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Supplementary Material

Metabolic analyses reveal common adaptations in two invasive *Haemophilus influenzae* strains

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Table S1: Oligonucleotide primers used in this study.

Target gene	Primer name	Primer sequence (5'-3')
<i>pfkA</i>	HI_QPO_pfkA_F	CAA TTT CTG ATG CGA CGA TAT ATT C
	HI_QPO_pflA_R	TCG ACC GTT TAC GTG ATA CAT
<i>zwf</i>	HI_QPO_zwf_F	GCC AGA ACC ATC ATA ATA GCC
	HI_QPO_zwf_R	GAA ACC GTT CAA AAC TTG CTC
<i>pykA</i>	HI_QPO_pykA_F	TTT GCT GCA TAC ATT GCA GAC AT
	HI_QPO_pykA_R	CAG TGG CAG CAAA TGG CTA G
<i>pflA</i>	HI_QPO_pflA_F	ATA CTG ATA GCG ATC ACG ATG TGC
	HI_QPO_pflA_R	CCC AAG GGT TTT CCA TTT ATG
<i>aceF</i>	HI_QPO_aceF_F	TTA TGT GAA GAC CGC AGT TAA AG
	HI_QPO_aceF_R	AAT TTA CTG AAA TCA ACT TTT GG
<i>ackA</i>	HI_QPO_ackA_F	GCG AAT ACC AAT TAA GTG AGC
	HI_QPO_ackA_R	CGT ATC GTT CAC GGT GGC GA
<i>ldhA</i>	HI_QPO_ldhA_F	TGA TAG TTT TCT GGC GTT GC
	HI_QPO_ldhA_R	TGA AAG GTT TTG GCA TGA ATA T
<i>dldD</i>	HI_QPO_dldD_F	AAG GTG TCT AAA CGA ACC GCA
	HI_QPO_dldD_R	CTA TGT TCG TCA AGT TGA TGA AG
<i>lldD</i>	HI_QPO_lllD_F	GCA AGA ATA GTT GGC ATT GAA AGT
	HI_QPO_lllD_R	GCG TAA TGT GAG TGA TTT GGA
<i>ndh</i>	HI_QPO_ndh_Fv2	CAG CCA AAT ACA CTT ATT ACC AAA GAT GG
	HI_QPO_ndh_Rv2	CCA CTA ATT GAT TGA TAC GAT TAA TTT C
<i>nqrB</i>	HI_QPO_nqrB_F	TGC CAA TGC AAC TTG GGG CTC
	HI_QPO_nqrB_R	ACT TCG TGA CCA CGA ACC AC
<i>cydA</i>	HI_QPO_cydA_Fv2	AGC GTT TTA ATA ATA AAC CGA AAC CAA GA
	HI_QPO_cydA_Rv2	CGT AGT GGT ATC CGT GCT TAT GAA TTA TTT A
<i>cydB</i>	HI_QPO_fdxG_Fv2	AAT CAA ATG GAA CGG TAA AAA CTG GAA C
	HI_QPO_fdxG_R	ACG GCA AAT AAA CGT CCT ACG CCT
<i>nrfA</i>	HI_QPO_nrfA_F	TTC AAA GTC AGG GTG TTG TGC
	HI_QPO_nrfA_R	TTG GGA TAA TGG TCA AAC CG
<i>frdA</i>	HI_QPO_frdA_F	CAG CAA ATA AAC CTT TGA TAC G
	HI_QPO_frdA_R	TGC TTA TGA AGG TGT AAA TCC A
<i>napA</i>	HI_QPO_napA_F	CGG AAA TGC ACC CAA TTT TGT G
	HI_QPO_napA_R	AAA CCG TGA TCG GCA AGT TCA AAA C
<i>torZ</i>	HI_QPO_torZ_Fv2	CAA CGT CGT GAT TTT CTG AAA AAA ACA
	HI_QPO_torZ_Rv2	GCA GTC ACT ACC GTT TTC ATT TCA GCT
<i>dmsA</i>	HI_QPO_dmsA_F	CGA ACC TGA TGA TCA AGA TTA TAT G
	HI_QPO_dmsA_R	AGT AAA CTG TGG TAG CCG TTG
<i>deoD</i>	HI_QPO_DeoD_F	CCA AGC ATA TTA CGA ACA TTC GT
	HI_QPO_DeoD_R	CCT GAA GGT GCA TTT GCT GAT
<i>gtp1</i>	HI_QPO_gtp1_F	CAT ATT GAA ACC GTT TGT ATC GC
	HI_QPO_gtp1_R	CCY GTA TCA ACT AAA TCA TCT AC
<i>gtp2</i>	HI_QPO_gtp2_F	AAT GCA AAA TTT GTC ACT GTA TT
	HI_QPO_gtp2_R	ACA AAG GTT AAA CCT AAA TCC CA
<i>guaA</i>	HI_QPO_guaA_F	CAT TAT CGA AGA TGC CGT TGC
	HI_QPO_guaA_R	TGC CGA TAG CAC GGT GTA AAA G
<i>guaB</i>	HI_QPO_guaB_F	CCC TTA CTT TTG ACG ATG TTC TAC T
	HI_QPO_guaB_R	GTA TCC ATT GCT GCT GAA AGC AT
<i>hpt</i>	HI_QPO_hpt_F	CYT TTA TGT TTA TGG CGG ATA T
	HI_QPO_hpt_R	TAT ATC GCC GTC CAA ATC TTT

<i>nadN</i>	HI_QPO_NadN_F HI_QPO_NadN_Rv2	GCG TCT ATT ACT CGT AAA ATT CCT AAG TGC TTT TTT ACG TTC ATC GCC AG
<i>OMLP pcp</i>	Hi_QPO_OMLP-pcpF Hi_QPO_OMLP-pcpR	CAA TTG GCG GTG GTC GTG GT TTA ATT ACA AGT TCA GCA CCG TTT ACT TGA C
<i>OMP26</i>	HI_QPO_OMP26F HI_QPO_OMP26R	C TGG CGA TCT GGG TGA TGT TGA AA AT CGC AAA AGT AAC CGC ACT TG
<i>OMPP2</i>	Hi_QPO_OMP2_F Hi_QPO_OMP2_R	TTC GTA TCT CCA GGT TTC CAA TAT GAA TTG TTT GTG AAG TTT ATG ATC TAC ACC GAA TA
<i>OMPP5</i>	Hi_QPO_OMP5_F Hi_QPO_OMP5_R	TGC TGG TTA CAC TGA CCG TAT TGG TT TTG CAG AGA TTG CGT CTG CTG C
<i>OMPP6</i>	Hi_QPO_OMP6_F Hi_QPO_OMP6_R	AGC AGC TGG CGT TGC ATT TAA ATA T CTG TTG CTG ATC TTC AAC AAC GTT ACA A
<i>gyrA</i>	HI_QPO_gyrA_F HI_QPO_gyrA_R	TTG GGC GTG CAT TAC CTG ACG TT CCC ACA ACA CGC GCT GAT TTT AC

Table S6 – Carbon substrates used by all three NTHi strains as determined using the Omnilog Phenotypic Microarray (Biolog). Data are shown in rel. absorbance values after normalization to the negative control well of the respective PM plate. The data are sorted from highest to lowest utilization rate for Hi2019.

	Hi2019	R2866	C188
Dihydroxyacetone	157	132	119
L-Lyxose	142	146	123
L-Lactic acid	132	153	123
D-Ribose	118	120	91
α -Hydroxybutyric acid	118	146	108
5-Keto-D-Gluconic acid	114	58	71
L-Arabinose	102	102	85
D-Xylose	98	101	79
D-Arabinose	98	60	68
Oxalomalic acid	96	91	116
Dihydroxyfumaric acid	87	127	118
2-Deoxy-D-Ribose	84	41	38
Glycolic acid	76	129	99
Palatinose	60	38	51
Uridine	58	119	124
D-Tagatose	53	19	33
Inosine	38	82	116
Pyruvic acid	35	86	71
D-Psicose	30	37	12
L-Rhamnose	27	38	40
L-Ornithine	19	16	46
Adenosine	18	67	85
Glucuronamide	17	26	9
D-Mannose	14	31	19
D-Glucose-6-Phosphate	12	50	53
D-Raffinose	12	1	2
α -D-Glucose	10	57	75
L-Malic acid	10	5	5
Gly-Pro	9	16	1
L-Fucose	9	11	22
Dulcitol	8	3	2
Thymidine	8	108	103
2'-Deoxyadenosine	8	46	35
Gentiobiose	7	4	4
Acetoacetic acid	6	6	22
D-Galacturonic acid	6	11	2
D-Galactose	2	9	34
α -Ketobutyric acid	2	37	21

Figure S1 Genomic comparison of *H. influenzae* strains R2866 and C188. **Panel A:** Mauve alignment of *H. influenzae* R2866, C188 and Hi2019 genomes to the HiRdKW20 reference genome. Contigs of the C188 genome were ordered using the R2866 genome as the reference before further alignments were carried out. **Panel B:** Comparison of the functional categories of unique genes found in strains C188 and R2866.

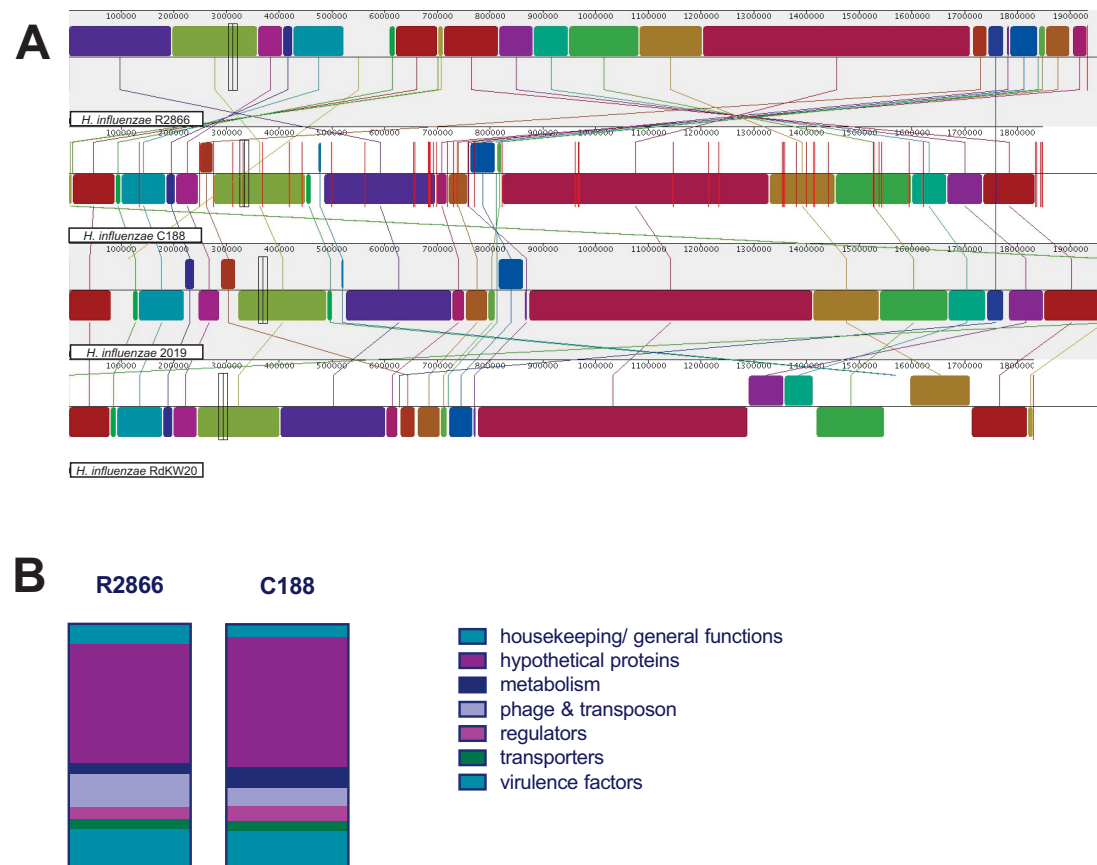
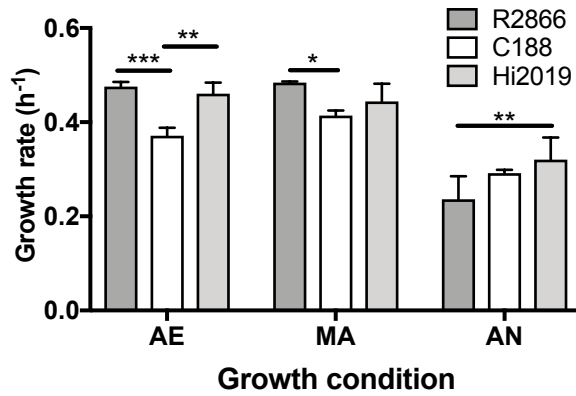


Figure S2 Comparison of growth rates in strains R2866, C188 and Hi2019 in response to changing oxygen tensions for Aerobic (AE) Microaerobic (MA) or Anaerobic (AN) growth. Adjusted p-values 2-way ANOVA: *** p=0.0007, ** p=0.0049-0.0029, * p=0.0184.

A Growth rates



B Invasive strain settling test

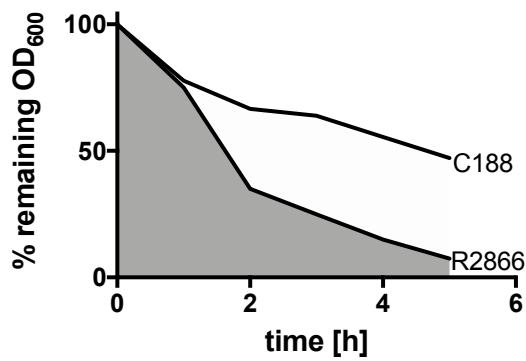
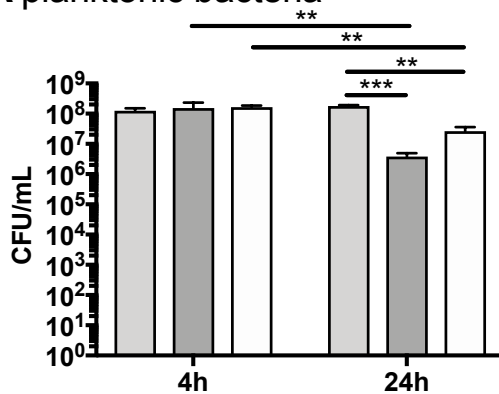
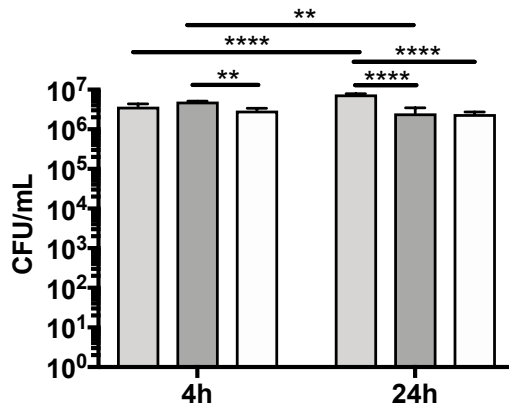


Figure S3 Co-infection assays showing populations of planktonic (A), total adherent (B) and intracellular (C) *H. influenzae* for NTHi strains 2019, C188 and R2866. The assays used the 16HBE14 cell line (bronchial epithelial cells) and at the 24h data point show reduced survival of the two invasive isolates under all conditions, including planktonic growth. Strain 2019 was used as a reference strain. Data were analysed using 2-Way ANOVA, adjusted p-values are Panel A: ** - p=0.0018-0.006, *** p=0.0008; Panel B: ** p=0.0062-0.0013, **** p<0.0001; Panel C: **** p<0.0001. Comparisons returning non-significant values are not shown. Data were compared both within the same timepoint (Hi2019, C188 R2866 after 4 or 24 hours) and for each strain between timepoints (e.g. Hi2019 after 4h vs after 24h).

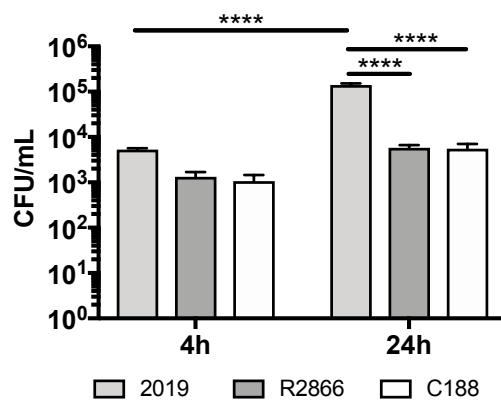
A planktonic bacteria



B total adherent bacteria



C total internalized bacteria



2019
 R2866
 C188