regulator                               |           | KanI                  | KacM     |            |                       |            |          |                       |           |
| Putative regulator                               | Orf3      | KanH                  |          |            |                       |            |          |                       |           |
| Putative regulator                               | Orf2      | KanG                  | KacN     |            |                       |            |          |                       |           |
| Putative regulator                               |           |                       |          |            |                       |            | GntR     | GenA                  | Sis24     |
| tRNA-ribosyltransferase                          |           |                       |          |            |                       | GacL       | GntV     | GenO                  | Sis3      |
| Trp-tRNA synthetase                              |           |                       |          |            |                       | GacM       | GntU     | Orf MecE04.2          | Sis2      |
| GTP cyclohydrolase I                             |           |                       |          |            |                       | GacA       | GntG     | GenW                  | Sis16     |
| Activator of Hsp90 ATPase                        |           |                       |          |            |                       |            |          |                       | Sis31     |
| I family protein                                 |           |                       |          |            |                       |            |          |                       |           |
| Putative serine repeat antigen                   |           |                       |          |            |                       |            |          |                       | Sis33     |
| Putative two-component system histidine kinase   |           |                       |          |            |                       |            |          | Orf MecG05.28c        | Sis34     |
| Putative two-component system response regulator |           |                       |          |            |                       |            |          | Orf MecG05.29c        | Sis35     |

<sup>a</sup> Representative annotations described in the main text are taken from the selected GenBank databases, which have been deposited by Piepersberg and co-worker at Bergische Universität Wuppertal in Germany; <sup>b</sup> Deposited as GenBank accession no. AM946392; <sup>c</sup> KanQ/KanB show substrate flexibility toward both pseudodisaccharides (paromamine and 2'-deamino-2'-hydroxyparomamine) and pseudotrisaccharides (kanamycins C and X) generating C6'-aminated pseudodisaccharides (neamine and 2'-deamino-2'-hydroxyneamine) and pseudotrisaccharides (kanamycins B and A), respectively.