

Supplement

Supplemental Table S1. Prevalence of *B. afzelii* and *B. garinii* RMS genes in patient isolates.

RMS genes	#1023	#1061	#1176	#1192	#1193	#1205	#1207	#1217	#1225	#1230	#1231	#1232	#1235	#1237	#1035	#1039	#1007	#1033	#1053	#1179	#1224	#1229	#1234	#1236
<i>bafPKo_I0015</i>																								
<i>bafPKo_Q0015</i>																								
<i>bafPKo_AA003</i>																								
<i>bafPKo_H0010</i>																								
	#1022	#1038	#1052	#1180	#1214	#1187	#1233	#1004	#1005	#1006	#1034	#1054	#1056	#1059	#1177	#1178	#1181	#1183	#1185	#1186	#1188	#1190	#1191	#1195
<i>bafPKo_I0015</i>																								
<i>bafPKo_Q0015</i>																								
<i>bafPKo_AA003</i>																								
<i>bafPKo_H0010</i>																								
	#1199	#1200	#1202	#1215	#1218	#1226	#1240	#1241	#1057	#1203	#1216	#1219	#1238	#1204	#1055	#1058	#1175	#1182	#1189	#1201	#1206	#1210	#1220	#1239
<i>bafPKo_I0015</i>																								
<i>bafPKo_Q0015</i>																								
<i>bafPKo_AA003</i>																								
<i>bafPKo_H0010</i>																								
	#1035	#1039	#1195	#1226																				
<i>bgaPBr_F0006</i>																								
<i>bgaPBr_H0006</i>																								
<i>bgaPBr_K0029</i>																								
<i>bgaFar04_F0013</i>																								

#isolate ID number. In dark grey are marked the positive RMS genes for each patient isolate. We observed for *B. afzelii* 16 isolates positive for one RMS gene, 15 for two RMS genes, 31 for three RMS genes, and 10 for four RMS genes. For *B. garinii*, we observed 2 isolates positive for two RMS genes and 3 isolates positive for three RMS genes.

Supplemental Table S2. Primers designed and used in this study

Primer	Sequence (5' to 3')	Genome location	Amplicon size
Real-time PCR primers for the detection of the RMS genes			
BafAA003_fwd_qPCR	AACCTAGCATTGCCGATTGTACAGA	lp28-7	81 bp
BafAA003_rev_qPCR	CCCTTAGAAGAATGTTGCTTGGA		
BafI0015_fwd_qPCR	AACCTGACTTTATGTTGTGTCTCA	lp28-4	97 bp
BafI0015_rev_qPCR	ATGACCCGAACCACAAGAATTATCA		
BafH0010_fwd_qPCR	ATGCTGAGTAATTTCTTGCCTGAT	lp28-3	115 bp
BafH0010_rev_qPCR	AGAGATACTTTATGAGAGACCCGAC		
BafQ0015_fwd_qPCR	AGAATGCAGTCCACCTAATTACTTT	lp32-10	120 bp

BafQ0015_rev_qPCR	TTTACGCCGACACTATATTCATTGA		
Bg_F0006_qPCR_fwd	GAGATGCGACACTACATATGGAGCT	lp28-9	128 bp
Bg_F0006_qPCR_rev	TTCTTGACTTTGCAACTGGAAGTGG		
Bg_F0013_qPCR_fwd	AACTATTAAAGGCGGTGAAGAAGGA	lp28-4	87 bp
Bg_F0013_qPCR_rev	TACTCATGCCTCTGAATGTGATGTT		
Bg_H0006_qPCR_fwd	TCTAGCTCGTACCATACTTTCTCTGTT	lp28-3	80 bp
Bg_H0006_qPCR_rev	ATTGATAATTCTTGTTGGGTCAGGGC		
Bg_K0029_qPCR_fwd	TCACCAGTATTTCTTCAAGTTCAC	lp36	83 bp
Bg_K0029_qPCR_rev	AGGATTATTTGCAGAAGACAAGGTT		
PCR primers for the amplification of complete RMS genes*			
BafAA003_fwd_KpnI	TTCAGGTACCATCTATTATGCAATCCAA	lp28-7	3269 bp
BafAA003_rev_BamHI	TTCAGGATCCATGCTATGATGAAAACATA		
BafI0015_fwd_BamHI	TTCAGGATCCTAATGAAAACATAATGATGTTG	lp28-4	3861 bp
BafI0015_rev_KpnI	TTCAGGTACCTTCCACCCTATTATACTAA		
BafH0010_Fwd_KpnI	GGGCAGGTACCTTATTTATGATAAAAAATT	lp28-3	3868 bp
BafH0010_Rev_BamHI	GCATGGATCCTAATGAAAACATAATGATATCG		
BafQ0015_fwd_BamHI	GGCAGGATCCTTATAAAGATGAACAGTAATG	lp32-10	3205 bp
BafQ0015_rev_KpnI	GGCTGGTACCTTATTCATTGAATTCTTTAAG		
Bg_F0006_SalI_fwd	GCTAGTCGACGGGAAAATATGATGTTATTA	lp28-9	3177 bp
Bg_F0006_KpnI_rev	GCTAGGTACCTGTGCTAAATACCAAATTC		
Bg_F0013_SalI_fwd	GGCTGGTCGACTAGTTTAGTTGATATTAA	lp28-4	3157 bp
Bg_F0013_KpnI_rev	GCTAGGTACCTAATTGTATAGCTAAGCTAC		
Bg_H0006_SalI_fwd	GGCTAGTCGACTGTAAGCATAAATAATGAA	lp28-3	3861 bp
Bg_H0006_KpnI_rev	GCTAGGTACCGTTAAGCTTAATTAAGCT		
Bg_K0029_SalI_fwd	GGCTAGTCGACTGTAAGCATAAATAATGAA	lp36	3855 bp
Bg_K0029_KpnI_rev	GCTAGGTACCAAGCTTAGTTAAGTTTGTTT		

*Restriction enzyme recognition site is in bold and underlined.

bbe02 (AE000785.1)	MTINDIVKTNPNISLYKQSKDFIKKENINKLKDFILIRKNLFSIDNNEANIESLLKYIFEELNYSVEQQKAGQIEGVESRVDILLFENDKDKASFNKMLKAKGQNEPIPIEDIL	120
bafPKo_H0010 (CP002944.1)	..KN....D.....E.E.....K..S.....RF.....G.....D.....VD.....E.....A...	120
bgaPBr_K0029 (CP001302.1)	..N.N....D..M....E.....S.....S.....V.....V.....RVD.....E.....LA...	120
bbe02 (AE000785.1)	IIAEVKRPTFSFDAKDKLKESEDQLYRYLNQYQKHVILSGKRVRLYDKSKVLYGKRYIEFNFXKIEEKEEYKEQEWFLFIYLIRKERYLKTNSVIEVEKEIAKEKEIIQKTLKEI	240
bafPKo_H0010 (CP002944.1)	L.V....S...NT...V..A.....M.....D.S.V.....I.....S.....R..	240
bgaPBr_K0029 (CP001302.1)	L.....S...NT.N.V.DA.....I.....D.S.....I.....I.....S.....R..	240
bbe02 (AE000785.1)	LYERPDDSIIVFKIAKNIIDYKFKLSGKEITQHILASILEESIIFILIRIFFIAYIEDNDIFKKILQENKLYRSSISFRYFFYDENTKKKLEYKIIITIFNLLDKGSDAIKFPVFNGLFSE	360
bafPKo_H0010 (CP002944.1)	..K...VI.....V.D.....E.....E.....N..G.....I.....A...	360
bgaPBr_K0029 (CP001302.1)	V.N.....E.....E.....N..G.....I.....A...	360
bbe02 (AE000785.1)	DKVKYLNNEGLLSISEIEEILVKMLFFEEKNIKDEKFKVYSRLDPKSPGELYETILLEYDLRIADTTVHRIIEDGVYLIRTEEELENKKVKNKIATYLGKNILYTSRSLDRKKSAYYTPDD	480
bafPKo_H0010 (CP002944.1)	S.....L.....I.....E..K.....V.....Q...V...Y.....	480
bgaPBr_K0029 (CP001302.1)	S.....L.....K...E..K.....V...F.....V...Y.....	480
bbe02 (AE000785.1)	LIDFMVISSIEEQLKTKSPLDIKIIDNSCGSGHFLISCLDYLTEKVVYELDKFEDVKELDEEYRVIIIESEEYDQDSISKELVLKRMMLKXCXYGVNDIPISVEITMLSLWINTFIFG	600
bafPKo_H0010 (CP002944.1)	K...A.FK.....K.....	600
bgaPBr_K0029 (CP001302.1)	R.....K...A.LK.....K.....	600
bbe02 (AE000785.1)	TPLSFIEHHIKAGNALLGYTKDEFFDIVKKNKFSGFSFLFKRIKEIITILEDIYQKIKGINDITKEDIEKSKKIYKEYEESKDIDNLRIFISLKIYLSLFDKSLNMEFSDIASVISLIE	720
bafPKo_H0010 (CP002944.1)	V.....T.....E.....T.....S.....S.....E.TEY.....KI.....TAI....	720
bgaPBr_K0029 (CP001302.1)	V.....T.N.....T.....N.....N.....T.....I.....	720
bbe02 (AE000785.1)	NILGNKTSSEDEKIEKIRKLSSYYKFFHYGIEFPDIQEGFDIVIGNPPWEKTKFNETEFFSKHPIYRKLGIKEQNIIEQILSKDNHPLSIEYNEEKNSIIAINNIYKFDKCFSTGG	840
bafPKo_H0010 (CP002944.1)	N.....L.....E.....S.....N.....K.....N.....G.....T.....D.S...	840
bgaPBr_K0029 (CP001302.1)	RA..K.....E.....I..D.S.....S.....S.....K..E.....G.....VNT.....S...D....	840
bbe02 (AE000785.1)	DPNLFYFVTFNLKLIKEGNLTLYLPSAIWNNESSRILRKHIFARYKNIYIYQFENKRFKDVHSSFKFAIFQLSNIKESTSSFKAFMIQSSDMILKEITRDLKSKDDAYKGIENI	960
bafPKo_H0010 (CP002944.1)	A.....PG.....T..SL.S.Y...E.....MN...S.....A.....N...Q...K...	960
bgaPBr_K0029 (CP001302.1)	PG.....T.YSL.S.Y...K.....NN...S.....E..D.R...V.....Q...N.....HG.....VS....N..NP.....	960
bbe02 (AE000785.1)	NQIKKLSPIQESIIEFKDNEEFTLINKMFSKFSALGEGYIDFKKGLDPSIHNRKSLKKECNKNLIFLYSGANIHQFNSRFFEDKDAKESKLLWIDKEDLEKVLKDNQYQTERVFYRA	1080
bafPKo_H0010 (CP002944.1)	D.....I.N...N.R...NS..V.R.E...GV..NLI--IH.A.Y..Y...FV.....N.....FF.....I.A..DRH..K.....	1078
bgaPBr_K0029 (CP001302.1)	D.....V...N.K..S.....Q..T.SKE...GG..HLI--KY.A.YE.Y..E.F.....N.....FF.....K.I..AEEDRH.....	1078
bbe02 (AE000785.1)	IASNTNERTMISTLSPGNICYVNSIYINDEKTPISLYKKLFIISIFNSFVDFLLRRFVDSNVLSKCLYQCPMPQPEEKEILSNPLYLNIAKNTSLIIARNDPENFKYLLYLEYFKFDKE	1200
bafPKo_H0010 (CP002944.1)	..R..D.....K.....M...Y...I.....LA.....KD...A...T..I.....LG.....H...S...	1198
bgaPBr_K0029 (CP001302.1)	K.....M...Y..I.....LA.....N.....C.....S.....D.....H.E.S...	1198
bbe02 (AE000785.1)	KVNKILKLDKEDEFFKEKENENNFIASLYSLAKEDFITLLGDFKALKKNGKGEDYISSLIKGYDNYLLNNKIF--YHK*	1278
bafPKo_H0010 (CP002944.1)	E.D...N..TK.....V.....T.....MV..N...V....-.....L.....E...RI..LNSTIQS.	1277
bgaPBr_K0029 (CP001302.1)	E....FN..A.....V.....T...RV..N..EV....-..V.....E...RI..LN-----,	1272

Figure S1. Alignment, obtained with Geneious Prime® 2023.1.1, of the *bbe02* gene of *Bbss* in comparison with *bafPKo_H0010* of *B. afzelii* and *bgaPBr_K0029* of *B. garinii*. Blast analysis identified a 93.6 % similarity between these three genes. Showing that this gene is conserved among Lyme borreliosis spirochetes.