

Establishment of a Multilocus Sequence Typing Scheme for *Pasteurella canis* Using Isolates from Infected Humans and Diseased Companion Animals

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Supplementary File

Multilocus sequence typing analysis and population biology of *Pasteurella canis* strains isolated from infected humans and diseased companion animals: A first investigation

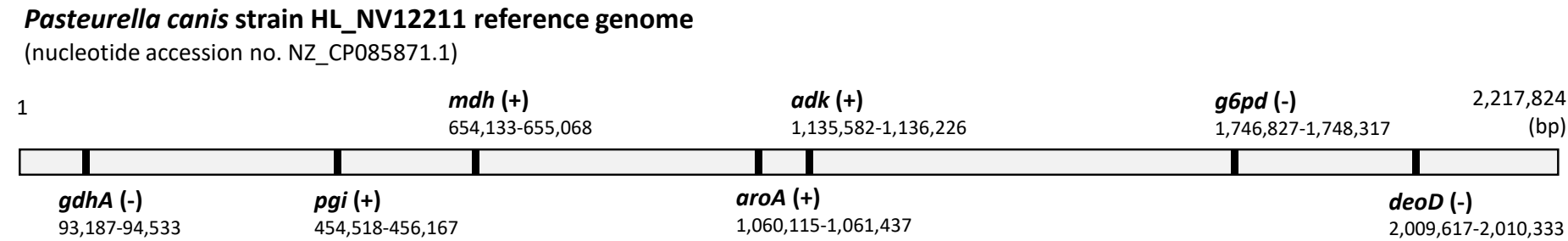
Supplementary Fig. 1. Seven house-keeping gene locations in *Pasteurella canis* strain HL_NV12211 reference genome.

Supplementary Table 1. PCR assays to determine genotypic traits of *Pasteurella canis* strains

Supplementary Table 2. Basic and development results of a multilocus sequence typing scheme for *Pasteurella canis*

Supplementary Fig. 2. Sequenced fragments and nucleotide substitutions in seven house-keeping genes for multilocus sequence typing using consensus sequences in *Pasteurella canis* strain HL_NV12211.

Supplementary Fig. 1. Seven house-keeping gene locations in *Pasteurella canis* strain HL_NV12211 reference genome.



We applied the graphic display of reference genome on National Center for Biotechnology Information site.
(+) or (-) indicate forward or reverse directions. Numerals show nucleotide start and end positions in the reference genome.
This figure means 7 house-keeping gene locations covering the whole area of reference genome.

Supplementary Table 1. PCR assays to determine genotypic traits of *Pasteurella canis* strains

Target gene (product)	Primer name*	Direction	Sequence (5'→3')	Length (mer)	T _m (°C) [†]	Annealing temp. (°C) [‡]	Amplicon size (bp)
<i>adk</i> (adenylate cyclase)	Pc_adk_F	Forward	CGGGRAAAGGAACACAAGC	19	52	57	550
	Pc_adk_R	Reverse	GAAGTATTGYGTATGCCCYGCT	22	56		
<i>aroA</i> (3-phosphoshikimate 1-carboxyvinyl transferase)	Pc_aroA_F	Forward	CTGCTCTTGACACAAGGTAAAAC	22	54	51	481
	Pc_aroA_R	Reverse	GCTTTTGCCATTGGAGAGACC	21	54		
<i>deoD</i> (purine nucleoside phosphorylase)	Pc_deoD_F	Forward	ATGCCAGGTGATCCACTACG	20	54	51	524
	Pc_deoD_R	Reverse	AATTCTGCTGCGACACCATAG	21	55		
<i>gdhA</i> (glutamate dehydrogenase)	Pc_gdhA_F	Forward	GCATGGACAGATGAYCAAGGTC	22	55	55	528
	Pc_gdhA_R	Reverse	ACCTGAGCCTGAAACTGCAAC	21	55		
<i>g6pd</i> (glucose-6-phosphate 1-dehydrogenase)	Pc_zwf_F [§]	Forward	TGAAGTAGCGAAAGTACTGCACTGC	25	60	51	677
	Pc_zwf_R	Reverse	TTCATTAAGCCACTCGGCTTACGC	24	60		
<i>mdh</i> (malate dehydrogenase)	Pc_mdh_F	Forward	AGTTGCAGTTCTAGGTGCCG	20	55	55	679
	Pc_mdh_R	Reverse	TAAAGTTGCTGAACCAACCGC	20	53		
<i>pgi</i> (phosphoglucose isomerase)	Pc_pgi_F	Forward	GTGATGCCAGAAGTGAACGC	20	54	57	704
	Pc_pgi_R	Reverse	TGGAAATACGCAGCAAAACGG	21	55		
<i>ompA</i> (outer membrane)	ompA-outer_F	Forward	AGAAATTCAGGCAAATTCAGCC	23	55	60	1348
	ompA-outer_R	Reverse	GCGACTAAGATCAATACAATCCAC	24	54		

protein A)

*The same primers were used for both PCR amplification and direct sequencing.

†Melting temp. (T_m) was calculated using nearest neighbor method.

‡To amplify seven house-keeping genes for multilocus sequence typing, we conducted 35 cycles consisting of denaturation at 98°C for 10 sec, annealing at each temp. for 15 sec, and extension at 72°C for 1 min.

To amplify *ompA*, we conducted 30 cycles consisting of denaturation at 94°C for 1 min, annealing at 60°C for 30 sec, and extension at 72°C for 1 min.

§When the single amplicon cannot be obtained using “Pc_zwf_F”, the alternative forward primer “alt_Pc_zwf_F” (CAGGAGCTGAGTCGTTAGGC; 20mer) may be used with “Pc_zwf_R”. Annealing temp. is 54°C, and alternative amplicon size is 829 bp.

Supplementary Table 2. Basic and development results of a multilocus sequence typing scheme for *Pasteurella canis*

Locus	Length of sequenced fragment (bp)	No. of alleles	No. of variable sites	Percentage of variable sites	dN/dS ratio*
<i>adk</i>	424	16	15	3.5	0.25
<i>aroA</i>	451	13	16	3.5	1.29
<i>deoD</i>	483	15	12	2.5	0.00
<i>gdhA</i>	439	18	39	8.9	0.22
<i>g6pd</i>	429	22	24	5.6	0.04
<i>mdh</i>	419	19	17	4.1	0.21
<i>pgi</i>	440	18	18	4.1	0.29

*dN/dS ratio means the ratio of nonsynonymous to synonymous polymorphisms for each locus.

Supplementary Fig. 2. Sequenced fragments and nucleotide substitutions in seven house-keeping genes for multilocus sequence typing using consensus sequences in *Pasteurella canis* strain HL_NV12211.

>HL_NV12211_adi_645 bp

ATGAAAATTATCCTATTAGGTGCACCAGGTGCGGGAAAAGGAACACAAGCACAATTTATTATGAATAAA**TTTAATATCCC**
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>HL_NV12211_aroA_1323 bp

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>HL_NV12211_*deoD*_717 bp

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>HL_NV12211_gdhA_1347 bp

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>HL_NV12211_mdh_936 bp

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>HL_NV12211_pgi_1650 bp

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Bold and black letters show sequenced fragments. In the fragments, gray shading indicates nucleotide substitutions. Out of the fragments, underlines show forward and reverse primer sequences.