

			20		40		60
Ngo (YP_207382)	M - - K T - - S -	I - R Y A - L L A -	AA - - L T - A A T	P A L - A D - - I T	V Y N G Q H K E A A	Q A V A D A F T R A	45
Nme (YP_003082738)	M - - K T - - S -	I - R Y A - L L A -	AA - - L T - A A T	P A L - A D - - I T	V Y N G Q H K E A A	Q A V A D A F T R A	45
Hin (AAB32110)	M - - Q F - - K H	F - K L A - T L A -	AA - - L A - F S A	N S F - A D - - I T	V Y N G Q H K E A A	A A V A K A F E Q E	46
Sma (AAA26573)	M - - K L R I S S	L G P V A - L L A -	S S M M L A - F G A	Q A A S A D Q G I V	I Y N A Q H E N L V	K S W V D G F T K D	54
Cje (YP_002343633)	M - - K K - - - -	I F - F M - F L T -	A - - - V S - F - L	- G - A S - E - L N	I Y S A R H Y N A D	F E I I K K F E E K	41
Bpe (NP_880337)	M P S T K R - - S S	L I P L L R A L A L	A G - - V A T F S A	Q A L A S D E - V S	L Y T T R E P K L I	Q P L L D A F A K D	55
		80		100		120	
Ngo (YP_207382)	T G I K V K L N S A	K G D Q L A G Q I K	E E G S R S P A D V	F Y S E Q I P A L A	T L S A A N L L E P	L P A S T I N E T R	105
Nme (YP_003082738)	T G I K V K L N S A	K G D Q L A G Q I K	E E G S R S P A D V	F Y S E Q I P A L A	T L S A A N L L E P	L P A S T I N E T R	105
Hin (AAB32110)	T G I K V T L N S G	K S A Q L A G Q L K	E E G D K T P A D V	F Y T E Q T A T F A	D L S E A G L L A P	I S E Q T I K Q T A	106
Sma (AAA26573)	T G I K V T L R N G	G D S E L G N Q L V	Q E G S A S P A D V	F L T E N S P A M V	L V D N A K L F A P	L D A A T L A Q V E	114
Cje (YP_002343633)	T G I K V N H T Q A	K A S E L I K R L S	L E G S N S P A D I	F I T A D I S N L T	E A K N L G L L S P	V S S K Y L E E F I	101
Bpe (NP_880337)	S G I K V N T V F V	K D G - L L E R V R	A E G D K S P A D V	L M T V D I G N L I	D L V N G G V T Q K	I Q S Q T L D S V V	114
		140		160		180	
Ngo (YP_207382)	G K G V P V A A K K	D W V A L S G R S R	V V V Y D T R K L S	E K D L E K S V L N	Y A T P K W K N R I	G Y V P T S G A F L	165
Nme (YP_003082738)	G K G V P V A A K K	D W V A L S G R S R	V V V Y D T R K L S	E K D L E K S V L N	Y A T P K W K N R I	G Y A P T S G A F L	165
Hin (AAB32110)	Q K G V P L A P K K	D W V A L S G R S R	V V V Y D H T K L S	E K D M E K S V L D	Y A T P K W K G K I	G Y V S T S G A F L	166
Sma (AAA26573)	P Q Y R P - S H G R	- W I G I A A R S T	V F V Y N P A K L S	D A Q L P K S L L D	L A K P E W K G F A	A A S P S G A D F L	172
Cje (YP_002343633)	P A H L R - D K D K	E W F A I T K R A R	I I A Y N K N T N I	D I S K M K N Y E D	L A K A E F K G E I	V M R S A T A P Y S	160
Bpe (NP_880337)	P A N L R - G A E G	S W Y A L S L R D R	V L Y V E K D L K L	D - S - F R - Y G D	L A D P K W K G K V	C I R S G Q H P Y N	170
		200		220		240	
Ngo (YP_207382)	E Q I V A - I V K L	K G E A A A L K W L	K G L K E Y - G - K	P Y A K N S V A L Q	A V E N G E I D A A	L I N N Y Y - W H A	221
Nme (YP_003082738)	E Q V V A - I V K L	K G E A A A L K W L	K G L K E Y - G - K	P Y A K N S V A L Q	A V E N G E I D A A	L I N N Y Y - W H A	221
Hin (AAB32110)	E Q V V A - L S K M	K G D K V A L N W L	K G L K E N - G - K	L Y A K N S V A L Q	A V E N G E V P A A	L I N N Y Y - W Y N	222
Sma (AAA26573)	A I V S A - L L E L	K G E K A T L A W L	K A M K T N - F - T	A Y K G N S T V M K	A V N A G Q V D S G	V I Y H Y Y - P F V	228
Cje (YP_002343633)	K T L L A S I I A N	D G N K E A K A W A	K G V L E N L A T N	P K G G D R D Q A R	Q V F A G E A K F A	V M N T Y Y I G L L	220
Bpe (NP_880337)	T A L V A A M I A H	D G A E A T E K W L	G V K A N L A R K	A A G G D R D V A R	D I L G G I C D I G	L A N A Y V V G H M	230
		260		280		300	
Ngo (YP_207382)	F A R E K G V Q - N	V - H T R L N F V R	- H - R D P - - G -	A L V T Y S G A A V	L K S S Q N K D E A	K K F V A F L A G K	274
Nme (YP_003082738)	F A R E K G V Q - N	V - H T R L N F V R	- H - R D P - - G -	A L V T Y S G A A V	L K S S Q N K D E A	K K F V A F L A S K	274
Hin (AAB32110)	L A K E K G V E - N	L - K S R L Y F V R	- H - Q D P - - G -	A L V S Y S G A A V	L K A S K N Q A E A	Q K F V D F L A S K	275
Sma (AAA26573)	D G A K T G E N - S	N - N I K L Y Y F K	- H - Q D P - - G -	A F V S I S G G G V	L A S S K H Q Q Q A	Q A F I K W I T G K	281
Cje (YP_002343633)	K N S K N P K D V E	V - G N S L G I I F	P N - Q D N R - G -	T H I N I S G I A M	T K S S K N Q D A A	K K F M E F M L S P	276
Bpe (NP_880337)	K N A E P G T D A R	K W G D A I K V V R	P T F A T A K D G G	T H V N I S G A A V	A A H A P N K A N A	V K L L E Y L V S E	290
		320		340		360	
Ngo (YP_207382)	E G Q R A L T A V R	A - E Y P L N P H V	V S T F N L E P I A	K L E A P Q V S A T	T V S E - K E H A T	R L L E Q A G M - K	331
Nme (YP_003082738)	E G Q R A L T A V R	A - E Y P L N P H V	V S T F N L E P I A	K L E A P Q V S A T	T V S E - K E H A T	R L L E Q A G M - K	331
Hin (AAB32110)	K G Q E A L V A V R	A - E Y P L R A D V	V S P F N L E P Y E	K L E A P V V S A T	T A Q D - K E H A T	K L I E E A G L - K	332
Sma (AAA26573)	Q G Q E I L R T N N	A F E Y A V G V G A	A S N P K L V P L K	D L D A P K V D A A	Q L N S - K K - V V	E L M T E A G L - L	338
Cje (YP_002343633)	E I Q K I L T D S N	Y - E F P I R N D V	E L S Q T V K D F G	T F K E D Q I P V S	K I A E N I K E A V	K I Y D E V G F - R	334
Bpe (NP_880337)	P A Q T L Y A Q A N	Y - E Y P V R A G V	K L D A V V A S F G	P L K V D T L P V A	E I A K Y R K Q A S	E L V D K V G F D N	349

Figure S1. Multiple protein sequence alignment of *B. pertussis* FbpA with ferric iron binding protein sequences from representative Gram-negative bacteria. A solute-binding protein family 1 signature sequence [(LIVA) X_2 (LIVW)(GAIV)(KDQ) X (FY)(ETN) X (DEA) $X_{(0,1,OR\ 2)}$ (GND)(IVPY)K(VI) X (VLP)] is underlined in the *B. pertussis* FbpA protein. Iron atom contact residues in the *B. pertussis* holo-FbpA protein are boxed. Source species of FbpA proteins: Ngo, *Neisseria gonorrhoeae*; Nme, *Neisseria meningitidis*; Hin, *Haemophilus influenzae*; Sma, *Serratia marcescens*; Cje, *Campylobacter jejuni*; Bpe, *Bordetella pertussis*. Accession numbers are shown in parentheses.

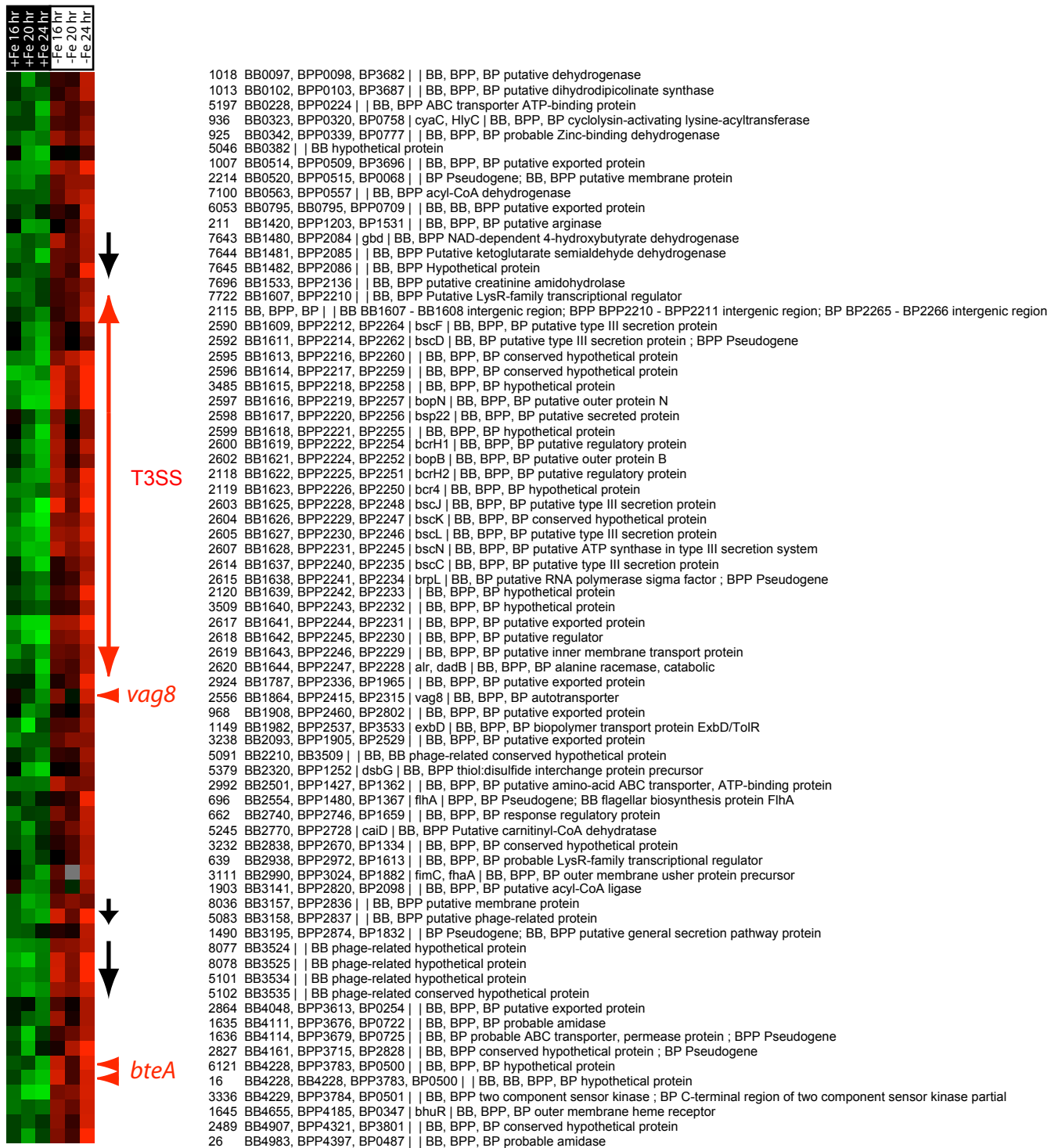


Figure S2. T3SS-related gene transcript abundance patterns in *B. bronchiseptica* RB50. T3SS-related gene transcript abundance patterns in *B. bronchiseptica* RB50 grown under iron replete and iron depleted conditions are shown. Rows correspond to array elements. Selected T3SS array elements are indicated on the right. Black arrows indicate other genes predicted to be cotranscribed. Some genes are represented by multiple array elements. Gray indicates missing data, and the elapsed time since the culture was started is indicated at the top.

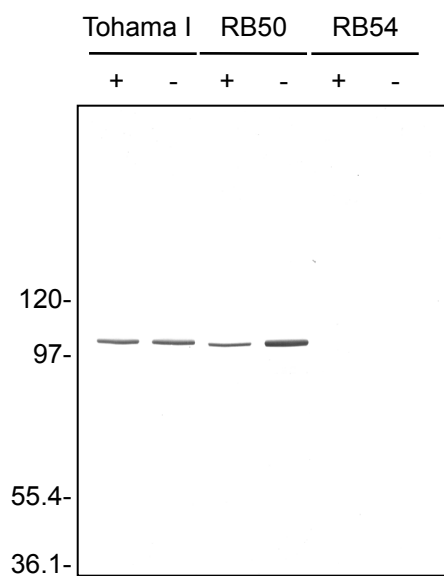


Figure S3. Vag8 production by *B. pertussis* Tohama I and *B. bronchiseptica* strains RB50 and Δ vagAS mutant RB54.

Total cell protein samples were prepared from bacteria harvested from iron-replete (+) and iron-depleted (-) SS medium and equivalent protein loads were analyzed by immunoblotting, employing a Vag8-specific monoclonal antibody. Sizes of molecular weight (MW) markers are shown in kDa.