

**Characterization of autochthonal *Hafnia* spp. strains isolated from Spanish soft raw
ewe's milk PDO cheeses to be used as adjunct culture**

Almudena V. Merchán^{1,2}, Santiago Ruiz-Moyano^{1,2,*}, María Vázquez Hernández^{1,2}, Alberto
Martín^{1,2}, María Jesús Lorenzo³, María José Benito^{1,2}

SUPPLEMENTAL MATERIAL

This article contains two supplementary Figures and five supplementary Tables

Figure S1. Cadaverine production of *Hafnia* spp. strains. Error bar reflect statistical significance difference calculated using Tukey's HSD test ($p \leq 0.05$).

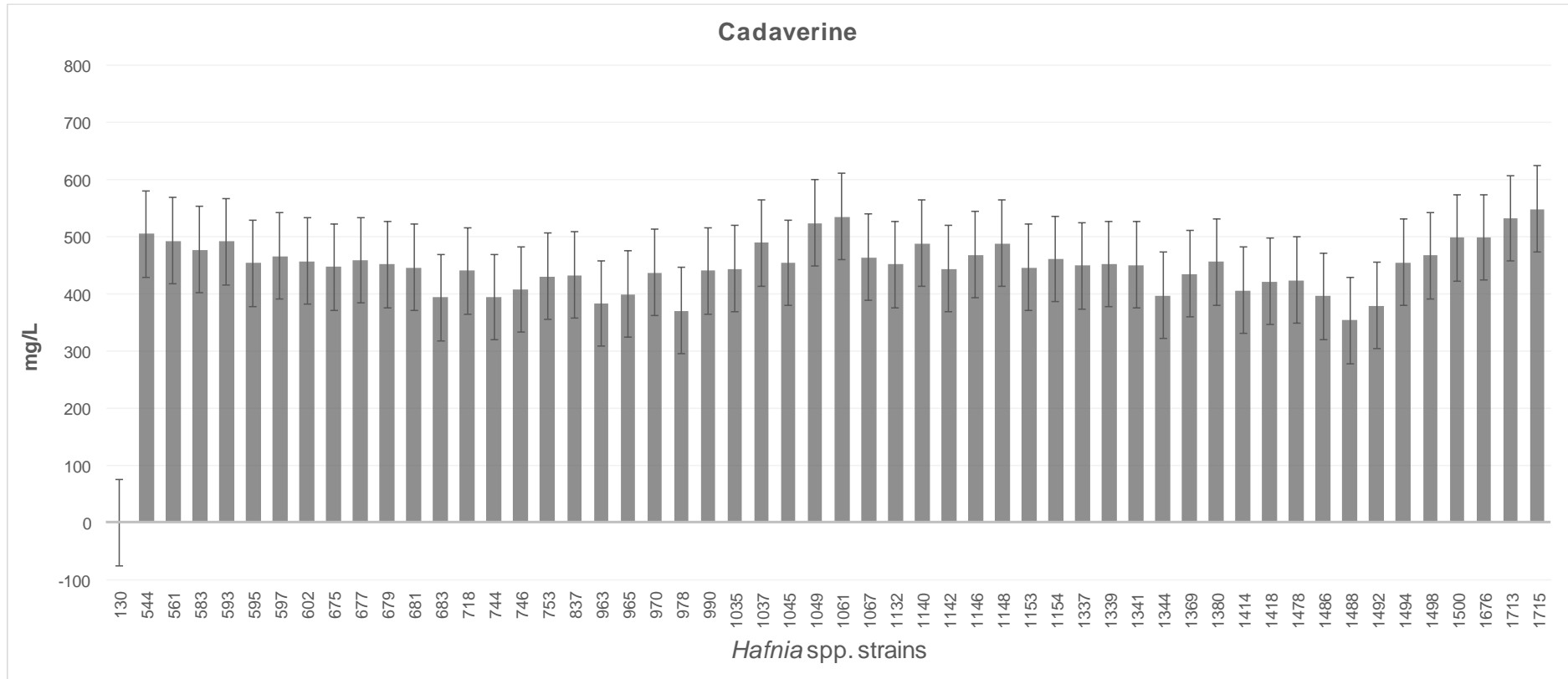


Figure S2. Putrescine production of *Hafnia* spp. strains. Error bar reflect statistical significance difference calculated using Tukey's HSD test ($p \leq 0.05$).

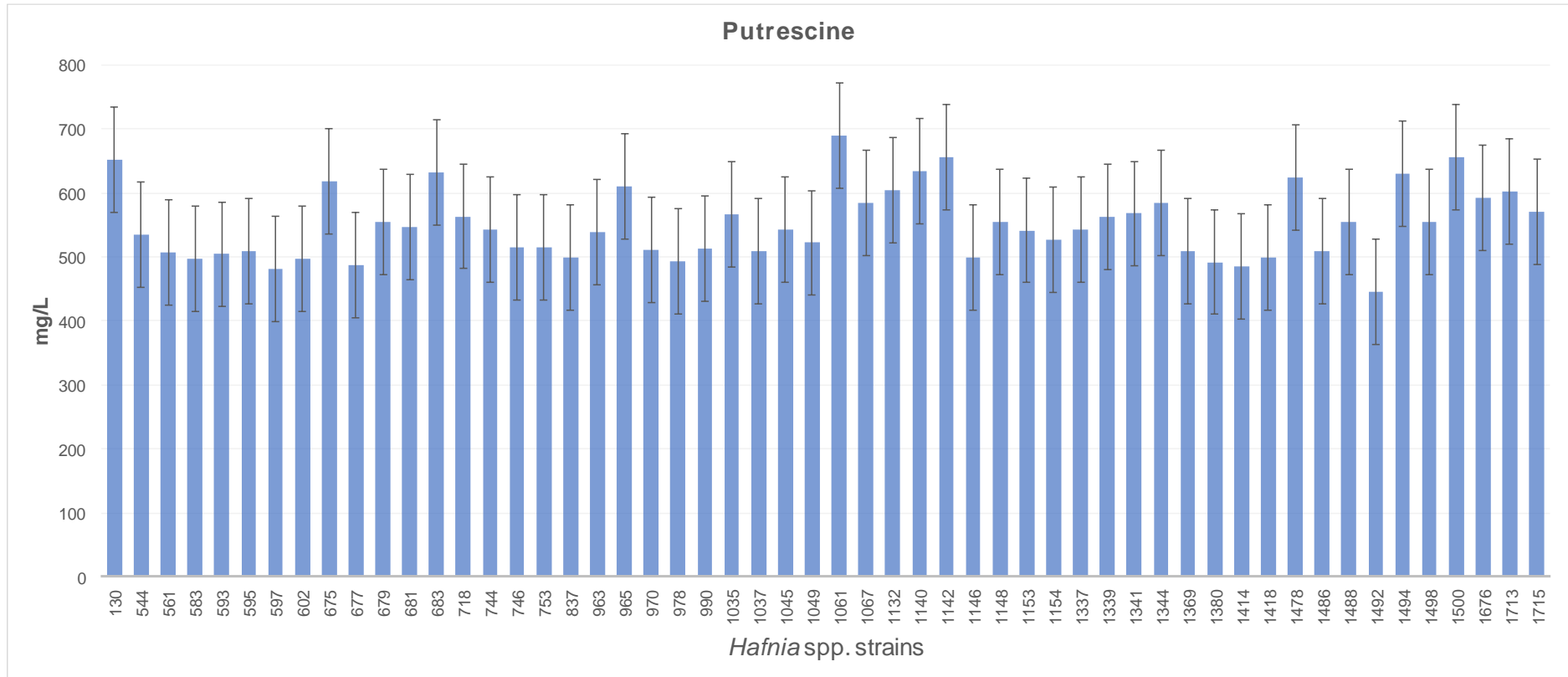


Table S1. *Hafnia alvei* and *Hafnia paralvei* selected strains, PDO origin and RAPD-PCR M13 profile.

IDENTIFICATION	ISOLATE CODE ¹	PDO	PROFILE M13
<i>H. alvei</i>	597, 602, 1148, 1153, 1154	"QUESO DE LA SERENA"	P1
	1486	"QUESO DE LA SERENA"	P2
	130	"TORTA DEL CASAR"	P3
	544	"QUESO DE LA SERENA"	
	675, 683 , 1035	"TORTA DEL CASAR"	P4
	595, 1067, 1142	"QUESO DE LA SERENA"	
	1037 , 1045 , 1341	"TORTA DEL CASAR"	P5
	970	"TORTA DEL CASAR"	P6
	1132	"QUESO DE LA SERENA"	
	<i>H. paralvei</i>	679 , 718, 837, 963, 978 , 990, 1049, 1339, 1369, 1380, 1418, 1713	"TORTA DEL CASAR"
561 , 583 , 593 , 744, 753, 1061, 1140, 1146, 1478, 1488, 1494, 1498		"QUESO DE LA SERENA"	
677 , 681 , 965, 1337, 1414		"TORTA DEL CASAR"	P8
310 , 1676 , 1715		"TORTA DEL CASAR"	P9
1492, 1500		"QUESO DE LA SERENA"	P10
1344		"TORTA DEL CASAR"	P11
746		"QUESO DE LA SERENA"	

¹Bold letter mark strains were selected base on their safety characteristics for studying their technological properties.

Table S2. Antibiotic class, subclass, general agent name and abbreviation used in this study.

Antibiotic Class	Antibiotic Subclass	Agents Included, Generical Names	Agent Abbreviation
Penicillins	Aminopenicillin	Ampicillin	AMP
	Ureidopenicillin	Piperacillin	PIP
β -Lactam/ β -lactamase inhibitor combinations		Ampicillin-sulbactam	SAM
		Amoxicillin-clavucanic acid	AMC
		Piperacillin-tazobactam	PTZ
		Tricarillin-clavucanic acid	TCL
Monobactams		Aztreonam	ATM
Cephems	Cephalosporin I	Cephalotin	CEP
		Cefazolin	CFZ
	Cephalosporin II	Cefuroxime	CXM
	Cephalosporin III	Cefotaxime	CTX
		Ceftazidime	CAZ
	Cephalosporin IV	Cefepime	FEP
	Cephamycin	Cefotetan	CTT
		Cefoxitin	FOX
Penems	Carbapenems	Dorapenem	DOR
		Imipinem	IMP
		Meropenem	MER
		Ertapenem	ETP
Aminoglycosides		Amikacin	AMK
		Gentamicina	GM
		Tobramicina	NN
Quinolones	Quinolone	Nalidixic acid	NA
	Fluoroquinolone	Ciprofloxacin	CIP
		Norfloxacin	NOR
		Lomefloxacin	LOM
		Levofloxacin	LEV
Tetracyclines		Tetracycline	TE
Phenicol		Chloramphenicol	C
Nitrofurans		Nitrofurantoin	F
Folate Pathway Inhibitors		Sulfisoxazole	G
		Trimethoprim-sulfamethoxazole	SXT
		Trimethoprim	W

Table S3. Conditions of temperature, pH and NaCl concentration for modelling *Hafnia* spp. growth under cheese ripening process.

Block	Temperature (°C)	%NaCl	pH
1	30	2	4.8
2	30	2	6
3	30	1	5.4
4	30	3	5.4
5	20	1	6
6	20	3	4.8
7	20	1	4.8
8	20	3	6
9	20	2	5.4
10	20	2	5.4
11	20	2	5.4
12	10	2	4.8
13	10	1	5.4
14	10	2	6
15	10	3	5.4
Factor combinations of ripening process			
Stage	Temperature (°C)	%NaCl	pH
Initial ripening (S1)	8	1	6
Early ripening (S2)	8	2	4.5
Mid ripening (S3)	10	3	5
Late ripening (S4)	12	3	5.5
End ripening (S5)	12	3	5.9

Table S4. Antibiotic susceptibility of *Hafnia alvei* strains, established according to the guidelines for *Enterobacteriaceae* of CSLI (2019) and EUCAST (2020). R: resistant; MS: moderately susceptible; S: susceptible.

CODE*,**	AMP	SAM	AMC	PTZ	TCL	PIP	ATM	FOX	CXM	CAZ	FEP	CTX	DOR	IMP	MER	ETP	AMK	GM	NN	NA	CIP	LEV	TE	C	F	W	
130	S	S	R	S	S	R	MS	R	S	S	S	S	R	S	R	R	S	S	R	S	S	S	S	S	S	S	
544	S	S	S	S	S	S	S	S	S	S	S	R	S	R	S	S	S	S	S	S	S	S	R	S	S	S	
595	R	R	R	R	MS	R	R	S	R	R	S	R	R	S	R	R	R	R	S	S	S	S	R	R	S	S	
597	R	R	R	R	R	R	R	S	R	R	S	R	R	R	R	R	R	S	R	MS	R	R	S	MS	S	MS	
602	R	R	R	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	MS	R	S	S	MS	S	MS	
675	R	R	R	R	MS	R	R	S	R	S	S	R	R	S	R	R	R	R	R	S	R	S	S	S	S	S	
683	R	R	R	R	S	S	S	S	R	R	S	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	
970	S	S	R	S	S	R	S	S	R	R	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
1035	R	R	R	R	R	R	R	S	R	R	S	R	R	R	R	R	R	S	S	S	S	S	S	S	S	S	
1037	R	S	R	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	R	S	S	S	S	S	S	
1045	S	R	R	R	S	S	S	S	R	R	S	R	S	S	S	S	S	S	S	MS	S	S	S	S	S	S	
1067	R	S	R	R	S	R	R	S	R	R	S	R	R	R	R	R	R	S	S	S	S	S	R	R	S	S	
1132	R	R	R	R	S	R	R	S	R	R	S	R	R	R	MS	MS	R	R	S	S	S	S	S	R	S	S	
1142	R	S	R	S	S	S	S	S	R	R	S	R	MS	R	S	S	R	S	S	S	S	S	R	S	S	S	
1148	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R	R	R	MS	R	S	S	R	S	S	
1153	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R	R	S	MS	R	R	S	MS	S	S	
1154	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R	R	R	S	R	S	R	MS	R	S	
1341	S	R	R	S	S	R	S	S	R	R	S	R	R	R	R	S	S	S	S	R	S	S	S	S	S	S	
1486	S	S	R	R	MS	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	MS	S	S	R	S	R	S

* All *H. alvei* are "R" to CFZ (cefazolin), CEP (cefalotin), G (sulfiacole)

** All *H. alvei* are "S" to CTT (cefotetan), NOR (norfloxacin), LOM (lomefloxacin), SXT (Trimethoprim-sulfamethoxazole)

1369	R	R	R	S	R	R	S	R	R	S	S	R	R	R	R	R	S	S	S	S	S	S	S	R	S
1380	R	R	R	S	R	R	S	R	R	S	S	R	S	R	R	R	S	S	S	S	S	S	S	R	S
1414	S	S	R	S	S	R	S	R	R	S	S	R	R	S	S	R	S	S	MS	S	S	MS	S	S	S
1418	R	R	R	MS	R	R	S	R	R	R	S	R	S	R	R	R	R	S	MS	S	S	S	S	S	S
1478	R	R	R	MS	R	S	R	R	R	S	S	R	R	R	R	R	R	S	MS	S	S	MS	S	S	R
1488	R	R	R	R	R	R	S	R	R	S	S	R	R	R	R	R	S	S	MS	S	S	S	S	S	S
1492	R	S	R	S	R	S	S	R	R	S	S	R	R	S	R	R	S	S	R	S	S	S	S	S	S
1494	R	R	R	S	R	R	S	R	R	S	S	R	R	R	MS	R	R	S	R	S	S	S	S	S	S
1498	R	R	R	R	R	R	S	R	R	S	S	R	R	R	R	R	S	R	R	R	S	S	S	R	S
1500	R	S	S	S	R	R	S	R	R	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S
1676	R	R	R	S	R	R	S	R	R	S	S	R	R	S	S	S	S	S	R	S	S	S	S	S	S
1713	R	R	R	S	S	S	S	R	R	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S
1715	R	R	S	S	S	S	S	R	R	S	S	R	S	S	S	S	S	S	R	S	S	S	S	S	S

** All *H.paralvei* are "R" to CFZ (cefazolin), CEP (cefalotin), G (sulfiaxole), AMC (amoxicilin/clavucanic acid), CTX (cefotaxime)

** All *H. paralvei* are "S" to CTT (cefotetan), NOR (norfloxacin), LOM (lomefloxacin), SXT (Trimethoprim sulfamethoxazole)