

Cholesterol degradation by *Gordonia cholesterolivorans*

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              GSGFGG              E
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Gcho1 : MA-----QTDFDVLIIGSGFGGSVSAALRLIEKGYRVGIIIEAGRRFEDDQFAKTSWRLNRFVWAPKFGLMGIQRV : 69
Gcho2 : MPEPRQSAQSEPTAAVDRTHASRTEYDVLVIGSGFGGSVTALRLSEKGYRVGVLEAGRRFEDSDFAKTSWDLRRLWAPKLGAYGIQRI : 90
Gbro  : MAAH-----HEPTHFVLIIGSGFGGSVSAALRLVEKGYRVGVIEAGRRFEDDFAKTSWRLNKWLWAPKLGLYGIQRI : 73
Mlep  : MK-----PDYDVLIIIGSGFGGSVSAALRLTEKGYRVGVLEAGRRFAEDDFAKTSWDLRKFLWAPKLGCGYGIQRI : 68
Nfar  : MTQ-----RETFDVLIVGSGFGGSVTALRLVEKGYRVGVLEAGRRFADHELPKTSWDVRKFLWAPALGCGYGIQRI : 71
Rjos  : MSKQ-----RATEYDVLIVGSGFGGSVTALRLVEKGYKVGILEAGRRYADADF AKTSWDLKKFLWAPALGFFGIQRV : 72
Scoe  : MPQ-----DAYDYDVLVVGSGFGGSVSAALRLTEKGYRVGVLEAGRRFTRESLPKNSWDLKNYLWAPKLGMEGIQRI : 71

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              GAGVGGGS
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Gcho1 : HMLKDVMMVLAGAGVGGGSLNYANTLYKPPPTFFFTDPQMNHITDWESELTPHYEQARRMLGVVTPNPTFTNSDRIMKEVADEMGVGDFTFTST : 159
Gcho2 : HLLRDCVILAGAGVGGGSLNYANTLYKPSGAFFEDPQMAHITDWEDELSPYYDQARRMLGVVTPNPHMTPADAIVKSVADDMGAGDTFIQT : 180
Gbro  : HALKDVMMILAGAGVGGGSLNYANTLYKPPPTFFFTDPQMRDITDWEDELSPYYEQARRMLGVVTPNPTVTESDRVHREVAEEMGAADTFTAT : 163
Mlep  : HLLRNVMILAGAGVGGGSLNYANTLYVPPPEPFFANQMAHITDWESELAHYDQARRMLGVVCPNPTFTDADRILKEWVDEMGGFDTFVPT : 158
Nfar  : HLLRDVILGAGAGVGGGSLNYANTLYVPPPEPFFRDPOMRDITDWEELTPYYEQAKMLGVVTPNPHMTPADEVFKKVAEDMGFGDTFVQT : 161
Rjos  : HLLRDCLILAGAGVGGGSLNYANTLYKPPASFFQDKQUSHITDWEDELTPYYEQARKMLGVVCPNPHMTPADELTKSVAEDMGVGDFTFIQT : 162
Scoe  : HLLGNVMMVLAGAGVGGGSLNYANTLYVPPKPFDDPQMRGITDWEELTPYYDQARRMLGVRLNPTMTPSDVHLKAAAERMGCDDTFHMA : 161

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Gcho1 : PVGVFFGAKTG--GE--GAPGETVPDPYFGGAGPERTACTECGACMTGCRVGAKNLTKNYLGLAERNGATIIDRTTVDRLEQRADGSWL : 245
Gcho2 : PVGVYFG-----EPGRIVADPYFGGVGPDRITGCIIECGECMTGCRHGAKNTLLKNYLGLAEKIGAVVHPLTTVTDLRLAGDGTWE : 259
Gbro  : PVGVFFGAKTG--FA--GEPGQKVPDPYFGGVGPPERTACTECGACMTGCRVGAKNLTKNYLGLAERAGAIIDRTTVQSLEQSSDGTWL : 249
Mlep  : PVGVFFGPD----GT--QTPGRIVADPYFGGVGPVRTGCLIECGCCMTGCRHGAKNTLVKNYLGLAESAGAQVPMTTVKGFELRSEGLWE : 242
Nfar  : PVGVFFG-----EPGKIVDPYFGGVGPDRITGCIIECGECMTGCRHGAKNTLVKNYLGLAEQAGAVVPMTTVTAIRPLPDGTWD : 240
Rjos  : PVGVFFG-----EAGKIVDPYFGGVGPDRITGCIIECGECMTGCRHGAKNTLLKNYLGLAEKAGAEIIPMTTVTGLREASDGTWD : 241
Scoe  : PVGVFFGDGEDADGTAKAGPGEQVPDPYFGGAGPDRKACNECGECMTGCRHGAKNTLNENYLGLAEKAGAVVHPMTTAVSVTDDSRGGFA : 251

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Gcho1 : VSAHGSSSWG--PFGA--RRRRFTADQVIVAAGTFNTQKIMHRAKG-STLPRLSDAMGVLTRTNSESILGAQAR-----SYDSSRDFT : 323
Gcho2 : VHTVRTGAM----MRK--RRRVFTARNVFLAAGTNGTQRLLHDLKDSGRLPELSSRLGELTRTNSESILGAIRY-----SVDESLDLT : 336
Gbro  : VGTHRSSSWG--PVGR--RARFTTAGQVIVAAGTFNTQRLLHHAKH-STLPQISDAMGVLTRTNSESILGAAAS-----HYDPRHDYS : 327
Mlep  : VHTVRTGSM----LRR--GRRFTTAAHLLLAAAGTNGTQRLLFRMRDQGKLPRLSQRLGVLTRTNSESIVGAGTL-----NVMPDRDLT : 319
Nfar  : VETKRTGKL----IGK--QPKTYTAGHVFLAAGTRGTQQLLFAMRDKGVLPKLSDRLGVLTRTNSESIVGAATK-----TLQPGQDFT : 317
Rjos  : VFTRRSGPR----KNR--NRKTYTAAANVFLAAGTNGTQHLLFDQKETGALPKISDRLGVLTRTNSESILGAAKN-----KVDPALELT : 318
Scoe  : VATLPTDRKKRGKRGKQQAGRTFTARRVFLAAGTYGTQTLHHRMKAGGQLPRLSDVVGELTRTNSEALVGAQTDDRRYRGATGEPRADFT : 341

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Gcho1 : EGVAITSSFHPTSMTHIEPVRYGKGSNAMAAYLQTLTLDGG----SVPNRLGQFLKNAVTNPLMLLRLFFVRKMSERTVIALVMQNMNNSL : 409
Gcho2 : RGVAITSSFYPAADTHIEPVRYGKGSNMGMLQSPMTDGD----QRLPRWLRFLGIMLRHPIATARILAVKGMERTIIALVMQNLDNSI : 422
Gbro  : HGVAITSSFHPDPTTHIEPVRYGKGSNATAAYLQTLTLDGG----TRANRFQFLRQVARNPLLLVRLLVVRKMSORTVIALVMQNMNNSL : 413
Mlep  : HGVAITSSIHPTSDTHIEPIRYGKGSNMGLLQTLMTDGGPEGTDVPRWRQLLHQASEDPRRMLRLINPRMSERTVIALVMQHLDNSI : 409
Nfar  : KGVAITSSIHPTPDTHIEPVRYGKGSNFMGLLQTLMDVGG----GRIPRWLKFLLLVLRHPLDLLSFLSTKMSERTIISLVMQHLDNSI : 403
Rjos  : KGVAITSSFHPTSDTHVEPVRYGKGSNSMALLQTLTLDGG----G--RRWMTFLKELAKDPTA-LKVLTPYKMSERTIIALVMQNLDNSI : 401
Scoe  : RGVAITSSIHPDANTHIEPVRYGKGSNSMGGLSILQVPYAGQTASGASRVLGF LGHAAKHPLLVLRSLSNRKMERTIIGLVMQSLDNSL : 431

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Gcho1 : TTFVRRKRGPF-KYVTSKQGI GEPNPTWIP EGN EATRRI AAKLP GGLAGGTWGDIFNMPLTAHYLGGCVISDDPATGVIDPYHRVNNYPTL : 498
Gcho2 : TTFTRRGLFGR-RLSSRQGHGRPNPTWIPAGNEATRRIAEKI-GGVPAGTWGEVFNVPMTAHFLGGCVIGENASTGVIDPYHRVHGYP TL : 510
Gbro : TTFVRRKRGPF-RYITSKQGHGEPNPTWIPKGNEATRRIASKLPGGMAGGTWGDIMNMPLTAHYLGGCAISDDPATGVIDAYHRVNNYPTL : 502
Mlep : TTFTKRGLGIRWYSSKQGN GEPNPSWIPIGNEVTRRLAAKI-DGVAGGTWGE LFNIP LTAHFLGGAVIGDNAEHGVIDPYHRVYGYPTL : 498
Nfar : TTYTKRGLFG-RKMTSKQGHGQPNPTWIPVGNQVTRKVAEEI-GGIAGGSWGEIFNIP LTAHFLGGAAIGADPEHGVIDPYHRVYGYPTL : 491
Rjos : TTYTKKGLFGRRKVT SKQGHGQPNPSWIPAGNEATRRIAEKI-DGRAGGTWGDVFNIP LTAHFLGGCTIIASEPSKGVIDPYHRVWGYPTL : 490
Scoe : TTHLKPAGVGKGLLTARQGHGSPMPKQIKAATDGASALAAEI-NGFAGSNVGELMGTPLTAHFLGGCPIGASRETGVIDPYHRLYGHFPGI : 520

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VVDGAAVSANLG
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Gcho1 : HITDGAAITANLGVNPSLSIC AQAERAISLWPNKGEADPRPAQGDAYRRLTPVAPKAPVWPADAPGALRLPITPVEK-----A : 576
Gcho2 : SVVDGSAVSANLGVNPSLTISAQAERAAALWPNKGETDSRPAQYLYRRVVEPVQPAAPAVPESAPGALRLPITPVGRP-----VNR : 591
Gbro : HVTDGAAVSANLGVNPSLTIC AQAERAMALWPNKGEQDMRPAQGRPYERISPCPPVAPVWPAPGALRLPITPVEHLA-----ERTSVG : 587
Mlep : YVVDGAAISANLGVNPSLSIAAQAERAASLWPNKGEQDQRPROGESYRRLAPIAPDHPVWPVPAEALGALRW-----L : 569
Nfar : SVVDGAAVSANLGVNPSLTIT AQAERAAAYWPNKGEQDKRPPQEGYRRIDPVPPVRPWPVPAEAPAALVLPISPVRRT EQ----DTPAAG : 577
Rjos : SVVDGSAVSANLGVNPSLTISAQAERAASLWPNKGEKDLRPAQEGYRRLNVPVAPIAPVWPEGAPAALVLPIVEIRNSAVVPDAQKHGVA : 580
Scoe : SVVDGAAVSANLGVNPSLTIT AQAERAMSYWPNKGESDPRPAQGAGYERVAAVAPRSPAVPERAFGALRLPFVEMPTV-----PPKK : 602

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FIG. S1: Comparison of amino acid sequences of some class I type cholesterol oxidases. The abbreviations of the bacterial strains as well as the accession numbers of the enzymes are as follows: Gcho1, *G. cholesterolivorans* (GU320250); Gcho2, *G. cholesterolivorans* (GU320251); Gbro, *G. bronchialis* (ACY20990); Mlep, *Mycobacterium leprae* (CAC29897); Nfar, *Nocardia facinica* (YP_118824); Rjos, *Rhodococcus jostii* (ABG97978); Scoe, *Streptomyces coelicolor* (CAB82020). Consensus lettering indicates regions of the protein that are shown to be important in interactions with the FAD cofactor; asterisks indicate amino acids that are reported to make actual hydrogen bonding contact to the cofactor; amino acids marked with a “+” indicate residues that are implicated as playing a role in cholesterol catalysis (according to Vrielink and Ghisla [33]).

TABLE S1. ORFs of the 8 kb genomic sequence of *G. cholesterolivorans* (accession number GU320250) and some of their homologues in other Gram-positive strains.

ORF start/end	Function (putative) Length (aa) / % G+C	Best four homologues in Blast search (% identity; locus_tag, species)	Accession number
hyp. protein 210/19	hypothetical protein 63 / 62.5	84%; Mvan_1510, <i>Mycobacterium vanbaalenii</i> PYR-1 84%; nfa8940, <i>Nocardia farcinica</i> IFM10152 82%; ROP_62580, <i>Rhodococcus opacus</i> B4 75%; Gbro_1725, <i>Gordonia bronchialis</i> DSM43247	YP_952348 YP_117103 YP_002783450 YP_003272880
IMP-DH 472/1983	inosine-5-monophosphate dehydrogenase; GuaB 503 / 69.6	86%; Gbro_1726, <i>Gordonia bronchialis</i> DSM43247 86%; ROP_62590, <i>Rhodococcus opacus</i> B4 83%; nfa8950, <i>Nocardia farcinica</i> IFM10152 82%; Mvan_1511, <i>Mycobacterium vanbaalenii</i> PYR-1	YP_003272881 YP_002783451 YP_117104 YP_952349
IMP-DH 2043/3212	inosine-5-monophosphate dehydrogenase 389 / 69.2	82%; Gbro_1727, <i>Gordonia bronchialis</i> DSM43247 75%; ROP_62600, <i>Rhodococcus opacus</i> B4 74%; Mvan_1512, <i>Mycobacterium vanbaalenii</i> PYR-1 73%; nfa8960, <i>Nocardia farcinica</i> IFM10152	YP_003272882 YP_002783452 YP_952350 YP_117105
hyp. protein 3290/3751	hypothetical protein 153 / 63.0	80%; KflaDRAFT_1454, <i>Kribbella flavida</i> DSM 17836 75%; SSAG_06863, <i>Streptomyces</i> sp. Mg1 72%; JNB_15918, <i>Janibacter</i> sp. HTCC2649 72%; AAur_4152, <i>Arthrobacter aurescens</i> TC1	ZP_03860764 ZP_05002561 ZP_00996518 YP_949819
Cyt. P450 3748/4971	cytochrome P450 407 / 69.0	69%; ROP_57840, <i>Rhodococcus opacus</i> B4 68%; RHA1_ro05719, <i>Rhodococcus jostii</i> RHA1	YP_002782976 YP_705656

		66%; Gbro_0988, <i>Gordonia bronchialis</i> DSM43247 66%; Mvan_4465, <i>Mycobacterium vanbaalenii</i> PYR-1	YP_003272192 YP_955246
TetR 4917/5561	TetR family transcriptional regulator protein 214 / 70.1	48%; RHA1_ro05718, <i>Rhodococcus jostii</i> RHA1 48%; ROP_57830, <i>Rhodococcus opacus</i> B4 46%; Gbro_0987, <i>Gordonia bronchialis</i> DSM43247 41%; Mvan_4466, <i>Mycobacterium vanbaalenii</i> PYR-1	YP_705655 YP_002782975 YP_003272191 YP_955247
ChoOx-1 5616/7346	Cholesterol oxidase, FAD dependent oxidoreductase 576 / 67.3	81%; Gbro_1728, <i>Gordonia bronchialis</i> DSM43247 66%; Mvan_1514, <i>Mycobacterium vanbaalenii</i> PYR-1 64%; RHA1_ro06201, <i>Rhodococcus jostii</i> RHA1 64%; ROP_62610, <i>Rhodococcus opacus</i> B4	YP_003272883 YP_952352 YP_706136 YP_002783453
Serine O-acetyl- transferase 8006/7425	Serine O-acetyltransferase; cysE 193 / 69.1	71%; Bcav_2610, <i>Beutenbergia cavernae</i> DSM 12333 62%; Achl_2196, <i>Arthrobacter chlorophenolicus</i> A6 61%; RHOER0001_1022, <i>Rhodococcus erythropolis</i> SK121 59%; ckrop_0418, <i>Corynebacterium kroppenstedtii</i>	YP_002882617 YP_002488253 ZP_04388353 YP_002905740

TABLE S2. ORFs of the 12.8 kb genomic sequence of *G. cholesterolivorans* (accession number GU320251) and some of their homologues in other Gram-positive strains.

ORF start/end	Function (putative) Length (aa) / % G+C	Best four homologues in Blast search (% identity; locus_tag, species)	Accession number
hyp. protein 19/522	hypothetical protein 167 / 65.7	84%; Mvan_1510, <i>Mycobacterium vanbaalenii</i> PYR-1 84%; nfa8940, <i>Nocardia farcinica</i> IFM10152 82%; ROP_62580, <i>Rhodococcus opacus</i> B4 75%; Gbro_1725, <i>Gordonia bronchialis</i> DSM43247	YP_952348 YP_117103 YP_002783450 YP_003272880
TetR 1143/529	TetR family transcriptional regulator protein 204 / 68.5	36%; Noca_0123, <i>Nocardioides</i> sp. JS614 33%; MAB_3596c, <i>Mycobacterium abscessus</i> 30%; RHA1_ro06321, <i>Rhodococcus jostii</i> RHA1 29%; Mvan_1745, <i>Mycobacterium vanbaalenii</i> PYR-1	YP_921355 YP_001704325 YP_706256 YP_952574
ChoOx-2 1375/3150	Cholesterol oxidase, FAD dependent oxidoreductase 591 / 67.7	74%; ROP_62610, <i>Rhodococcus opacus</i> B4 74%; RHA1_ro06201, <i>Rhodococcus jostii</i> RHA1 71%; MSMEG_1604, <i>Mycobacterium smegmatis</i> mc2 155 65%; Gbro_1728, <i>Gordonia bronchialis</i> DSM43247	YP_002783453 YP_706136 YP_885983 YP_003272883
supA 3147/3920	supA, yrbEA 257 / 64.3	69%; ROP_46930, <i>Rhodococcus opacus</i> B4 61%; Mvan_5204, <i>Mycobacterium vanbaalenii</i> PYR-1 59%; RHA1_ro04696, <i>Rhodococcus jostii</i> RHA1 58%; Gbro_3952, <i>Gordonia bronchialis</i> DSM43247	YP_002781885 YP_955981 YP_704640 YP_003275019
supB 3954/4817	supB, yrbEB 287 / 67.7	69%; ROP_46920, <i>Rhodococcus opacus</i> B4 59%; RHA1_ro04697, <i>Rhodococcus jostii</i> RHA1	YP_002781884 YP_704641

		57%; Gbro_3951, <i>Gordonia bronchialis</i> DSM43247 56%; Mvan_5203, <i>Mycobacterium vanbaalenii</i> PYR-1	YP_003275018 YP_955980
mceA 4814/5980	mceA 388 / 67.4	44%; ROP_46910, <i>Rhodococcus opacus</i> B4 33%; RHA1_ro04698, <i>Rhodococcus jostii</i> RHA1 33%; Gbro_3950, <i>Gordonia bronchialis</i> DSM43247 32%; Mvan_5202, <i>Mycobacterium vanbaalenii</i> PYR-1	YP_002781883 YP_704642 YP_003275017 YP_955979
mceB 5977/7014	mceB 345 / 66.4	31%; Mvan_5201, <i>Mycobacterium vanbaalenii</i> PYR-1 50%; ROP_46900, <i>Rhodococcus opacus</i> B4 39%; RHA1_ro04699, <i>Rhodococcus jostii</i> RHA1 37%; Gbro_3949, <i>Gordonia bronchialis</i> DSM43247	YP_955978 YP_002781882 YP_704643 YP_003275016
mceC 7011/8003	mceC 330 / 68.5	46%; ROP_46890, <i>Rhodococcus opacus</i> B4 33%; RHA1_ro04700, <i>Rhodococcus jostii</i> RHA1 32%; Mvan_5200, <i>Mycobacterium vanbaalenii</i> PYR-1 31%; Gbro_3948, <i>Gordonia bronchialis</i> DSM43247	YP_002781881 YP_704644 YP_955977 YP_003275015
mceD 8000/9121	mceD 373 / 68.2	47%; ROP_46880, <i>Rhodococcus opacus</i> B4 41%; Mvan_5199, <i>Mycobacterium vanbaalenii</i> PYR-1 40%; RHA1_ro04701, <i>Rhodococcus jostii</i> RHA1 35%; Gbro_3947, <i>Gordonia bronchialis</i> DSM43247	YP_002781880 YP_955976 YP_704645 YP_003275014
mceE 9121/10221	mceE 366 / 66.6	52%; RHA1_ro02748, <i>Rhodococcus jostii</i> RHA1 48%; ROP_46870, <i>Rhodococcus opacus</i> B4 34%; Gbro_3946, <i>Gordonia bronchialis</i> DSM43247 32%; Mvan_5198, <i>Mycobacterium vanbaalenii</i> PYR-1	YP_702711 YP_002781879 YP_003275013 YP_955975
mceF 10218/11630	mceF 470 / 69.9	49%; RHA1_ro02747, <i>Rhodococcus jostii</i> RHA1 44%; ROP_46860, <i>Rhodococcus opacus</i> B4	YP_702710 YP_002781878

		34%; Mvan_5197, <i>Mycobacterium vanbaalenii</i> PYR-1	YP_955974
		30%; Gbro_3945, <i>Gordonia bronchialis</i> DSM43247	YP_003275012
hyp. protein- 12306/12785	hyp. protein 159 / 68.8	46%; ROP_46840, <i>Rhodococcus opacus</i> B4	YP_002781876
		43%; RHA1_ro02745, <i>Rhodococcus jostii</i> RHA1	YP_702708
		27%; Mvan_5195, <i>Mycobacterium vanbaalenii</i> PYR-1	YP_955972
		26%; Gbro_3943, <i>Gordonia bronchialis</i> DSM43247	YP_003275010

TABLE S3. Primers and conditions applied for RT-PCR and mutagenesis studies^a

Primer pairs	Sequence	Application
ChoOx-1-Fw ChoOx-1-Rv	CAGAGCTGACGCCCCATTAC TCGGCCTCACCCCTTGTTT	Amplification of a 1264 bp fragment from the gene <i>choox-1</i> for RT-PCR study
ChoOx-2-Fw ChoOx-2-Rv	GATCTGCGGGCGGTTTCCTCT ATCACCCCGGTTCGACGCATTCT	Amplification of a 1280 bp fragment from the gene <i>choox-2</i> for RT-PCR study
CH118 CH119	<u>GCTCTAGAGCAGCGTCAGCGAGTGGTCC</u> <u>GGAATTCCAGGAACAGCAGCACGAAGC</u>	<i>choox-2</i> gene amplification product of 3442 bp including <i>Xba</i> I (CH118) and <i>Eco</i> RI (CH119) restriction sites used for apramycin cassette introduction for mutagenesis study
Apra-Fw Apra-Rv	GCAGAGCAGGATTCCCGTTGAG ATTGCACTCCACCGCTGATGAC	Amplification of a 436 bp fragment within the apramycin (<i>apra</i>) resistance gene (originated from pIJ773) for mutagenesis study
Apra-Fw CH11	GCAGAGCAGGATTCCCGTTGAG GGAGTCGACCGCTACCAACG	Amplification of a 3300 bp fragment including a part of the <i>apra</i> resistance gene and surrounding genes for mutagenesis study
Apra-Fw CH13	GCAGAGCAGGATTCCCGTTGAG GATCTGCGGGCGGTTTCCTCT	Amplification of a 2445 bp fragment including a part of the <i>apra</i> resistance gene and surrounding genes for mutagenesis study
CH11 CH14	GGAGTCGACCGCTACCAACG ATCACCCCGGTTCGACGCATTCT	Amplification of a 3510 bp fragment ^b including the complete <i>apra</i> resistance gene and surrounding genes for mutagenesis study
CH13 CH15a	GATCTGCGGGCGGTTTCCTCT AGATCCCCGCCTTCAGTT	Amplification of a 3560 bp fragment ^b including the complete <i>apra</i> resistance gene and surrounding genes for mutagenesis study

^a PCR conditions: annealing temp., 55°C; elongation temp., 72°C (< 3 kb) or 68°C (> 3kb); PCR cycles applied, 30.

^b The expected fragment length is for the mutant strain only; for the wild type strain the expected fragment is 1385 bp shorter due to exclusion of the apramycin gene.