

Supplementary information

The neomycin biosynthetic gene cluster of *Streptomyces fradiae* NCIMB 8233: genetic and biochemical evidence for the roles of two glycosyltransferases and a deacetylase

Qingzhi Fan,^{a,b} Fanglu Huang,^b Peter F. Leadlay^a and Jonathan B. Spencer^{b,c}

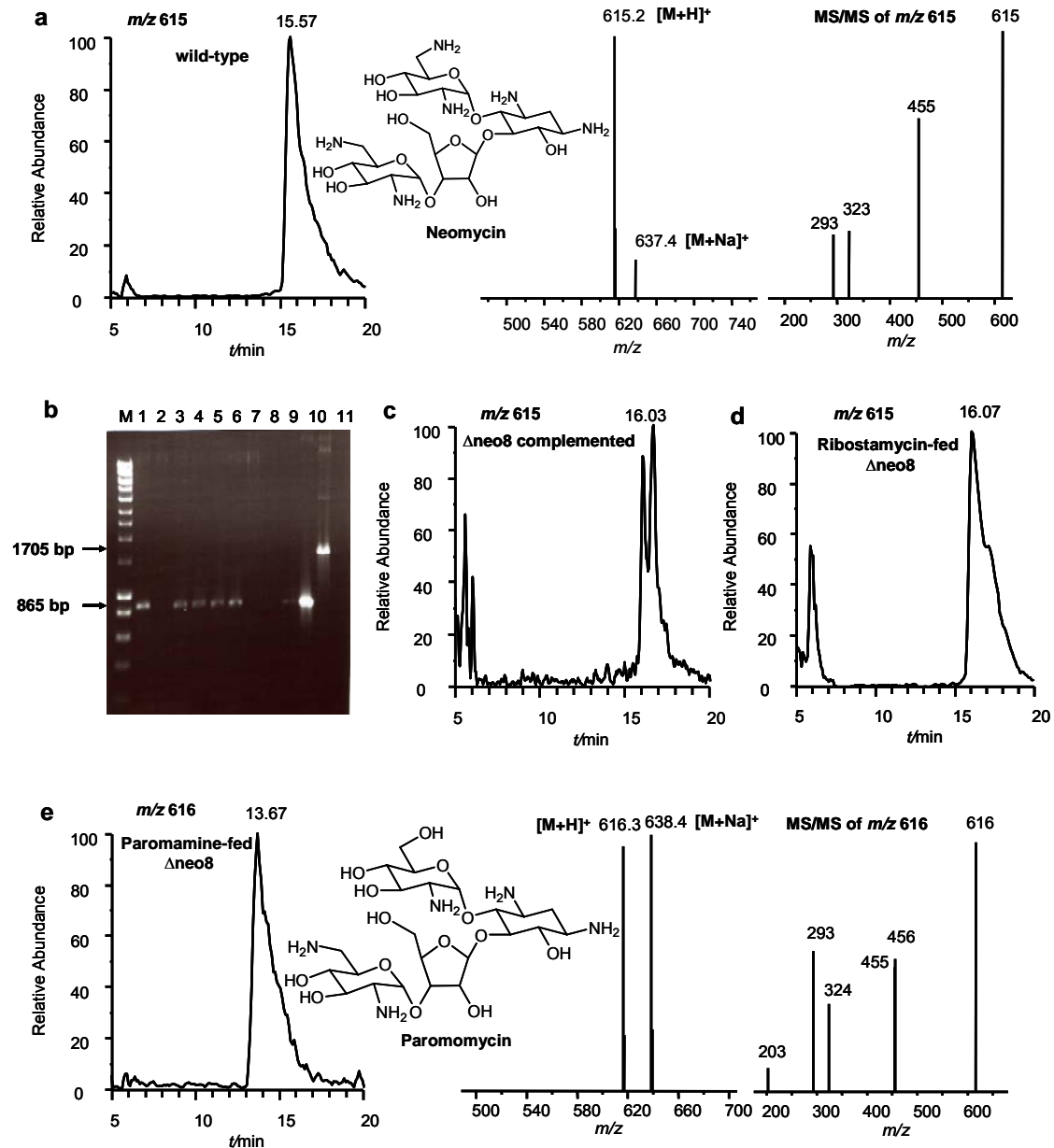


Figure S1. LC-MS analysis of the Δ neo8 mutant strain and its verification. (a) LC-MS analysis of neomycin from purified *Streptomyces fradiae* wild-type supernatant, as a control. (b) PCR screening for Δ neo8 mutant: M, Marker; Lane 1-9, verification of mutant candidates (band at 865 bp); Lane 10, plasmid pQZ27, as positive control; Lane 11, plasmid pQZ25, as negative control. (c) Restoration of neomycin production in the pQZ60-complemented Δ neo8 mutant. (d) Restoration of neomycin production upon feeding ribostamycin. The peak at 6 min corresponds to diphosphorylated ribostamycin. This arises likely from the action of *neo1*, the phosphotransferase resistance gene. (e) Production of paromomycin upon feeding paromamine.

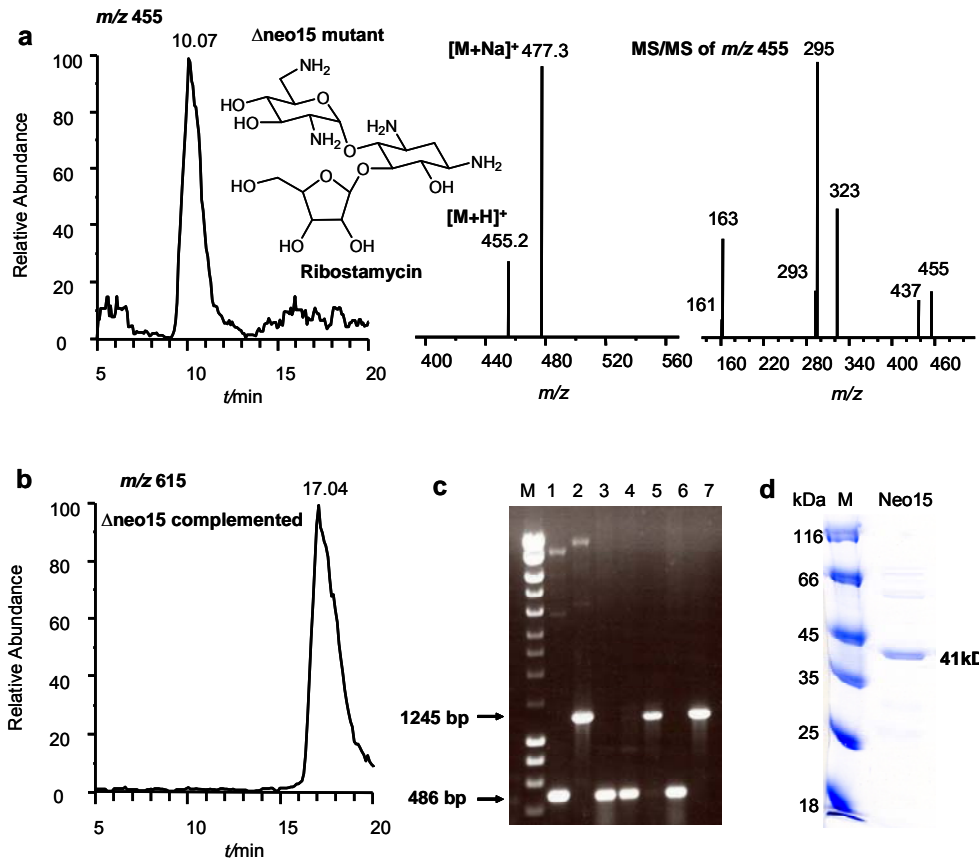


Figure S2. LC-MS analysis of the Δ neo15 mutant strain, its verification and Neo15 protein expression. (a) Accumulation of ribostamycin in the purified supernatant of Δ neo15. (b) Restoration of neomycin production in the pQZ62-complemented Δ neo15 mutant. (c) PCR screening for Δ neo15 mutant: M, Marker; Lane 1, plasmid pQZ36, as positive control; Lane 2, plasmid pQZ35, as negative control; Lane 3-6, verification of mutant candidates (band at 486 bp); Lane 7, wild-type genomic DNA control. (d) SDS-PAGE analysis of purified Neo15 protein.

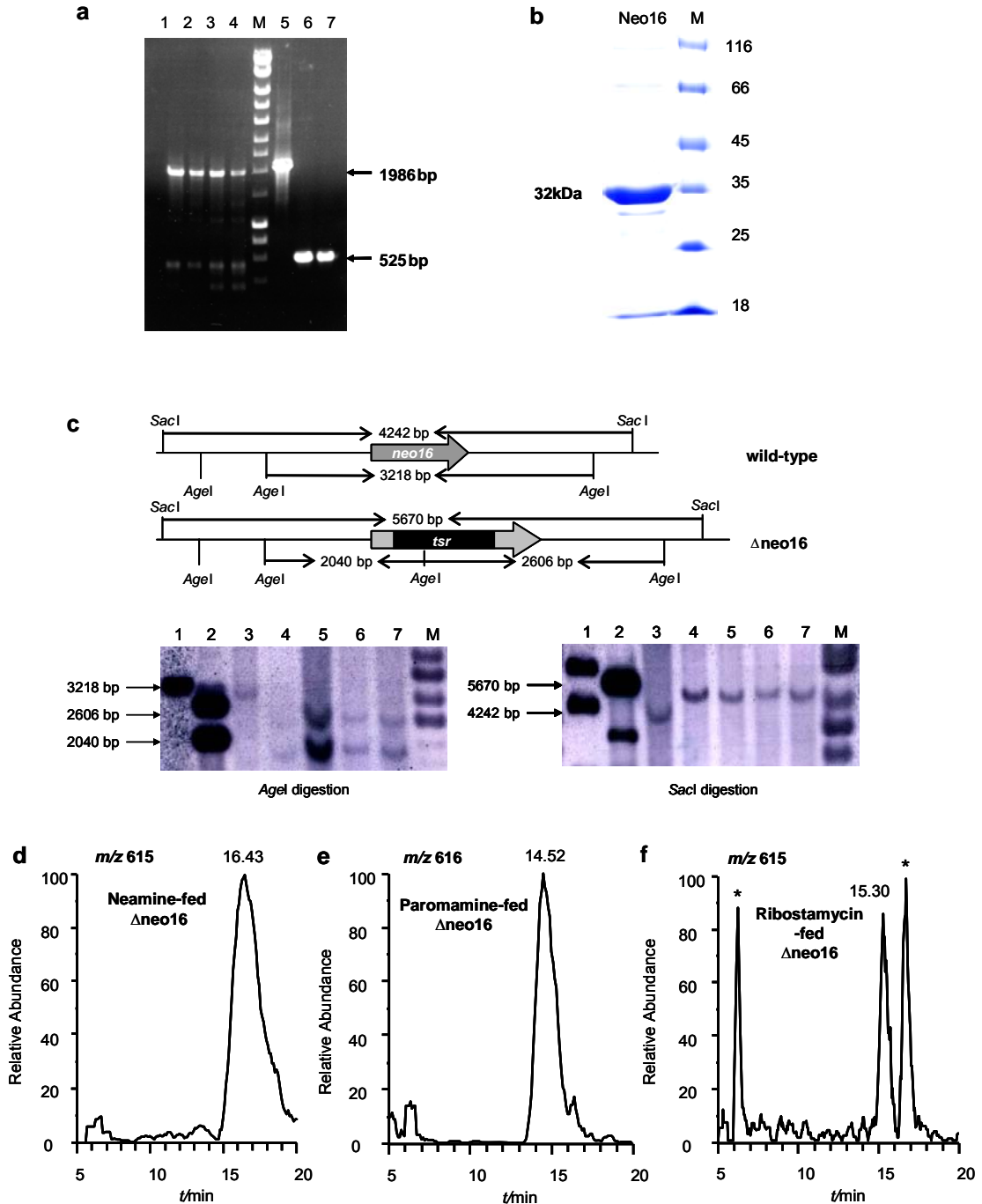


Figure S3. LC-MS analysis of the Δ neo16 mutant strain, its verification and Neo16 protein expression. (a) PCR screening for Δ neo16 mutant: Lane 1-4, verification of mutant candidates (band at 1986 bp); M, Marker; Lane 5, plasmid pQZ22, as positive control; Lane 6, plasmid pQZ15, as negative control; Lane 7, wild-type genomic DNA control. (b) SDS-PAGE analysis of purified Neo16 protein. (c) Southern blot analysis of Δ neo16 with *AgeI* digestion and *SacI* digestion respectively using the *AgeI*-digested fragments

(2606 bp and 2040 bp) as probe: Lane 1, plasmid pQZ15, as negative control; Lane 2, plasmid pQZ16, as positive control; Lane 3, wild-type genomic DNA control; Lane 4-7, verification of mutant candidates (band at 2606 bp and 2040 bp for *Agel* digestion; band at 5670 bp for *SacI* digestion. (d) Restoration of neomycin production upon feeding neamine. (e) Production of paromomycin upon feeding paromamine. (f) Production of neomycin upon feeding ribostamycin. Starred peaks (*) are not aminoglycoside-related compounds as judged by MS/MS.

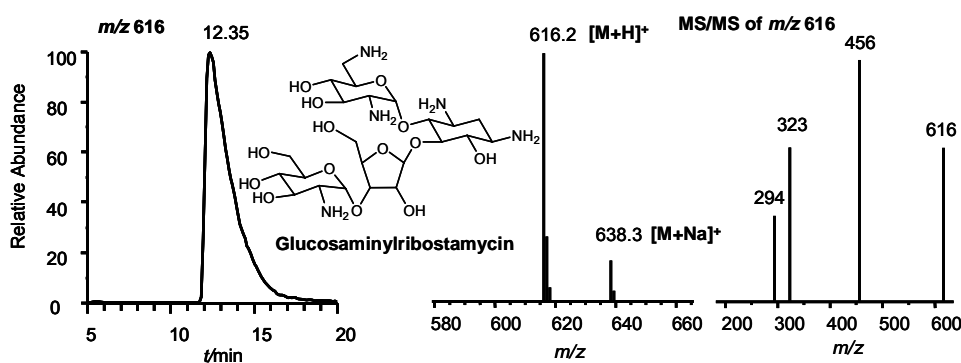


Figure S4. LC-MS analysis of BtrD deacetylase activity *in vitro*. BtrD substitutes efficiently for Neo16 in deacetylation of *N*-acetylglucosaminylribostamycin to form glucosaminylribostamycin.

Table S1. Neomycin and paromomycin production of wild-type and mutant cultures supplemented with different intermediates of the biosynthetic pathway

Sample ID	Culture	Compound fed to the culture	Yield of neomycin / mg L ⁻¹	Average yield of neomycin/ mg L ⁻¹	Yield of neomycin as a percentage of wild-type neomycin production / %	Yield of paromomycin / mg L ⁻¹	Average yield of paromomycin / mg L ⁻¹	Yield of paromomycin as a percentage of wild-type neomycin production / %
WT1	wild-type	Nil	0.689	0.666	100	-		
WT2	wild-type	Nil	0.644					
WT4	wild-type	paromamine	0.200		30	0.258		39
WT3	wild-type	ribostamycin	0.255		38	-		
N8-1	Δ neo8	neamine	0.232	0.179	27	-		
N8-2	Δ neo8	neamine	0.125					
N8-3	Δ neo8	paromamine	0.172	0.275	41	0.368	0.772	116
N8-4	Δ neo8	paromamine	0.370			0.982		
N8-5	Δ neo8	paromamine	0.282			0.966		
N8-6	Δ neo8	ribostamycin	0.214	0.216	32	-		
N8-7	Δ neo8	ribostamycin	0.217					
N16-1	Δ neo16	neamine	0.096	0.088	13	-		
N16-2	Δ neo16	neamine	0.032					
N16-3	Δ neo16	neamine	0.137					
N16-4	Δ neo16	paromamine	0.004	0.002	0	0.400	0.454	68
N16-5	Δ neo16	paromamine	0			0.509		
N16-6	Δ neo16	ribostamycin	0.010	0.004	1	-		
N16-7	Δ neo16	ribostamycin	0.006					
N16-8	Δ neo16	ribostamycin	0.001					
N16-9	Δ neo16	ribostamycin	0.001					
N16-10	Δ neo16	ribostamycin	0.003					
N16-11	Δ neo16	ribostamycin	0.002					
N16-12	Δ neo16	ribostamycin	0.004					