GenEpi BioTrain – Block 2 Wave 3

Gene-by-gene approach Part 2: cgMLST applications using EnteroBase

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French National Reference Center for *E. coli*, *Shigella* and *Salmonella*

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Intended Learning Objectives

Specific objectives of this session:

1. Comprehend the principles and applications in epidemiological surveillance and outbreak investigation of gene-by-gene approaches, including cgMLST and hierarchical clustering

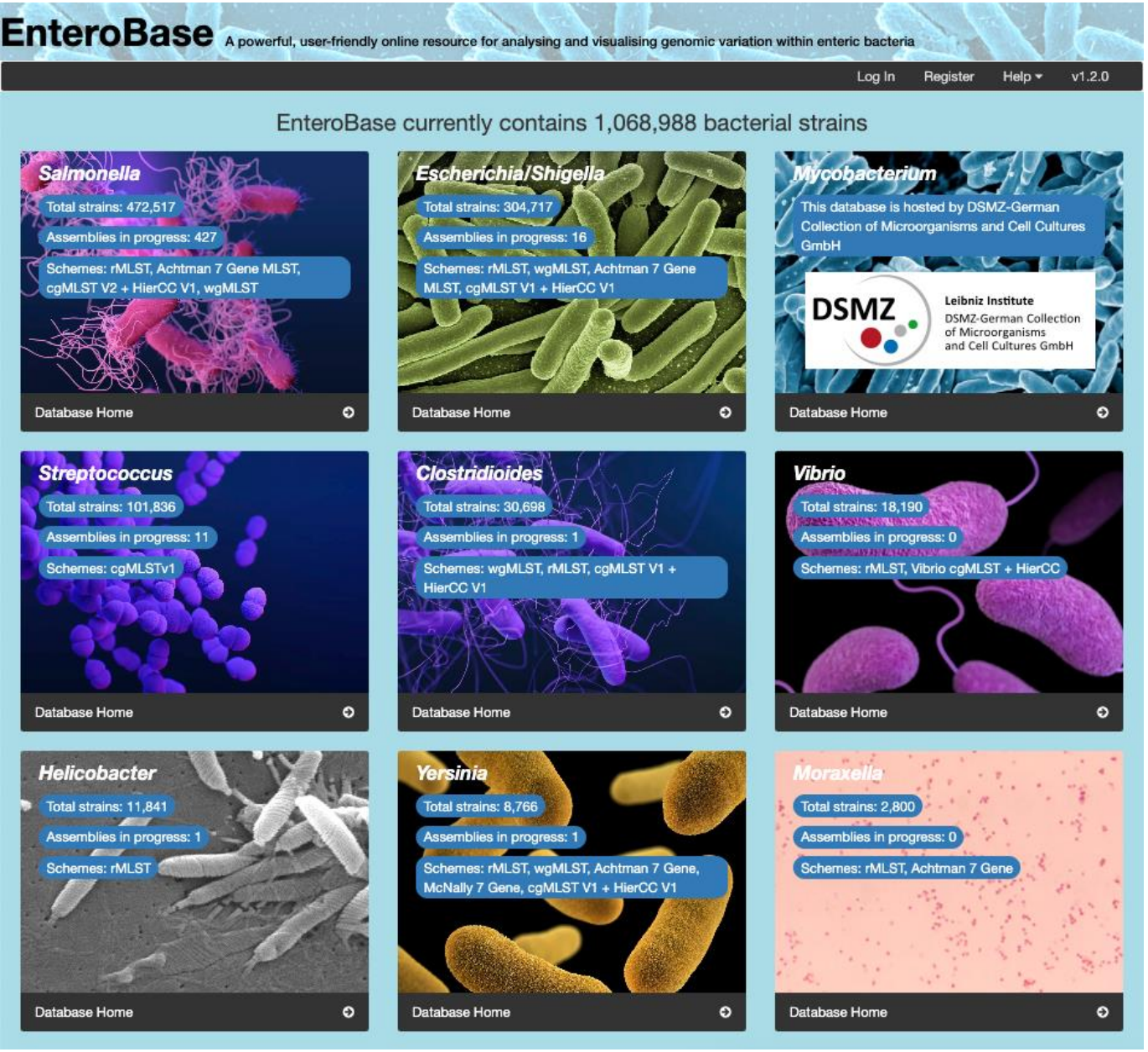
2. Correlate previously acquired knowledge on genome sequencing and the biology of *E. coli* and *Shigella* with the tools available on EnteroBase and their importance for pathogen characterisation

3. Understand how the different features present on EnteroBase can be used for epidemiological surveillance and outbreak investigation

Outline

This session consists of the following elements

1. Important concepts applied to EnteroBase
2. Submitting and searching genomes on EnteroBase
3. “Experimental Data” on *E. coli*, *Shigella* and *Salmonella*
4. “Custom View”
5. Tree visualization and data export
6. Data sharing



“A powerful, user-friendly online resource for analysing and visualising genomic variation within enteric bacteria”

Includes several databases with various tools, sometimes specific for each pathogen

Theory and important concepts on EnteroBase

Hierarchical Clustering (HierCC)

cgMLST

- Evaluation of the allelic variation of a combination of genes belonging to the core genome
- *E. coli/Shigella*: 2513 alleles
- *Salmonella* spp.: 3002 alleles
- Most genomes present a unique allelic profile (cgST)

HierCC

- Classification of the relationship of a given genome based on the allelic distance to an ancestral strain, already present in the database
- 13 classification levels
- HC0 – no difference
- HC5 – maximum 5 allele differences (genomic surveillance)
- …
- HC2350 – different species within the *Escherichia* genus
- HC2850 – sub-species of *Salmonella*

Zhou, Charlesworth & Achtman, *Bioinformatics*. 2021;37(20):3645-3646.

Hierarchical Clustering (HierCC)

Escherichia/Shigella

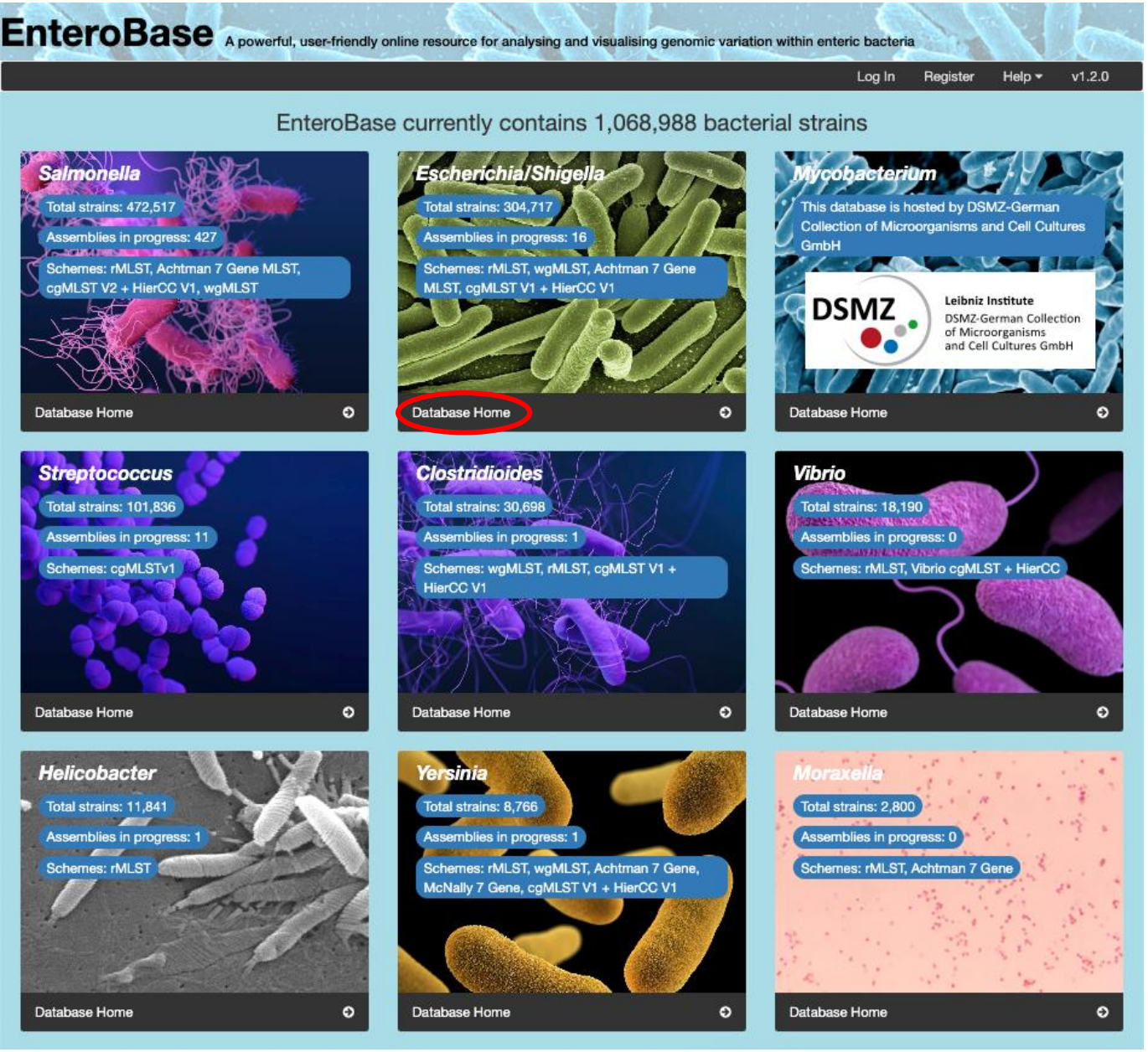
- HC0 – identical strains
- HC5 – most used threshold for genomic surveillance
- HC100 – most serotypes of *E. coli*
- HC400 – some clonal groups
- HC1100 – clonal complexes - equivalent to 7-MLST CCs
- HC2000 – superlineages
- HC2350 – species

HierCC

- HC0 – identical strains
- HC5 – most used threshold for genomic surveillance
- HC50 – evolutive group
- HC2000 – superlineages (~ serotype)
- HC2850 – sub-species

Zhou, Charlesworth & Achtman, *Bioinformatics*. 2021;37(20):3645-3646.

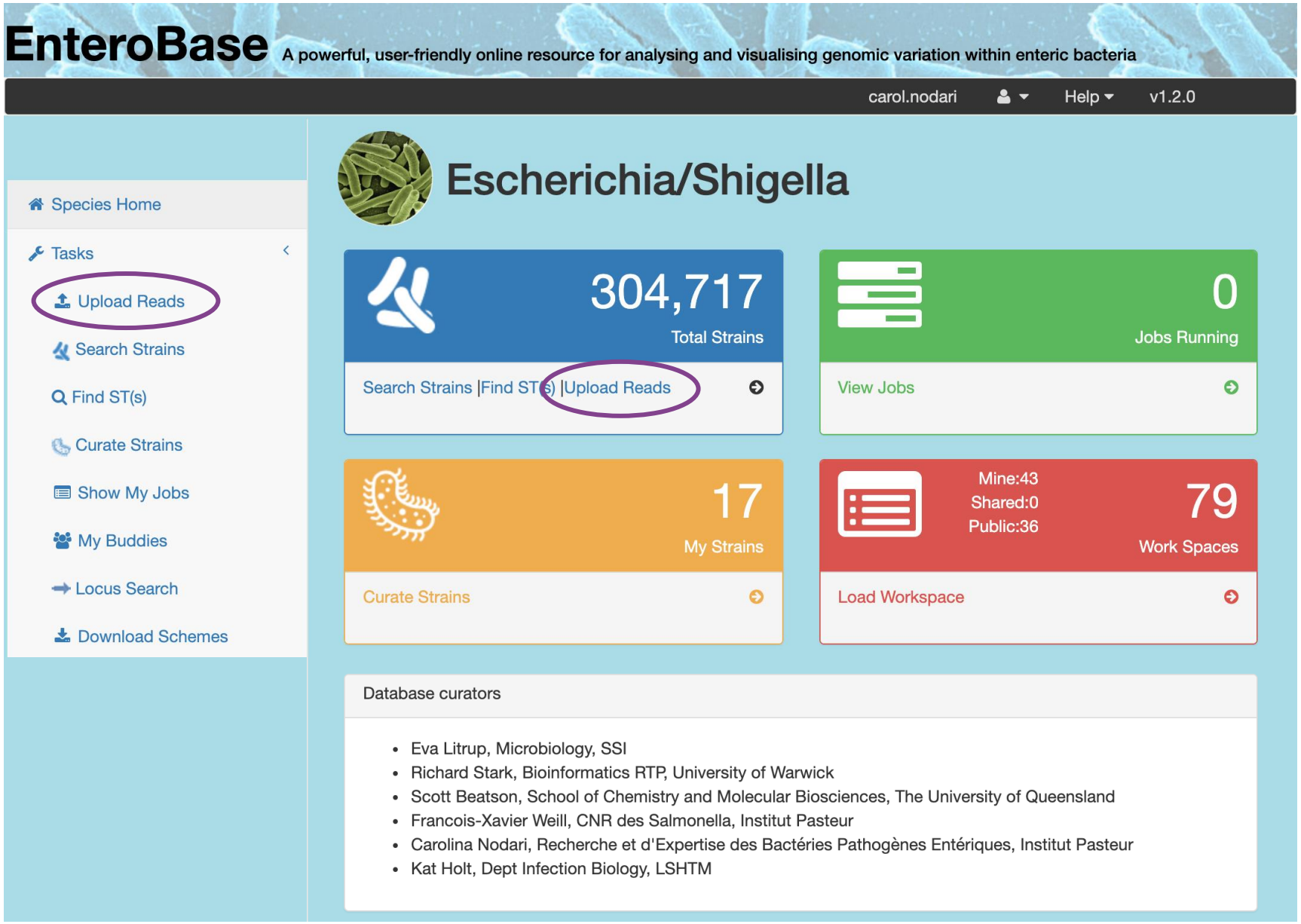
Submitting and searching genomes on EnteroBase



Submitting genomes

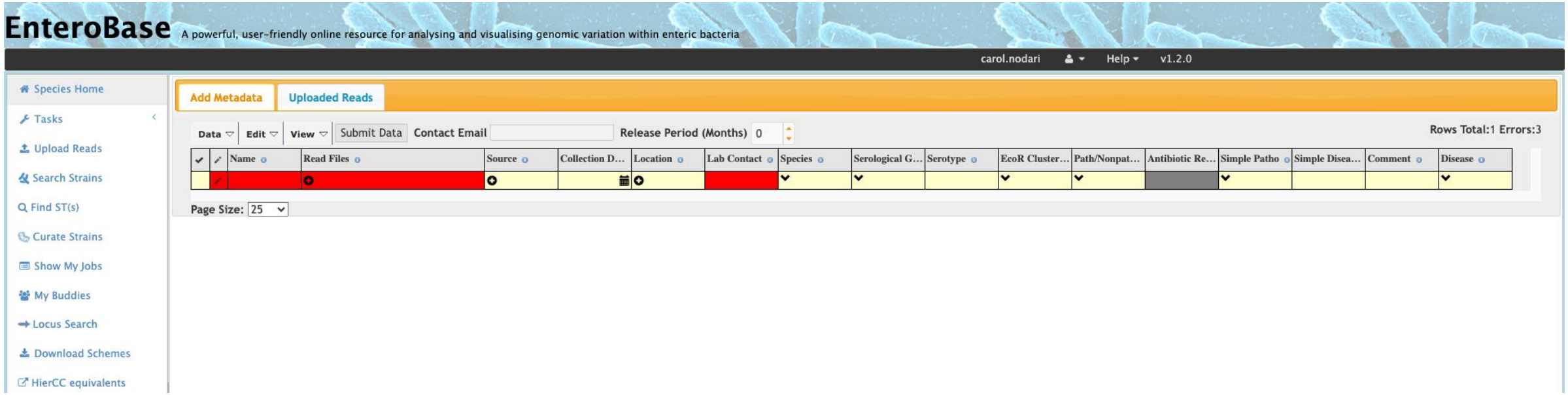
- Start with the basics: choose the **database** that corresponds to the pathogen of interest (submitting in the wrong database will make your data fail QC)

- **Important:** you must have an account and be logged-in to be able to upload and download data to EnteroBase


EnteroBase
A powerful, user-friendly online resource for analysing and visualising genomic variation within enteric bacteria
carol.nodari
Help
v1.2.0
Species Home
Tasks
Upload Reads
Search Strains
Find ST(s)
Curate Strains
Show My Jobs
My Buddies
Locus Search
Download Schemes
Escherichia/Shigella
304,717
Total Strains
Search Strains |Find ST(s) |Upload Reads
0
Jobs Running
View Jobs
17
My Strains
Curate Strains
Mine:43
Shared:0
Public:36
79
Work Spaces
Load Workspace
Database curators
Eva Litrup, Microbiology, SSI
Richard Stark, Bioinformatics RTP, University of Warwick
Scott Beatson, School of Chemistry and Molecular Biosciences, The University of Queensland
Francois-Xavier Weill, CNR des Salmonella, Institut Pasteur
Carolina Nodari, Recherche et d'Expertise des Bactéries Pathogènes Entériques, Institut Pasteur
Kat Holt, Dept Infection Biology, LSHTM

Genome submission

When uploading genomes, it is possible to include a variety of metadata, including country, date of isolation and niche source (human, food, environment), and a minimal of information is required to be able to submit genomes in the database



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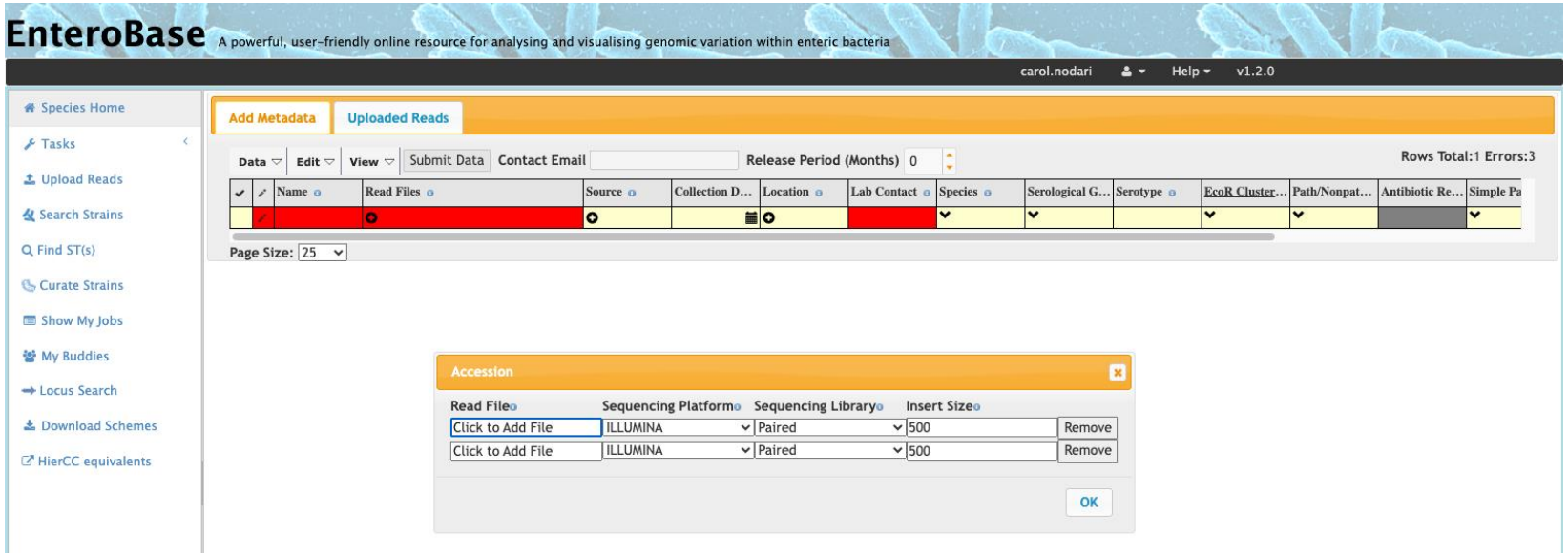


- By clicking on the "read files" field, a window will pop up. One file should be chosen for each line.

- **Important:** for the moment, it is only possible to submit Illumina genomic data.

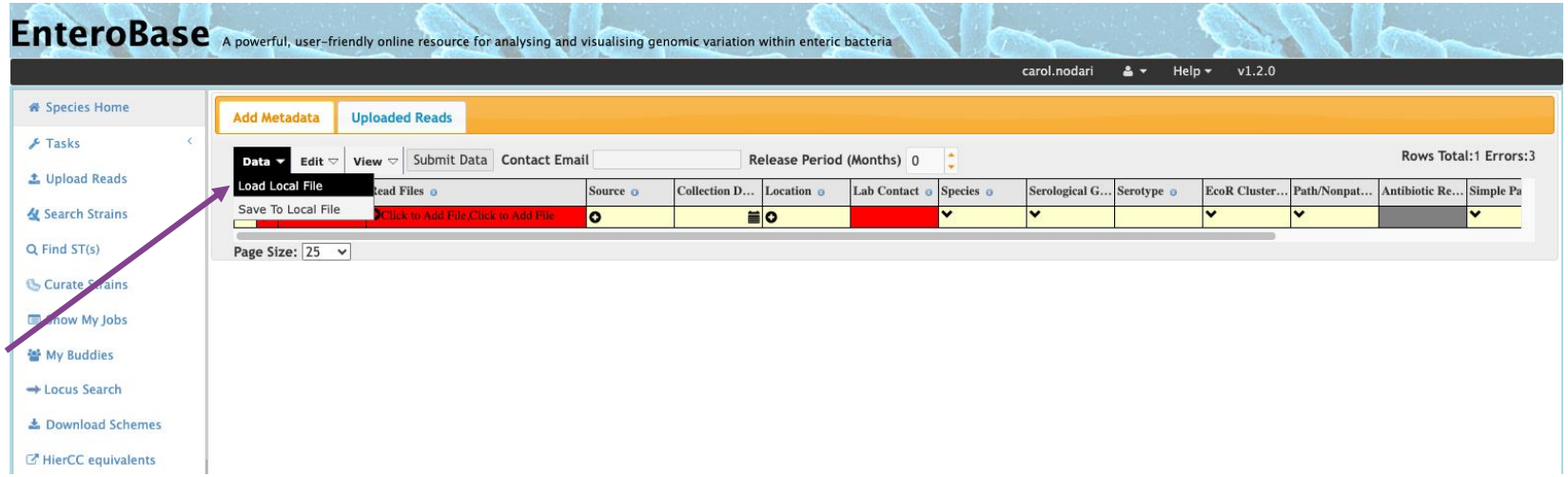
Genome submission - batch upload

- Genomes and their associated metadata can be uploaded in batches.

- A tab-separated **.txt** file containing all the metadata, as well and the names of the read files can be uploaded using the "Data > Load local file" menu.

- **Important:** in the "Read files" column, files names *.R1.fastq(.gz) and *.R2.fastq(.gz) must be separated by a **comma** and no white spaces.

	A	B	C	D	E	F	G	H	I
1	Name	Species	Serotype	Collection Year	Continent	Country	Source	Lab Contact	Read Files
2	202403595	Escherichia coli		2024	Europe	France	Human	F.X.Weill, Institut Pasteur	Ec202403595_S28.1.fastq.gz,Ec202403595_S28.2.fastq.gz
3	202403596	Escherichia coli		2024	Europe	France	Human	F.X.Weill, Institut Pasteur	Ec202403596_S29.1.fastq.gz,Ec202403596_S29.2.fastq.gz
4	202403597	Escherichia coli		2024	Europe	France	Human	F.X.Weill, Institut Pasteur	Ec202403597_S30.1.fastq.gz,Ec202403597_S30.2.fastq.gz
5									
6									



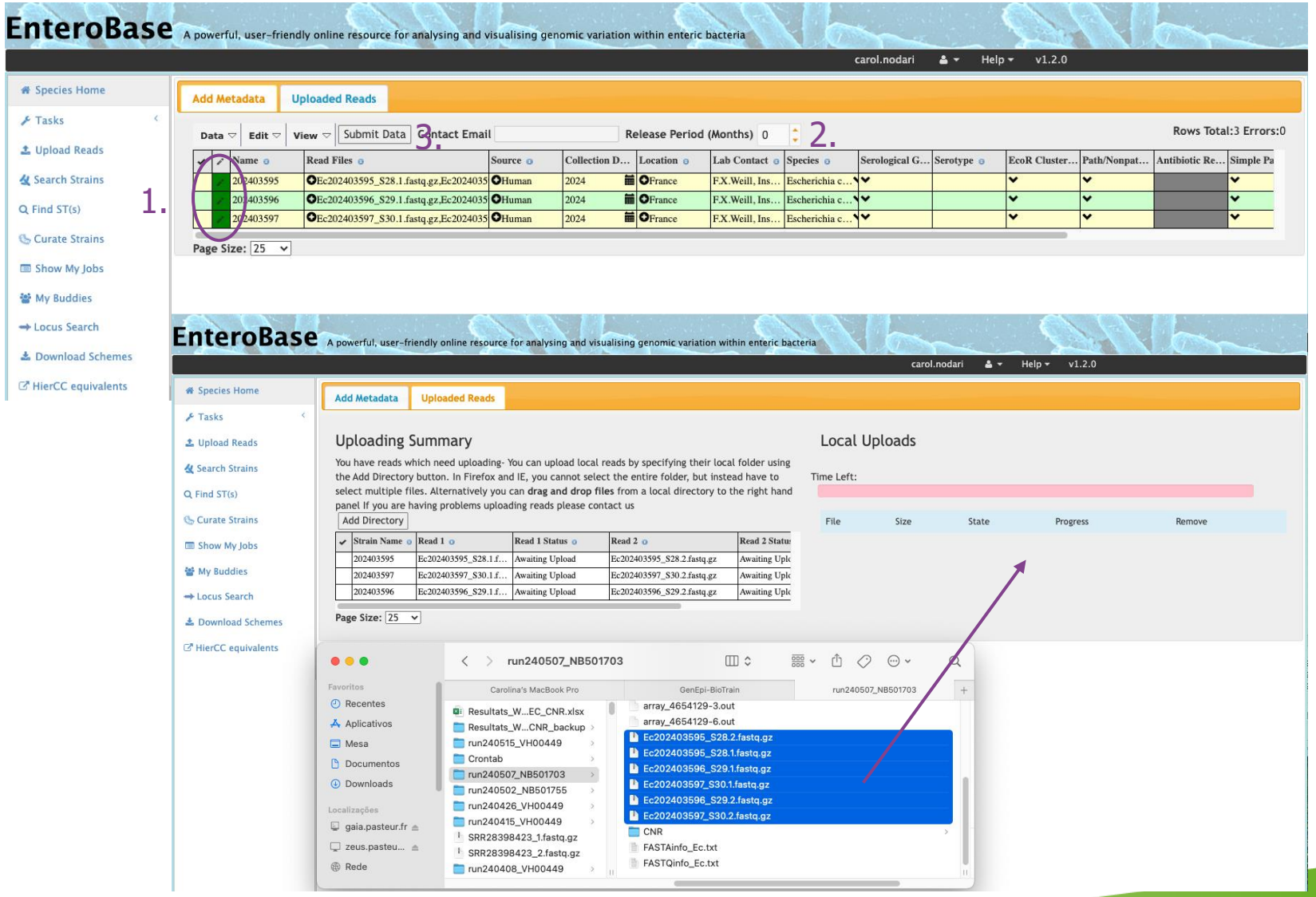
Genome submission - batch upload

1. If all data was correctly submitted, the second column will become green. Fields with errors will be marked in red.

2. Choose the time to make data public (up to 12 months).

3. Submit the metadata.

4. In the "Uploaded reads" tab you will be able to add the read files by choosing a folder containing them or by dragging and dropping the files directly on the right-side panel.

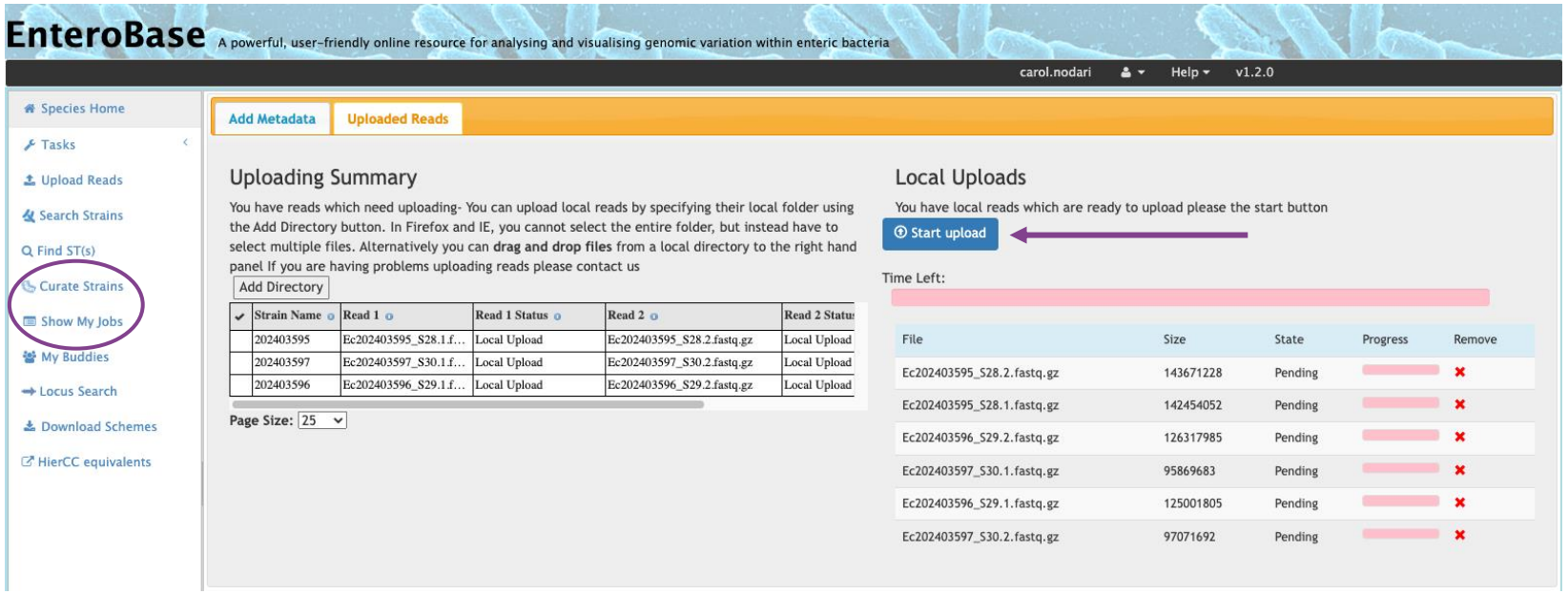


Genome submission - batch upload

5. Start upload to begin transferring the data.

6. The submitted genomic data will be QCed, assembled, annotated and assigned a cgMLST and HierCC profile.

7. You will be able to follow the status of your submission on the "Curate Strains" and "Show My Jobs" menus.

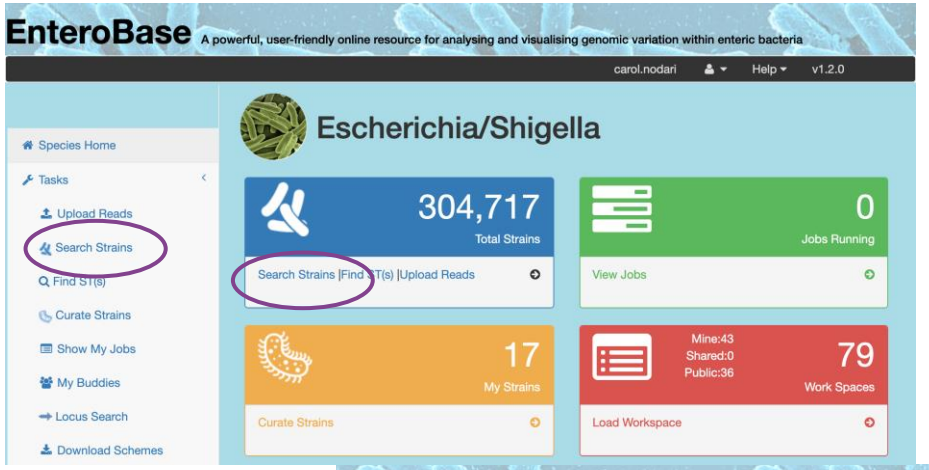


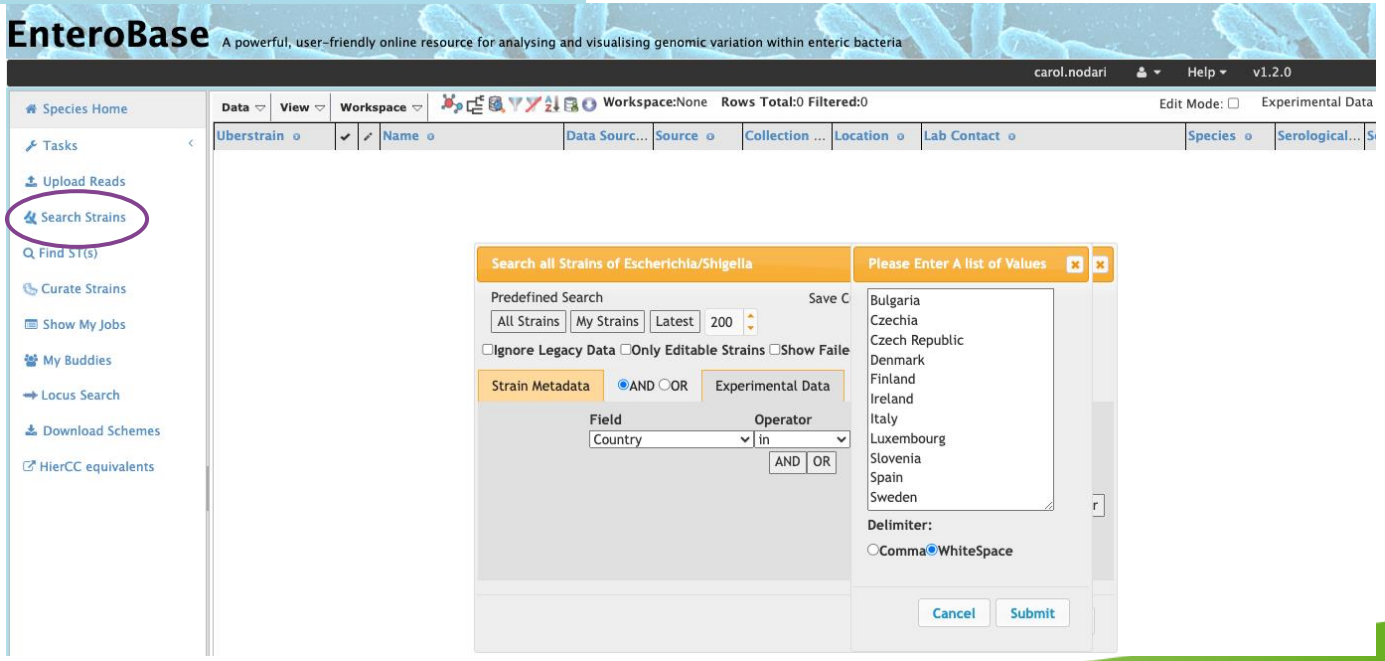
Searching genomes on EnteroBase

- Genomes of pathogens of interest present in **public databases** can be searched using Enterobase with the "Search Strains" tool.

- The research can be done based on strain **metadata** (isolation place or date, BioProject or SRA number on GenBank,…) or "**experimental data**", i.e., data derived from the genomes (ST, serotype, *E. coli* pathotype,…)

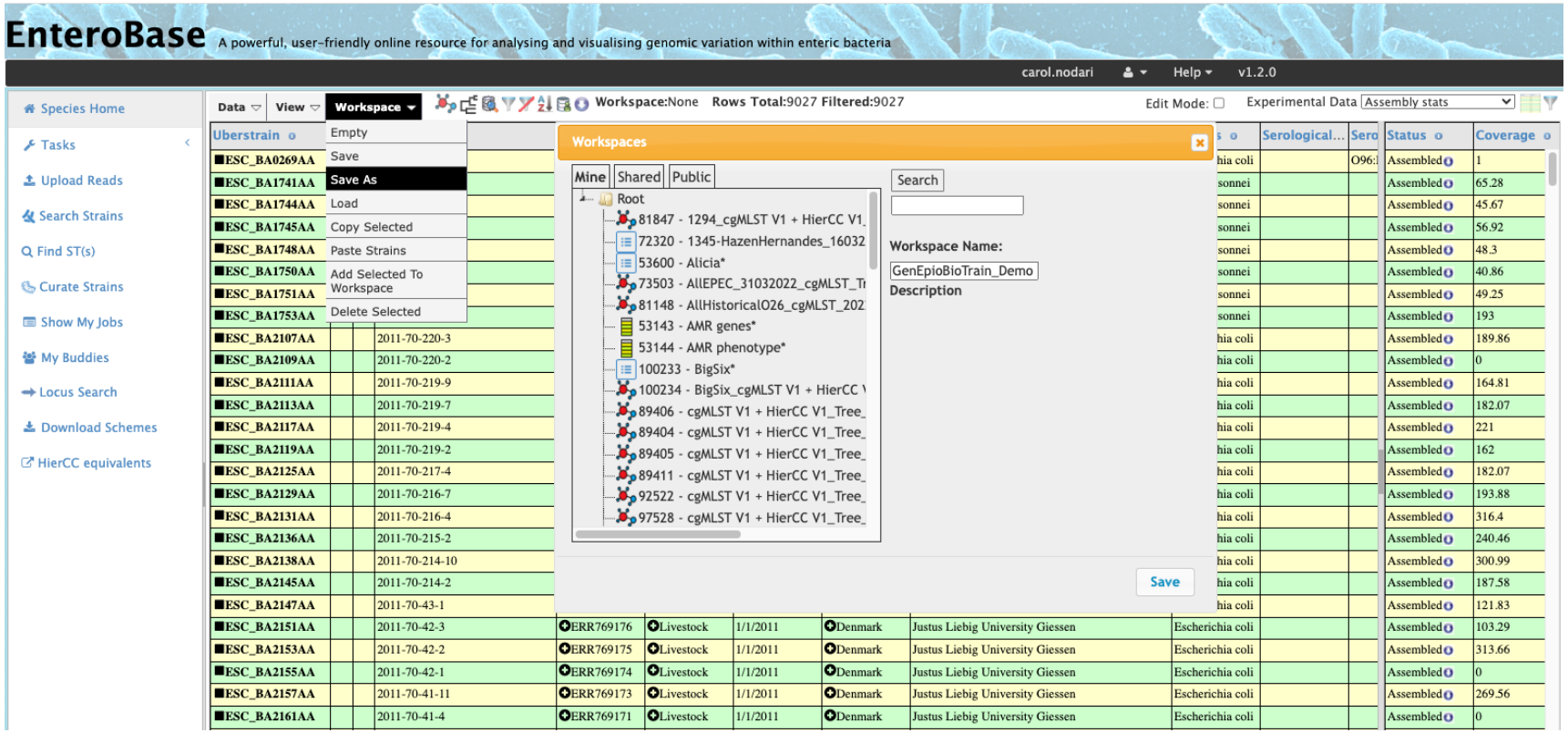
- **Hint**: by using the operator "in", you can look into a **list** of values. Remember to write country or city names **in English**.





Searching genomes on EnteroBase

- Research results can be saved in "workspaces", which can be shared later.
- You can also add the results of future searches in the same "workspace" using the "Add selected to workspace" option.
- The list of results of a search and/or a workspace are split in two parts: the left side contains the metadata, while the right side contains the experimental data.
- The experimental data shown by default are the "assembly status", but several other options are available, with some of them being exclusive of a specific pathogen.



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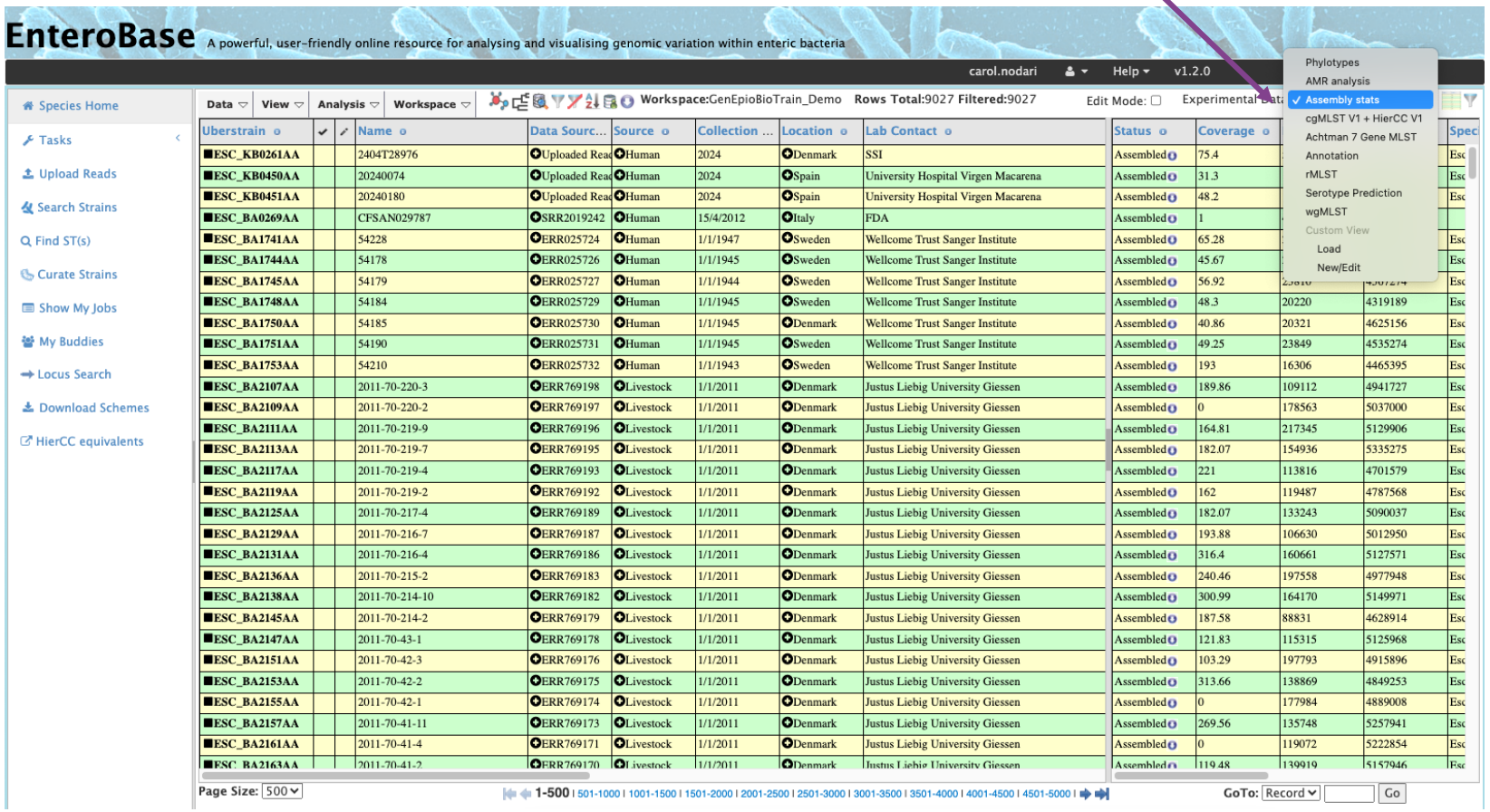
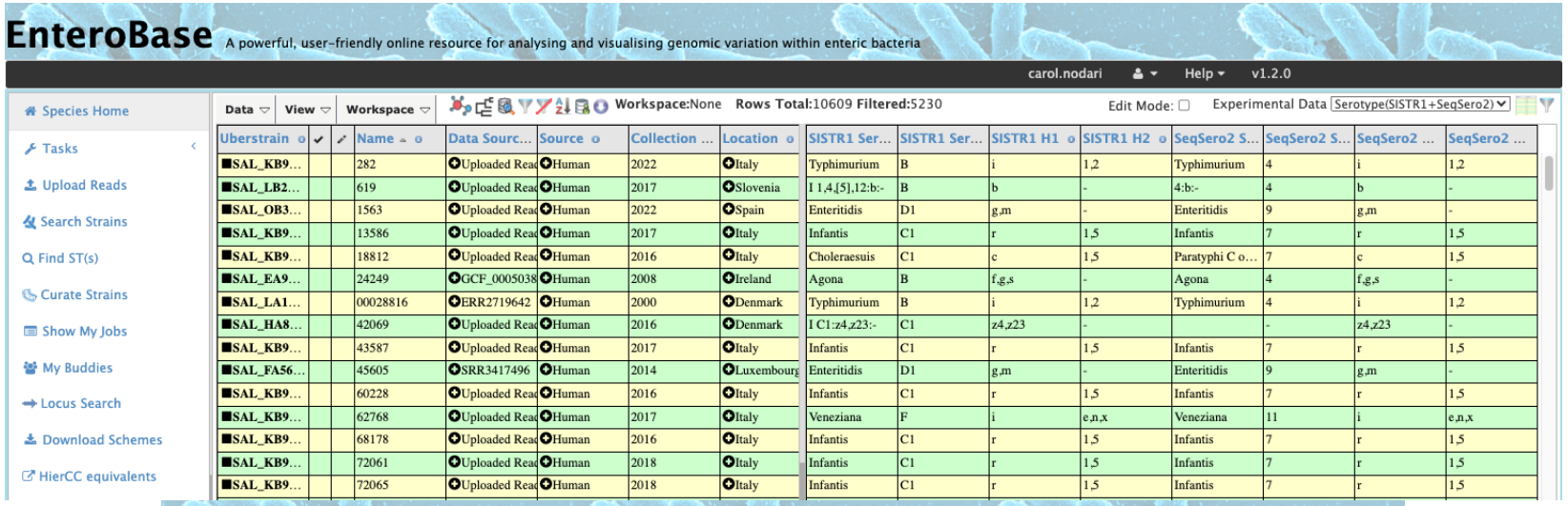


Uberstrain	Name	Data Sourc...	Source	Collection ...	Location	Lab Contact	Status	Coverage			Spec
ESC_KB0261AA	2404T28976	Uploaded Reac	Human	2024	Denmark	SSI	Assembled	75.4			Esc
ESC_KB0450AA	20240074	Uploaded Reac	Human	2024	Spain	University Hospital Virgen Macarena	Assembled	31.3			Esc
ESC_KB0451AA	20240180	Uploaded Reac	Human	2024	Spain	University Hospital Virgen Macarena	Assembled	48.2			Esc
ESC_BA0269AA	CFSAN029787	SRR2019242	Human	15/4/2012	Italy	FDA	Assembled	1			
ESC_BA1741AA	54228	ERR025724	Human	1/1/1947	Sweden	Wellcome Trust Sanger Institute	Assembled	65.28			Esc
ESC_BA1744AA	54178	ERR025726	Human	1/1/1945	Sweden	Wellcome Trust Sanger Institute	Assembled	45.67			Esc
ESC_BA1745AA	54179	ERR025727	Human	1/1/1944	Sweden	Wellcome Trust Sanger Institute	Assembled	56.92	25810	4507274	Esc
ESC_BA1748AA	54184	ERR025729	Human	1/1/1945	Sweden	Wellcome Trust Sanger Institute	Assembled	48.3	20220	4319189	Esc
ESC_BA1750AA	54185	ERR025730	Human	1/1/1945	Denmark	Wellcome Trust Sanger Institute	Assembled	40.86	20321	4625156	Esc
ESC_BA1751AA	54190	ERR025731	Human	1/1/1945	Sweden	Wellcome Trust Sanger Institute	Assembled	49.25	23849	4535274	Esc
ESC_BA1753AA	54210	ERR025732	Human	1/1/1943	Sweden	Wellcome Trust Sanger Institute	Assembled	193	16306	4465395	Esc
ESC_BA2107AA	2011-70-220-3	ERR769198	Livestock	1/1/2011	Denmark	Justus Liebig University Giessen	Assembled	189.86	109112	4941727	Esc
ESC_BA2109AA	2011-70-220-2	ERR769197	Livestock	1/1/2011	Denmark	Justus Liebig University Giessen	Assembled	0	178563	5037000	Esc
ESC_BA2111AA	2011-70-219-9	ERR769196	Livestock	1/1/2011	Denmark	Justus Liebig University Giessen	Assembled	164.81	217345	5129906	Esc
ESC_BA2113AA	2011-70-219-7	ERR769195	Livestock	1/1/2011	Denmark	Justus Liebig University Giessen	Assembled	182.07	154936	5335275	Esc
ESC_BA2117AA	2011-70-219-4	ERR769193	Livestock	1/1/2011	Denmark	Justus Liebig University Giessen	Assembled	221	113816	4701579	Esc
ESC_BA2119AA	2011-70-219-2	ERR769192	Livestock	1/1/2011	Denmark	Justus Liebig University Giessen	Assembled	162	119487	4787568	Esc
ESC_BA2125AA	2011-70-217-4	ERR769189	Livestock	1/1/2011	Denmark	Justus Liebig University Giessen	Assembled	182.07	133243	5090037	Esc
ESC_BA2129AA	2011-70-216-7	ERR769187	Livestock	1/1/2011	Denmark	Justus Liebig University Giessen	Assembled	193.88	106630	5012950	Esc
ESC_BA2131AA	2011-70-216-4	ERR769186	Livestock	1/1/2011	Denmark	Justus Liebig University Giessen	Assembled	316.4	160661	5127571	Esc
ESC_BA2136AA	2011-70-215-2	ERR769183	Livestock	1/1/2011	Denmark	Justus Liebig University Giessen	Assembled	240.46	197558	4977948	Esc
ESC_BA2138AA	2011-70-214-10	ERR769182	Livestock	1/1/2011	Denmark	Justus Liebig University Giessen	Assembled	300.99	164170	5149971	Esc
ESC_BA2145AA	2011-70-214-2	ERR769179	Livestock	1/1/2011	Denmark	Justus Liebig University Giessen	Assembled	187.58	88831	4628914	Esc
ESC_BA2147AA	2011-70-43-1	ERR769178	Livestock	1/1/2011	Denmark	Justus Liebig University Giessen	Assembled	121.83	115315	5125968	Esc
ESC_BA2151AA	2011-70-42-3	ERR769176	Livestock	1/1/2011	Denmark	Justus Liebig University Giessen	Assembled	103.29	197793	4915896	Esc
ESC_BA2153AA	2011-70-42-2	ERR769175	Livestock	1/1/2011	Denmark	Justus Liebig University Giessen	Assembled	313.66	138869	4849253	Esc
ESC_BA2155AA	2011-70-42-1	ERR769174	Livestock	1/1/2011	Denmark	Justus Liebig University Giessen	Assembled	0	177984	4889008	Esc
ESC_BA2157AA	2011-70-41-11	ERR769173	Livestock	1/1/2011	Denmark	Justus Liebig University Giessen	Assembled	269.56	135748	5257941	Esc
ESC_BA2161AA	2011-70-41-4	ERR769171	Livestock	1/1/2011	Denmark	Justus Liebig University Giessen	Assembled	0	119072	5222854	Esc
ESC_BA2163AA	2011-70-41-2	ERR769170	Livestock	1/1/2011	Denmark	Justus Liebig University Giessen	Assembled	119.48	139919	5157946	Esc

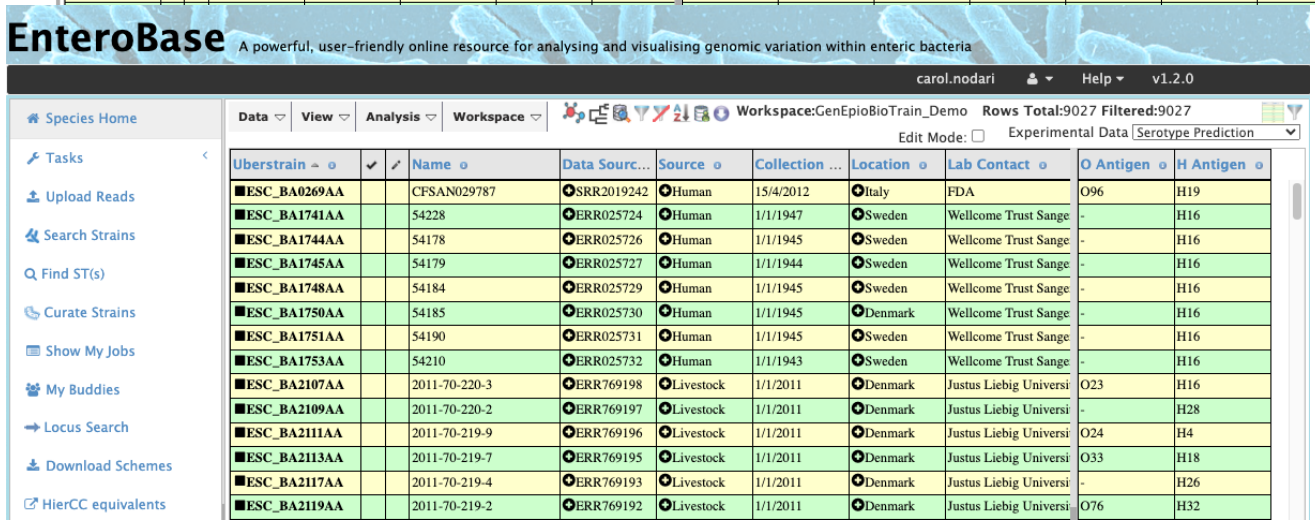
“Experimental Data” on *E. coli*, *Shigella* and *Salmonella*

Experimental Data

- With the assembled genomes, a lot of data can be retrieved from the genomes.
- Among the features included in EnteroBase, we can find **serotype** prediction tools, which are specific for each pathogen.
- Recently, an **AMR analysis** using AMRFinderPlus has been implemented is currently been tested in small sets of genomes.
- Both 7 genes **MLST** and **cgMLST** can also be retrieved, as well as the **HierCC** information.
- **Pathotypes** of *E. coli*, except for EAEC, and *Shigella* serogroups can also be identified in the menu "Phylotypes".



Uberstrain	Name	Data Sourc...	Source	Collection ...	Location	SISTR1 Ser...	SISTR1 Ser...	SISTR1 H1	SISTR1 H2	SeqSero2 S...	SeqSero2 S...	SeqSero2 ...	SeqSero2 ...
SAL_KB9...	282	Uploaded Read	Human	2022	Italy	Typhimurium	B	i	1,2	Typhimurium	4	i	1,2
SAL_LB2...	619	Uploaded Read	Human	2017	Slovenia	I 1,4,[5],12:b:-	B	b	-	4:b:-	4	b	-
SAL_OB3...	1563	Uploaded Read	Human	2022	Spain	Enteritidis	D1	g,m	-	Enteritidis	9	g,m	-
SAL_KB9...	13586	Uploaded Read	Human	2017	Italy	Infantis	C1	r	1,5	Infantis	7	r	1,5
SAL_KB9...	18812	Uploaded Read	Human	2016	Italy	Choleraesuis	C1	c	1,5	Paratyphi C o...	7	c	1,5
SAL_EA9...	24249	GCF_0005038	Human	2008	Ireland	Agona	B	f,g,s	-	Agona	4	f,g,s	-
SAL_LA1...	00028816	ERR2719642	Human	2000	Denmark	Typhimurium	B	i	1,2	Typhimurium	4	i	1,2
SAL_HA8...	42069	Uploaded Read	Human	2016	Denmark	I C1:z4,z23:-	C1	z4,z23	-		-	z4,z23	-
SAL_KB9...	43587	Uploaded Read	Human	2017	Italy	Infantis	C1	r	1,5	Infantis	7	r	1,5
SAL_FA56...	45605	SRR3417496	Human	2014	Luxembourg	Enteritidis	D1	g,m	-	Enteritidis	9	g,m	-
SAL_KB9...	60228	Uploaded Read	Human	2016	Italy	Infantis	C1	r	1,5	Infantis	7	r	1,5
SAL_KB9...	62768	Uploaded Read	Human	2017	Italy	Veneziana	F	i	e,n,x	Veneziana	11	i	e,n,x
SAL_KB9...	68178	Uploaded Read	Human	2016	Italy	Infantis	C1	r	1,5	Infantis	7	r	1,5
SAL_KB9...	72061	Uploaded Read	Human	2018	Italy	Infantis	C1	r	1,5	Infantis	7	r	1,5
SAL_KB9...	72065	Uploaded Read	Human	2018	Italy	Infantis	C1	r	1,5	Infantis	7	r	1,5



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ESC_BA0269AA	CFSAN029787	SRR2019242	Human	15/4/2012	Italy	FDA	O96	H19
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ESC_BA1744AA	54178	ERR025726	Human	1/1/1945	Sweden	Wellcome Trust Sange	-	H16
ESC_BA1745AA	54179	ERR025727	Human	1/1/1944	Sweden	Wellcome Trust Sange	-	H16
ESC_BA1748AA	54184	ERR025729	Human	1/1/1945	Sweden	Wellcome Trust Sange	-	H16
ESC_BA1750AA	54185	ERR025730	Human	1/1/1945	Denmark	Wellcome Trust Sange	-	H16
ESC_BA1751AA	54190	ERR025731	Human	1/1/1945	Sweden	Wellcome Trust Sange	-	H16
ESC_BA1753AA	54210	ERR025732	Human	1/1/1943	Sweden	Wellcome Trust Sange	-	H16
ESC_BA2107AA	2011-70-220-3	ERR769198	Livestock	1/1/2011	Denmark	Justus Liebig Universi	O23	H16
ESC_BA2109AA	2011-70-220-2	ERR769197	Livestock	1/1/2011	Denmark	Justus Liebig Universi	-	H28
ESC_BA2111AA	2011-70-219-9	ERR769196	Livestock	1/1/2011	Denmark	Justus Liebig Universi	O24	H4
ESC_BA2113AA	2011-70-219-7	ERR769195	Livestock	1/1/2011	Denmark	Justus Liebig Universi	O33	H18
ESC_BA2117AA	2011-70-219-4	ERR769193	Livestock	1/1/2011	Denmark	Justus Liebig Universi	-	H26
ESC_BA2119AA	2011-70-219-2	ERR769192	Livestock	1/1/2011	Denmark	Justus Liebig Universi	O76	H32

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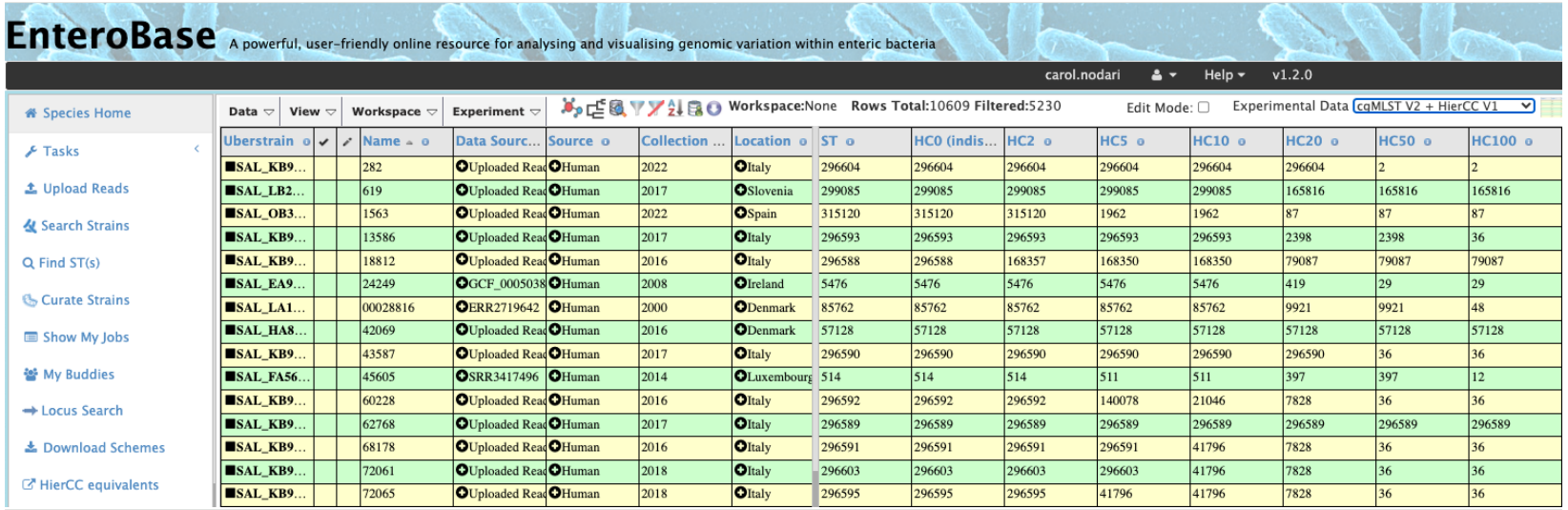
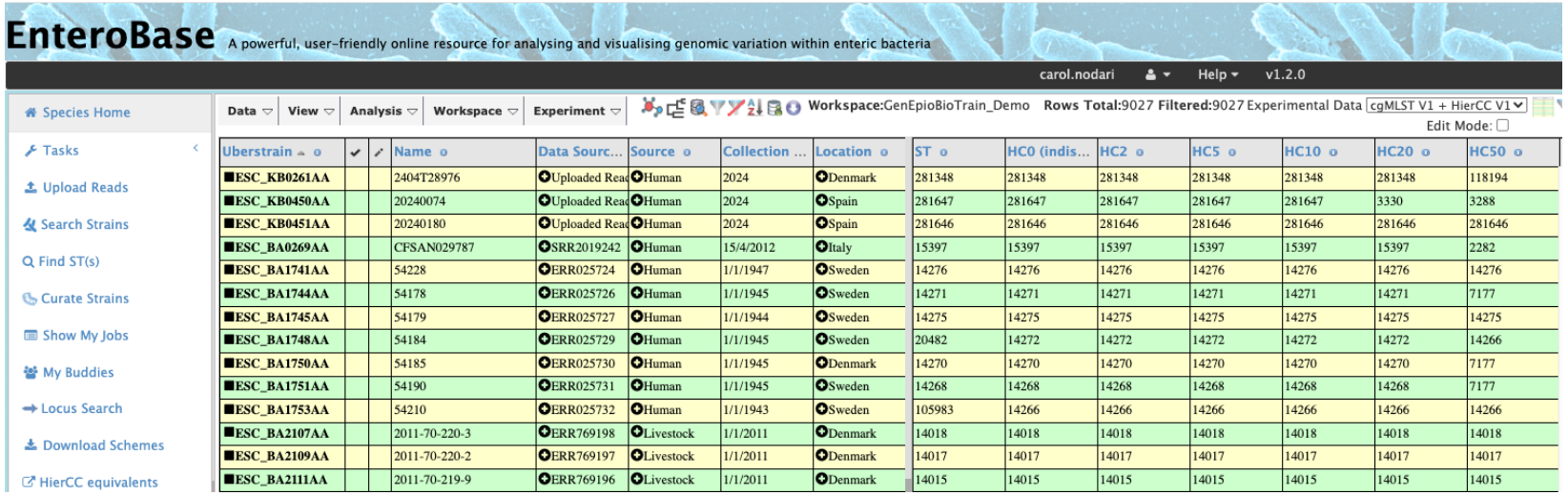


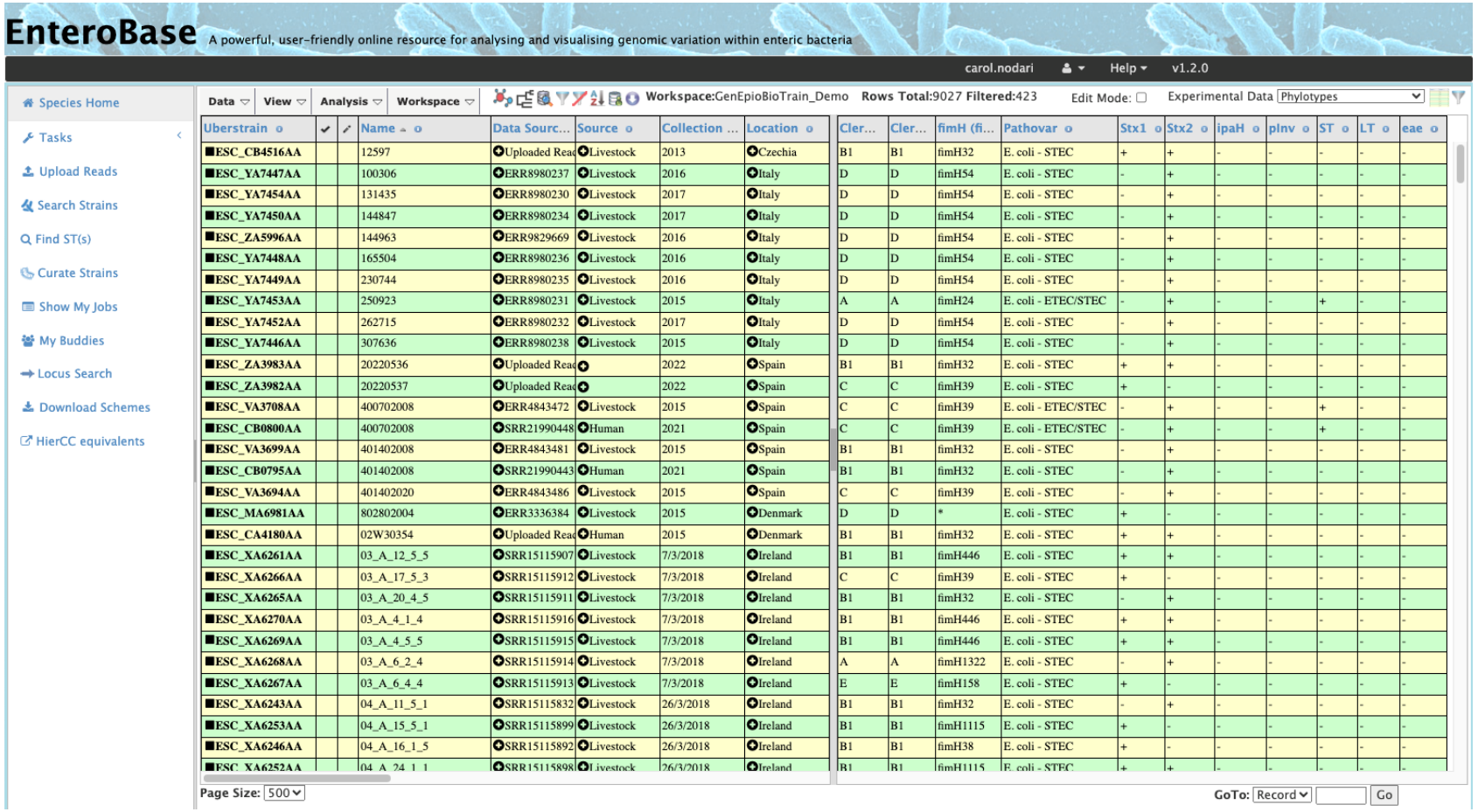
Uberstrain	Name	Data Sourc...	Source	Collection ...	Location	ST	HC0 (indis...	HC2	HC5	HC10	HC20	HC50	HC100
SAL_KB9...	282	Uploaded Reac	Human	2022	Italy	296604	296604	296604	296604	296604	296604	2	2
SAL_LB2...	619	Uploaded Reac	Human	2017	Slovenia	299085	299085	299085	299085	299085	165816	165816	165816
SAL_OB3...	1563	Uploaded Reac	Human	2022	Spain	315120	315120	315120	1962	1962	87	87	87
SAL_KB9...	13586	Uploaded Reac	Human	2017	Italy	296593	296593	296593	296593	296593	2398	2398	36
SAL_KB9...	18812	Uploaded Reac	Human	2016	Italy	296588	296588	168357	168350	168350	79087	79087	79087
SAL_EA9...	24249	GCF_0005038	Human	2008	Ireland	5476	5476	5476	5476	5476	419	29	29
SAL_LA1...	00028816	ERR2719642	Human	2000	Denmark	85762	85762	85762	85762	85762	9921	9921	48
SAL_HA8...	42069	Uploaded Reac	Human	2016	Denmark	57128	57128	57128	57128	57128	57128	57128	57128
SAL_KB9...	43587	Uploaded Reac	Human	2017	Italy	296590	296590	296590	296590	296590	296590	36	36
SAL_FA56...	45605	SRR3417496	Human	2014	Luxembourg	514	514	514	511	511	397	397	12
SAL_KB9...	60228	Uploaded Reac	Human	2016	Italy	296592	296592	296592	140078	21046	7828	36	36
SAL_KB9...	62768	Uploaded Reac	Human	2017	Italy	296589	296589	296589	296589	296589	296589	296589	296589
SAL_KB9...	68178	Uploaded Reac	Human	2016	Italy	296591	296591	296591	296591	41796	7828	36	36
SAL_KB9...	72061	Uploaded Reac	Human	2018	Italy	296603	296603	296603	296603	41796	7828	36	36
SAL_KB9...	72065	Uploaded Reac	Human	2018	Italy	296595	296595	296595	41796	41796	7828	36	36



Uberstrain	Name	Data Sourc...	Source	Collection ...	Location	ST	HC0 (indis...	HC2	HC5	HC10	HC20	HC50
ESC_KB0261AA	2404T28976	Uploaded Reac	Human	2024	Denmark	281348	281348	281348	281348	281348	281348	118194
ESC_KB0450AA	20240074	Uploaded Reac	Human	2024	Spain	281647	281647	281647	281647	281647	3330	3288
ESC_KB0451AA	20240180	Uploaded Reac	Human	2024	Spain	281646	281646	281646	281646	281646	281646	281646
ESC_BA0269AA	CFSAN029787	SRR2019242	Human	15/4/2012	Italy	15397	15397	15397	15397	15397	15397	2282
ESC_BA1741AA	54228	ERR025724	Human	1/1/1947	Sweden	14276	14276	14276	14276	14276	14276	14276
ESC_BA1744AA	54178	ERR025726	Human	1/1/1945	Sweden	14271	14271	14271	14271	14271	14271	7177
ESC_BA1745AA	54179	ERR025727	Human	1/1/1944	Sweden	14275	14275	14275	14275	14275	14275	14275
ESC_BA1748AA	54184	ERR025729	Human	1/1/1945	Sweden	20482	14272	14272	14272	14272	14272	14266
ESC_BA1750AA	54185	ERR025730	Human	1/1/1945	Denmark	14270	14270	14270	14270	14270	14270	7177
ESC_BA1751AA	54190	ERR025731	Human	1/1/1945	Sweden	14268	14268	14268	14268	14268	14268	7177
ESC_BA1753AA	54210	ERR025732	Human	1/1/1943	Sweden	105983	14266	14266	14266	14266	14266	14266
ESC_BA2107AA	2011-70-220-3	ERR769198	Livestock	1/1/2011	Denmark	14018	14018	14018	14018	14018	14018	14018
ESC_BA2109AA	2011-70-220-2	ERR769197	Livestock	1/1/2011	Denmark	14017	14017	14017	14017	14017	14017	14017
ESC_BA2111AA	2011-70-219-9	ERR769196	Livestock	1/1/2011	Denmark	14015	14015	14015	14015	14015	14015	14015

Experimental Data

- With the assembled genomes, a lot of data can be retrieved from the genomes.
- Among the features included in EnteroBase, we can find **serotype** prediction tools, which are specific for each pathogen.
- Recently, an **AMR analysis** using AMRFinderPlus has been implemented is currently been tested in small sets of genomes.
- Both 7 genes **MLST** and **cgMLST** can also be retrieved, as well as the **HierCC** information.
- **Pathotypes** of *E. coli*, except for EAEC, and *Shigella* serogroups can also be identified in the menu "Phylotypes".

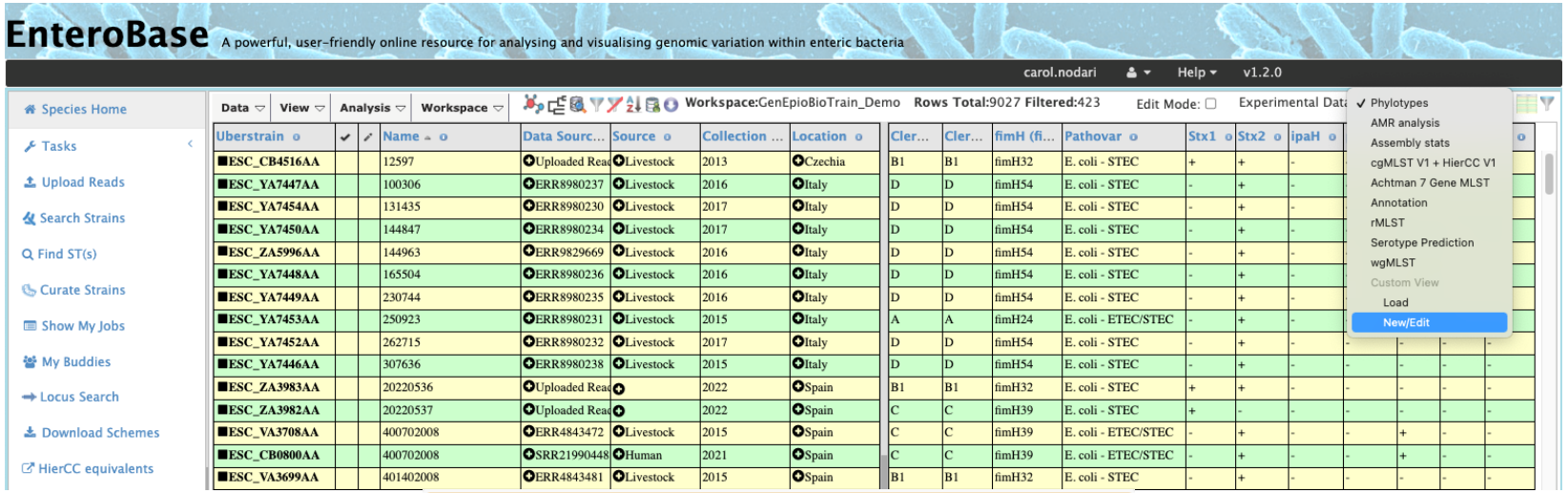


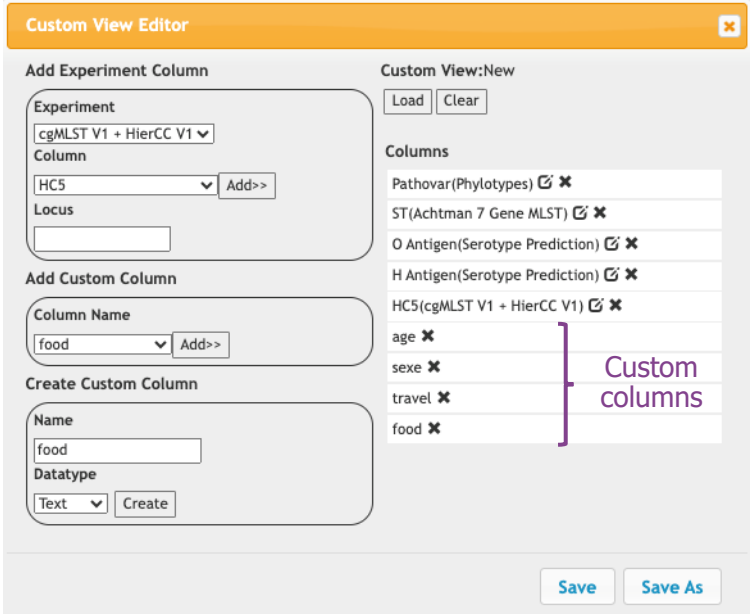
Uberstrain	Name	Data Sourc...	Source	Collection ...	Location	Cler...	Cler...	fimH (fi...	Pathovar	Stx1	Stx2	ipaH	plnv	ST	LT	eae
ESC_CB4516AA	12597	Uploaded Read	Livestock	2013	Czechia	B1	B1	fimH32	E. coli - STEC	+	+	-	-	-	-	-
ESC_YA7447AA	100306	ERR8980237	Livestock	2016	Italy	D	D	fimH54	E. coli - STEC	-	+	-	-	-	-	-
ESC_YA7454AA	131435	ERR8980230	Livestock	2017	Italy	D	D	fimH54	E. coli - STEC	-	+	-	-	-	-	-
ESC_YA7450AA	144847	ERR8980234	Livestock	2017	Italy	D	D	fimH54	E. coli - STEC	-	+	-	-	-	-	-
ESC_ZA5996AA	144963	ERR9829669	Livestock	2016	Italy	D	D	fimH54	E. coli - STEC	-	+	-	-	-	-	-
ESC_YA7448AA	165504	ERR8980236	Livestock	2016	Italy	D	D	fimH54	E. coli - STEC	-	+	-	-	-	-	-
ESC_YA7449AA	230744	ERR8980235	Livestock	2016	Italy	D	D	fimH54	E. coli - STEC	-	+	-	-	-	-	-
ESC_YA7453AA	250923	ERR8980231	Livestock	2015	Italy	A	A	fimH24	E. coli - ETEC/STEC	-	+	-	-	+	-	-
ESC_YA7452AA	262715	ERR8980232	Livestock	2017	Italy	D	D	fimH54	E. coli - STEC	-	+	-	-	-	-	-
ESC_YA7446AA	307636	ERR8980238	Livestock	2015	Italy	D	D	fimH54	E. coli - STEC	-	+	-	-	-	-	-
ESC_ZA3983AA	20220536	Uploaded Read		2022	Spain	B1	B1	fimH32	E. coli - STEC	+	+	-	-	-	-	-
ESC_ZA3982AA	20220537	Uploaded Read		2022	Spain	C	C	fimH39	E. coli - STEC	+	-	-	-	-	-	-
ESC_VA3708AA	400702008	ERR4843472	Livestock	2015	Spain	C	C	fimH39	E. coli - ETEC/STEC	-	+	-	-	+	-	-
ESC_CB0800AA	400702008	SRR21990448	Human	2021	Spain	C	C	fimH39	E. coli - ETEC/STEC	-	+	-	-	+	-	-
ESC_VA3699AA	401402008	ERR4843481	Livestock	2015	Spain	B1	B1	fimH32	E. coli - STEC	-	+	-	-	-	-	-
ESC_CB0795AA	401402008	SRR21990443	Human	2021	Spain	B1	B1	fimH32	E. coli - STEC	-	+	-	-	-	-	-
ESC_VA3694AA	401402020	ERR4843486	Livestock	2015	Spain	C	C	fimH39	E. coli - STEC	-	+	-	-	-	-	-
ESC_MA6981AA	802802004	ERR3336384	Livestock	2015	Denmark	D	D	*	E. coli - STEC	+	-	-	-	-	-	-
ESC_CA4180AA	02W30354	Uploaded Read	Human	2015	Denmark	B1	B1	fimH32	E. coli - STEC	+	+	-	-	-	-	-
ESC_XA6261AA	03_A_12_5_5	SRR15115907	Livestock	7/3/2018	Ireland	B1	B1	fimH446	E. coli - STEC	+	+	-	-	-	-	-
ESC_XA6266AA	03_A_17_5_3	SRR15115912	Livestock	7/3/2018	Ireland	C	C	fimH39	E. coli - STEC	+	-	-	-	-	-	-
ESC_XA6265AA	03_A_20_4_5	SRR15115911	Livestock	7/3/2018	Ireland	B1	B1	fimH32	E. coli - STEC	-	+	-	-	-	-	-
ESC_XA6270AA	03_A_4_1_4	SRR15115916	Livestock	7/3/2018	Ireland	B1	B1	fimH446	E. coli - STEC	+	+	-	-	-	-	-
ESC_XA6269AA	03_A_4_5_5	SRR15115915	Livestock	7/3/2018	Ireland	B1	B1	fimH446	E. coli - STEC	+	+	-	-	-	-	-
ESC_XA6268AA	03_A_6_2_4	SRR15115914	Livestock	7/3/2018	Ireland	A	A	fimH1322	E. coli - STEC	-	+	-	-	-	-	-
ESC_XA6267AA	03_A_6_4_4	SRR15115913	Livestock	7/3/2018	Ireland	E	E	fimH158	E. coli - STEC	+	-	-	-	-	-	-
ESC_XA6243AA	04_A_11_5_1	SRR15115832	Livestock	26/3/2018	Ireland	B1	B1	fimH32	E. coli - STEC	-	+	-	-	-	-	-
ESC_XA6253AA	04_A_15_5_1	SRR15115899	Livestock	26/3/2018	Ireland	B1	B1	fimH1115	E. coli - STEC	+	-	-	-	-	-	-
ESC_XA6246AA	04_A_16_1_5	SRR15115892	Livestock	26/3/2018	Ireland	B1	B1	fimH38	E. coli - STEC	+	-	-	-	-	-	-
ESC_XA6252AA	04_A_24_1_1	SRR15115898	Livestock	26/3/2018	Ireland	B1	B1	fimH1115	E. coli - STEC	+	+	-	-	-	-	-

“Custom View”

Custom View

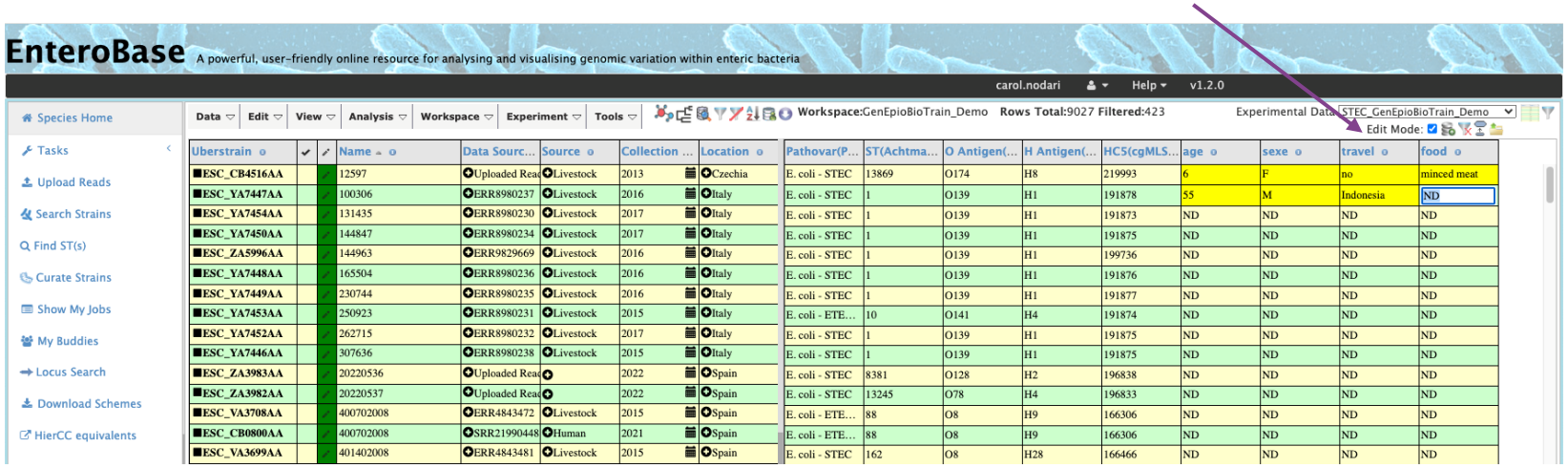
- To facilitate navigating between the data provided in the different menus, it is possible to create "**Custom views**" with chosen information.
- It is also possible to create and add data to "**Custom columns**" that are not public and/or part of the analysis pipeline from Enterobase.
- Unless specified by the user, the custom views and information in the custom columns remain **private**.
- Custom views can be **saved** and reused in future workspaces and **shared** with other (trusted) users.





Custom View

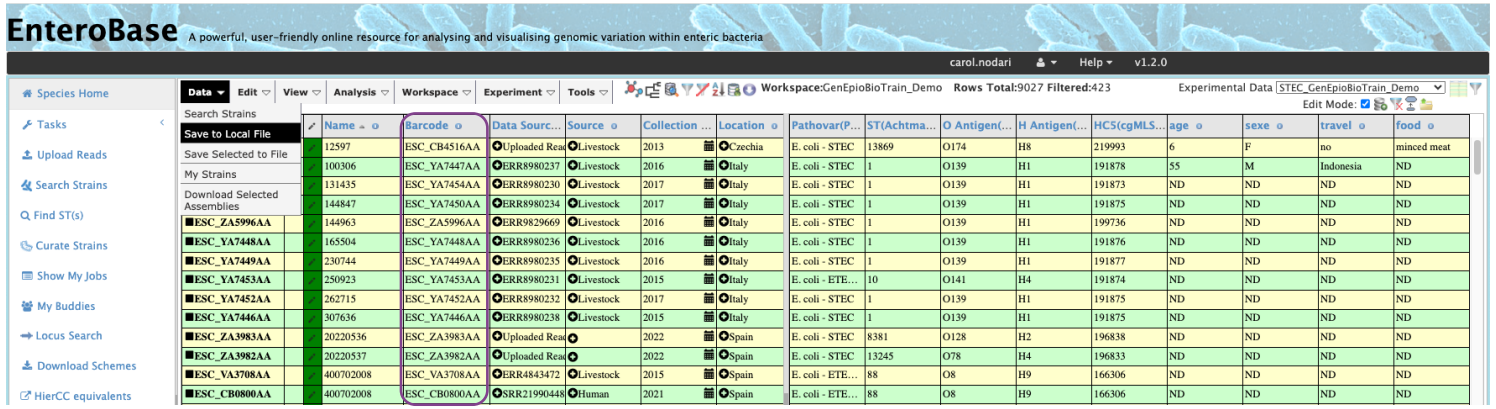
- With “Edit mode” activated, it is possible to include/update information in the custom columns. Unsaved changes will be shown in yellow.
- (Edit mode also needs to be activated to update metadata on the left side part of the table).
- Use “Experiment > Save changes” or right-click on the cells to save the added information.



Uberstrain	Name	Data Sourc...	Source	Collection ...	Location	Pathovar(P...	ST(Achtma...	O Antigen(...	H Antigen(...	HC5(cgMLS...	age	sexe	travel	food
ESC_CB4516AA	12597	Uploaded Reac	Livestock	2013	Czechia	E. coli - STEC	13869	O174	H8	219993	6	F	no	minced meat
ESC_YA7447AA	100306	ERR8980237	Livestock	2016	Italy	E. coli - STEC	1	O139	H1	191878	55	M	Indonesia	ND
ESC_YA7454AA	131435	ERR8980230	Livestock	2017	Italy	E. coli - STEC	1	O139	H1	191873	ND	ND	ND	ND
ESC_YA7450AA	144847	ERR8980234	Livestock	2017	Italy	E. coli - STEC	1	O139	H1	191875	ND	ND	ND	ND
ESC_ZA5996AA	144963	ERR9829669	Livestock	2016	Italy	E. coli - STEC	1	O139	H1	199736	ND	ND	ND	ND
ESC_YA7448AA	165504	ERR8980236	Livestock	2016	Italy	E. coli - STEC	1	O139	H1	191876	ND	ND	ND	ND
ESC_YA7449AA	230744	ERR8980235	Livestock	2016	Italy	E. coli - STEC	1	O139	H1	191877	ND	ND	ND	ND
ESC_YA7453AA	250923	ERR8980231	Livestock	2015	Italy	E. coli - ETE...	10	O141	H4	191874	ND	ND	ND	ND
ESC_YA7452AA	262715	ERR8980232	Livestock	2017	Italy	E. coli - STEC	1	O139	H1	191875	ND	ND	ND	ND
ESC_YA7446AA	307636	ERR8980238	Livestock	2015	Italy	E. coli - STEC	1	O139	H1	191875	ND	ND	ND	ND
ESC_ZA3983AA	20220536	Uploaded Reac		2022	Spain	E. coli - STEC	8381	O128	H2	196838	ND	ND	ND	ND
ESC_ZA3982AA	20220537	Uploaded Reac		2022	Spain	E. coli - STEC	13245	O78	H4	196833	ND	ND	ND	ND
ESC_VA3708AA	400702008	ERR4843472	Livestock	2015	Spain	E. coli - ETE...	88	O8	H9	166306	ND	ND	ND	ND
ESC_CB0800AA	400702008	SRR21990448	Human	2021	Spain	E. coli - ETE...	88	O8	H9	166306	ND	ND	ND	ND
ESC_VA3699AA	401402008	ERR4843481	Livestock	2015	Spain	E. coli - STEC	162	O8	H28	166466	ND	ND	ND	ND

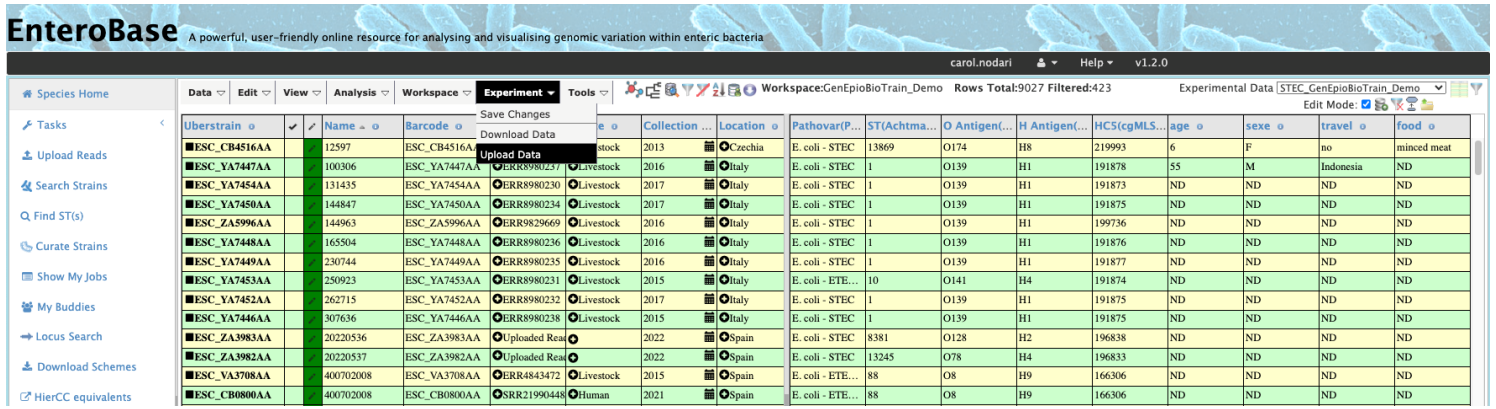
Custom View

- **Information** (metadata and/or data for custom columns) can be added in **batches** into EnteroBase.
- To allow correct correlation between the data and the strain, each strain on Enterobase contains a unique identifier – a "**Barcode**".
- **Strain barcodes** (which are different from assembly barcodes) can be found in the **left side panel** and **downloaded** to a local **.txt** file.
- After **updating** the local file with the information to be added, it can be **uploaded** again.



Name	Barcode	Data Sourc...	Source	Collection ...	Location	Pathovar(P...	ST(Achtma...	O Antigen(...	H Antigen(...	HC5(cgMLS...	age	sexe	travel	food
12597	ESC_CB4516AA	Uploaded Rea	Livestock	2013	Czechia	E. coli - STEC	13869	O174	H8	219993	6	F	no	minced meat
100306	ESC_YA7447AA	ERR8980237	Livestock	2016	Italy	E. coli - STEC	1	O139	H1	191878	55	M	Indonesia	ND
131435	ESC_YA7454AA	ERR8980230	Livestock	2017	Italy	E. coli - STEC	1	O139	H1	191873	ND	ND	ND	ND
144847	ESC_YA7450AA	ERR8980234	Livestock	2017	Italy	E. coli - STEC	1	O139	H1	191875	ND	ND	ND	ND
144963	ESC_ZA5996AA	ERR9829669	Livestock	2016	Italy	E. coli - STEC	1	O139	H1	199736	ND	ND	ND	ND
165504	ESC_YA7448AA	ERR8980236	Livestock	2016	Italy	E. coli - STEC	1	O139	H1	191876	ND	ND	ND	ND
230744	ESC_YA7449AA	ERR8980235	Livestock	2016	Italy	E. coli - STEC	1	O139	H1	191877	ND	ND	ND	ND
250923	ESC_YA7453AA	ERR8980231	Livestock	2015	Italy	E. coli - ETE...	10	O141	H4	191874	ND	ND	ND	ND
262715	ESC_YA7452AA	ERR8980232	Livestock	2017	Italy	E. coli - STEC	1	O139	H1	191875	ND	ND	ND	ND
307636	ESC_YA7446AA	ERR8980238	Livestock	2015	Italy	E. coli - STEC	1	O139	H1	191875	ND	ND	ND	ND
20220536	ESC_ZA3983AA	Uploaded Rea		2022	Spain	E. coli - STEC	8381	O128	H2	196838	ND	ND	ND	ND
20220537	ESC_ZA3982AA	Uploaded Rea		2022	Spain	E. coli - STEC	13245	O78	H4	196833	ND	ND	ND	ND
400702008	ESC_VA3708AA	ERR4843472	Livestock	2015	Spain	E. coli - ETE...	88	O8	H9	166306	ND	ND	ND	ND
400702008	ESC_CB0800AA	SRR21990448	Human	2021	Spain	E. coli - ETE...	88	O8	H9	166306	ND	ND	ND	ND

	A	B	C	AJ	AK	AL	AM	AN	AO	AP	AQ	AR
1	Uberstrain	Name	Barcode	Pathovar(Phylotypes	ST(Achtman 7 Gene	O Antigen(Serotype	H Antigen(Serotype	HC5(cgMLST V1 + H	age	sexe	travel	food
2	ESC_CB4516AA	12597	ESC_CB4516AA	E. coli - STEC	13869	O174	H8	219993	6	F	no	minced meat
3	ESC_YA7447AA	100306	ESC_YA7447AA	E. coli - STEC	1	O139	H1	191878	55	M	Indonesia	ND
4	ESC_YA7454AA	131435	ESC_YA7454AA	E. coli - STEC	1	O139	H1	191873	ND	ND	ND	ND
5	ESC_YA7450AA	144847	ESC_YA7450AA	E. coli - STEC	1	O139	H1	191875	ND	ND	ND	ND
6	ESC_ZA5996AA	144963	ESC_ZA5996AA	E. coli - STEC	1	O139	H1	199736	ND	ND	ND	ND
7	ESC_YA7448AA	165504	ESC_YA7448AA	E. coli - STEC	1	O139	H1	191876	ND	ND	ND	ND
8	ESC_YA7449AA	230744	ESC_YA7449AA	E. coli - STEC	1	O139	H1	191877	ND	ND	ND	ND
9	ESC_YA7453AA	250923	ESC_YA7453AA	E. coli - ETEC/STEC	10	O141	H4	191874	ND	ND	ND	ND
10	ESC_YA7452AA	262715	ESC_YA7452AA	E. coli - STEC	1	O139	H1	191875	ND	ND	ND	ND
11	ESC_YA7446AA	307636	ESC_YA7446AA	E. coli - STEC	1	O139	H1	191875	ND	ND	ND	ND



Uberstrain	Name	Barcode	Collection ...	Location	Pathovar(P...	ST(Achtma...	O Antigen(...	H Antigen(...	HC5(cgMLS...	age	sexe	travel	food
ESC_CB4516AA	12597	ESC_CB4516AA	2013	Czechia	E. coli - STEC	13869	O174	H8	219993	6	F	no	minced meat
ESC_YA7447AA	100306	ESC_YA7447AA	2016	Italy	E. coli - STEC	1	O139	H1	191878	55	M	Indonesia	ND
ESC_YA7454AA	131435	ESC_YA7454AA	2017	Italy	E. coli - STEC	1	O139	H1	191873	ND	ND	ND	ND
ESC_YA7450AA	144847	ESC_YA7450AA	2017	Italy	E. coli - STEC	1	O139	H1	191875	ND	ND	ND	ND
ESC_ZA5996AA	144963	ESC_ZA5996AA	2016	Italy	E. coli - STEC	1	O139	H1	199736	ND	ND	ND	ND
ESC_YA7448AA	165504	ESC_YA7448AA	2016	Italy	E. coli - STEC	1	O139	H1	191876	ND	ND	ND	ND
ESC_YA7449AA	230744	ESC_YA7449AA	2016	Italy	E. coli - STEC	1	O139	H1	191877	ND	ND	ND	ND
ESC_YA7453AA	250923	ESC_YA7453AA	2015	Italy	E. coli - ETE...	10	O141	H4	191874	ND	ND	ND	ND
ESC_YA7452AA	262715	ESC_YA7452AA	2017	Italy	E. coli - STEC	1	O139	H1	191875	ND	ND	ND	ND
ESC_YA7446AA	307636	ESC_YA7446AA	2015	Italy	E. coli - STEC	1	O139	H1	191875	ND	ND	ND	ND
ESC_ZA3983AA	20220536	ESC_ZA3983AA	2022	Spain	E. coli - STEC	8381	O128	H2	196838	ND	ND	ND	ND
ESC_ZA3982AA	20220537	ESC_ZA3982AA	2022	Spain	E. coli - STEC	13245	O78	H4	196833	ND	ND	ND	ND
ESC_VA3708AA	400702008	ESC_VA3708AA	2015	Spain	E. coli - ETE...	88	O8	H9	166306	ND	ND	ND	ND
ESC_CB0800AA	400702008	ESC_CB0800AA	2021	Spain	E. coli - ETE...	88	O8	H9	166306	ND	ND	ND	ND

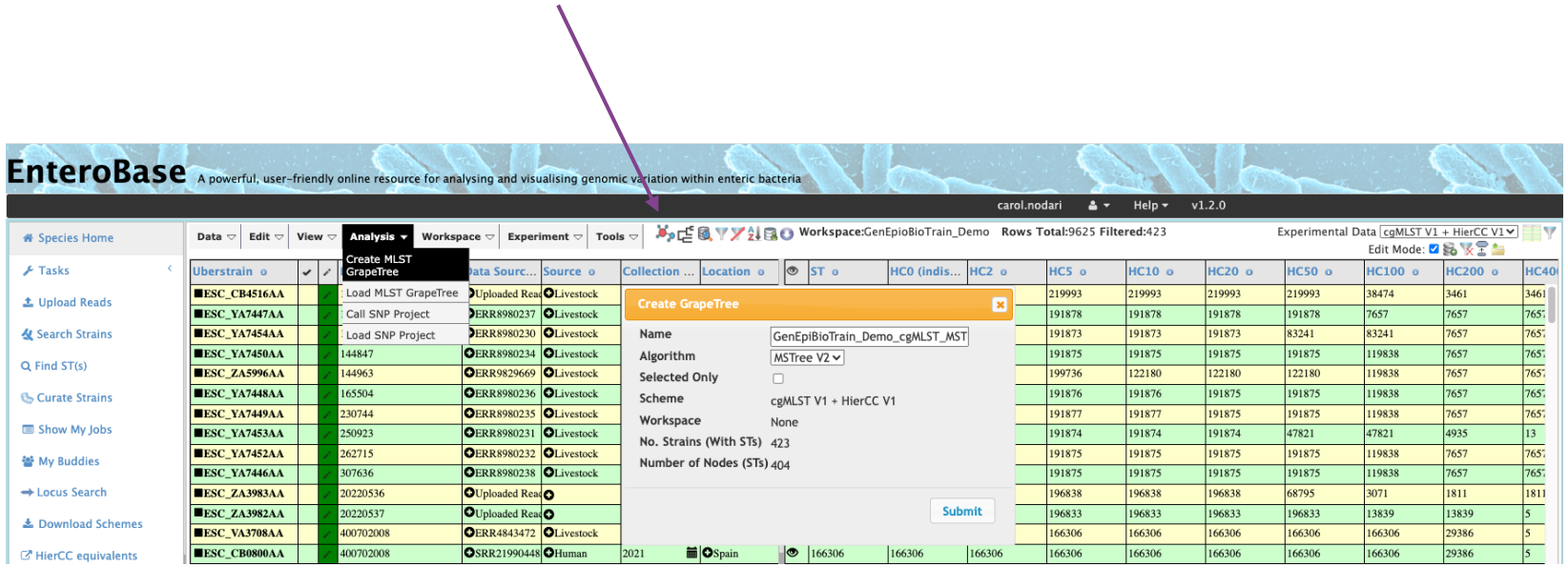
Tree visualization and data export

cgMLST-based MS tree

- Different tools are available on EnteroBase to visualize **phylogenetic relationships** between genomes in a **workspace**.

- It is possible to generate **MS trees** directly on Enterobase based on the **cgMLST** + HierCC data.

- For **epidemiological** purposes, it is recommended to build a **minimum-spanning tree** (MS tree).



cgMLST-based MS tree

- By default, tree nodes are **coloured** using the "**Country**" metadata available. Other options are available using the menu "Tree Layout > Node style > Colour by".

- To easily visualize **allele distances**, use "Tree Layout > Branch style > Show labels".

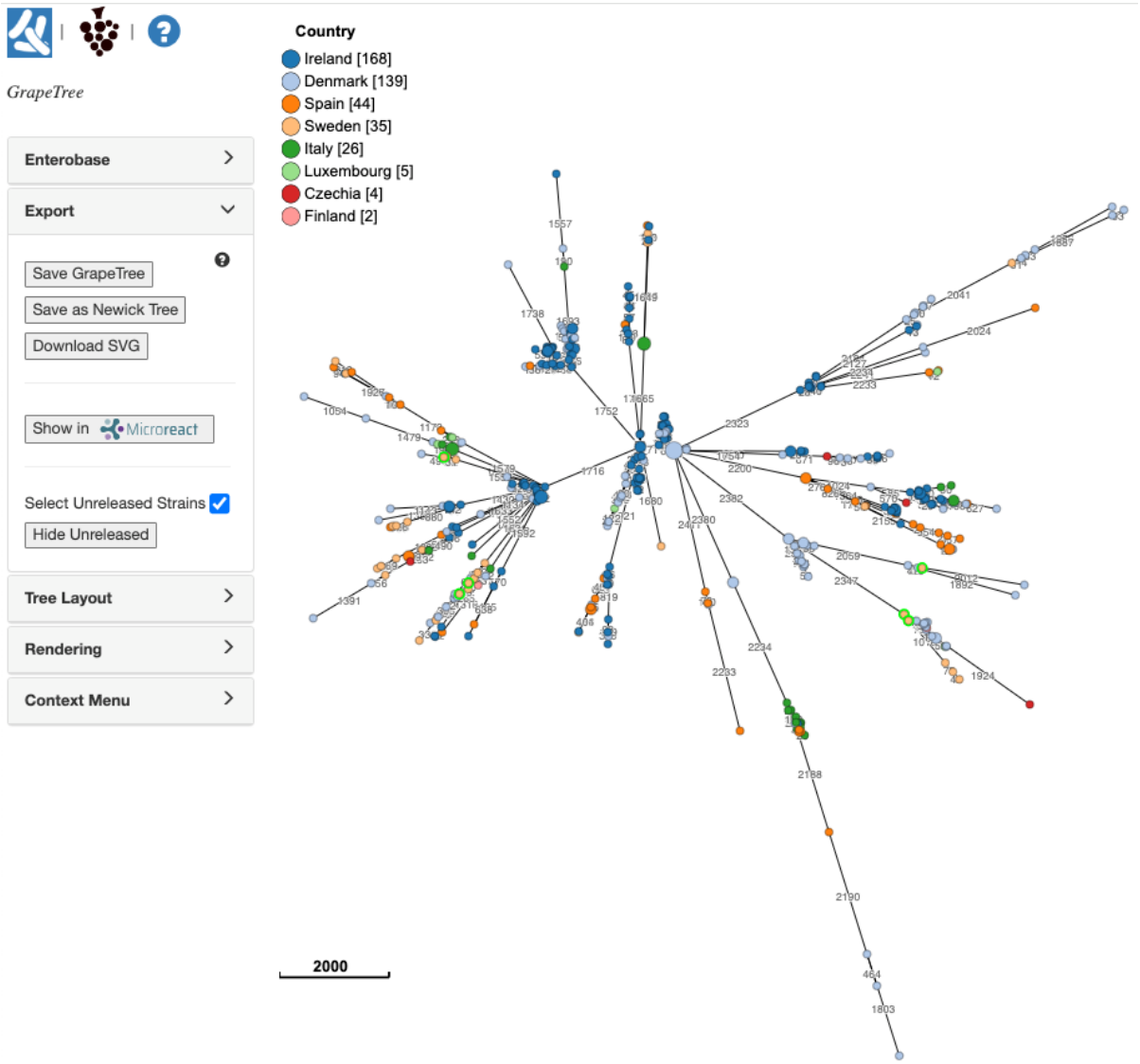
- **Experimental and custom data** can also be used to colour nodes, by they first need to be imported using the "Enterobase > Import fields" button.

cgMLST-based MS tree

- Trees can also be **exported** in a variety of formats using the different the buttons in the "Export" menu.

- To be able to properly download the tree, **all strains present in the tree need to belong to you or be public**. To **check and remove any unreleased strains** from the tree, use "Select Unreleased Strains > Hide Unreleased".

- Unfortunately, the option "Show in Microreact" is no longer functional.

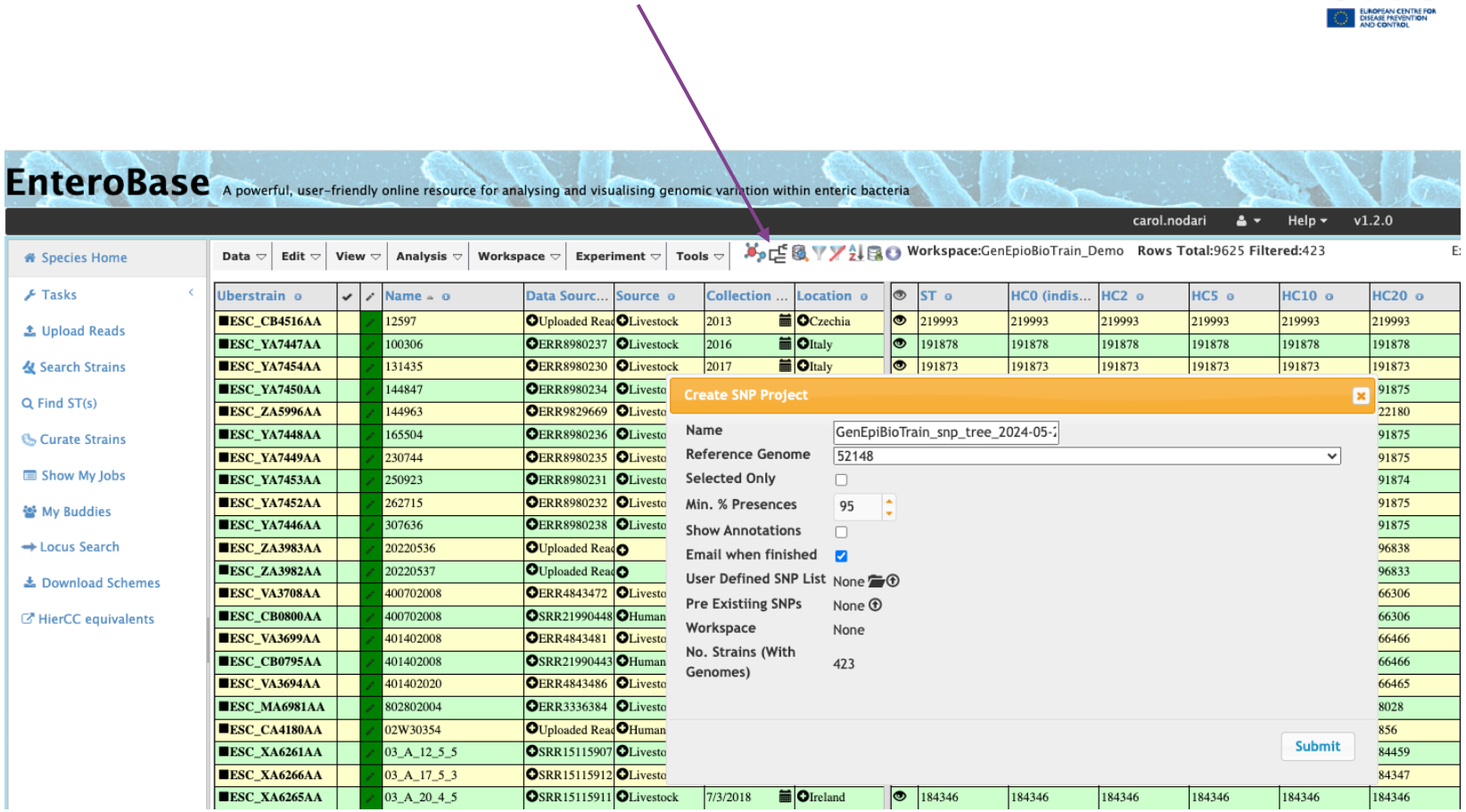


SNP tree

- SNP trees can also be built with **a selection or all the genomes** from a workspace using the **dendrogram** icon.

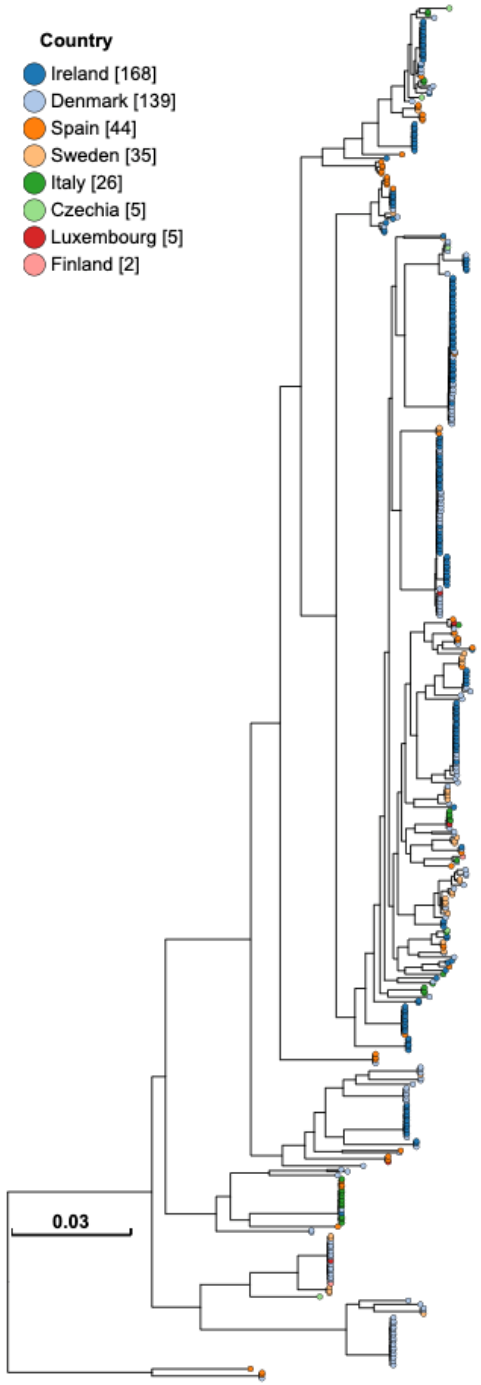
- The **reference genome** used for the SNP calling **must be part of the workspace** and should preferably be a complete genome (but draft genomes can also be used).

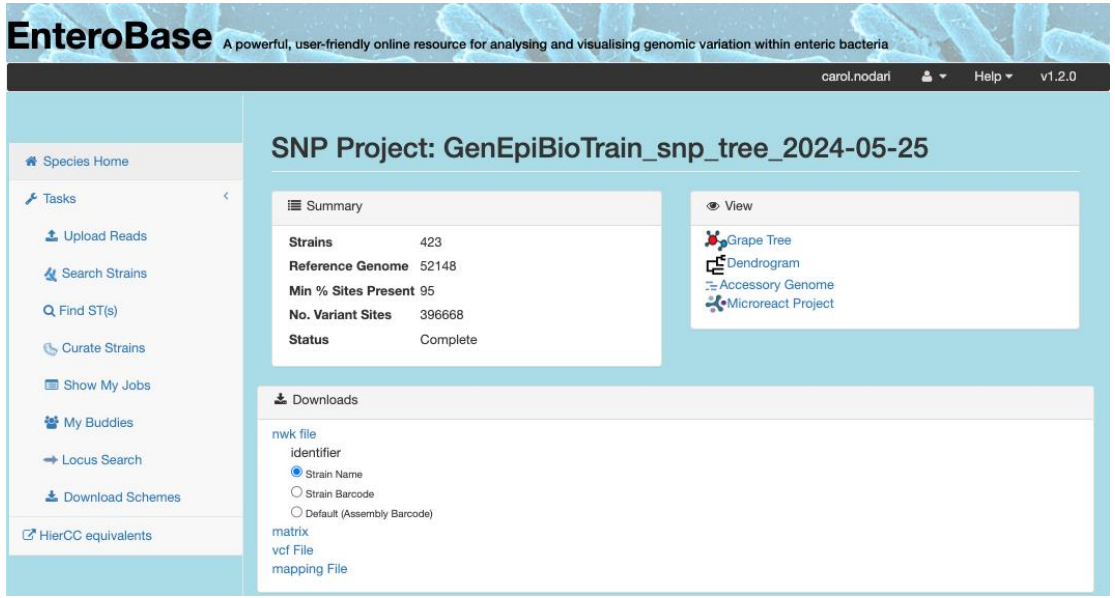
- Since this job usually **takes some time**, it is possible to receive an email alert once it is completed.



SNP tree

- Node colouring and tree exports work similarly to the MS trees.
- It is also possible to **download a SNP matrix** (similar to a .vcf file) showing the location of the SNPs in each genome present in the tree.
- SNP projects on EnteroBase **do not remove recombining regions**, so it is not unusual to observe abnormally long branches.





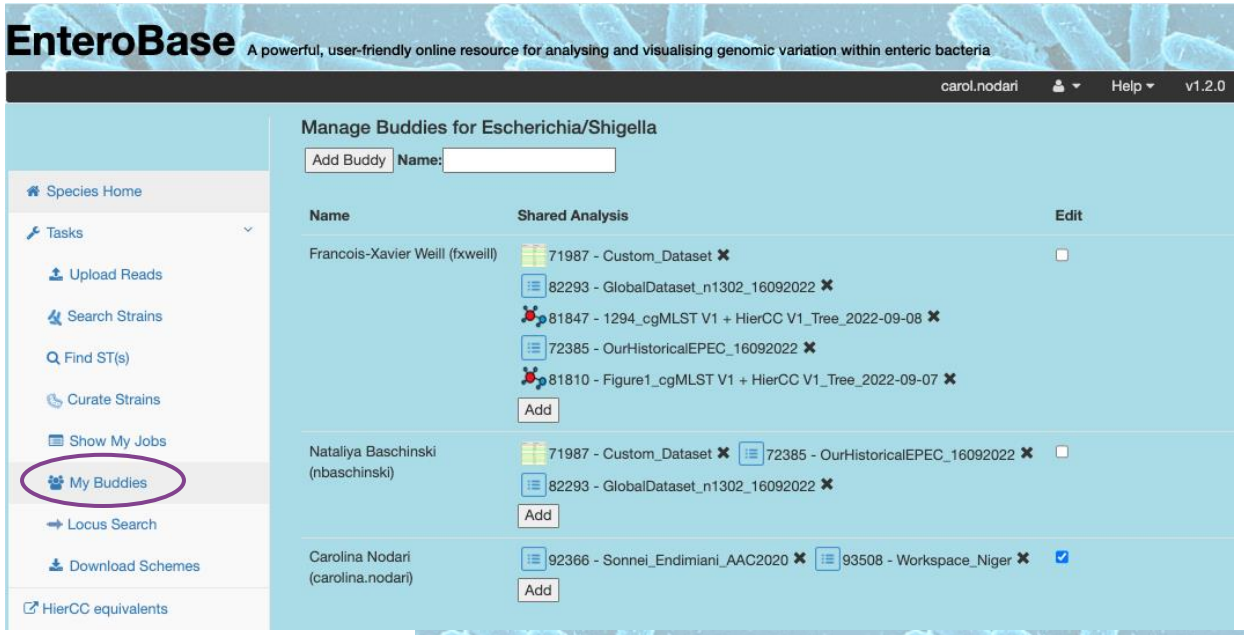
Data sharing

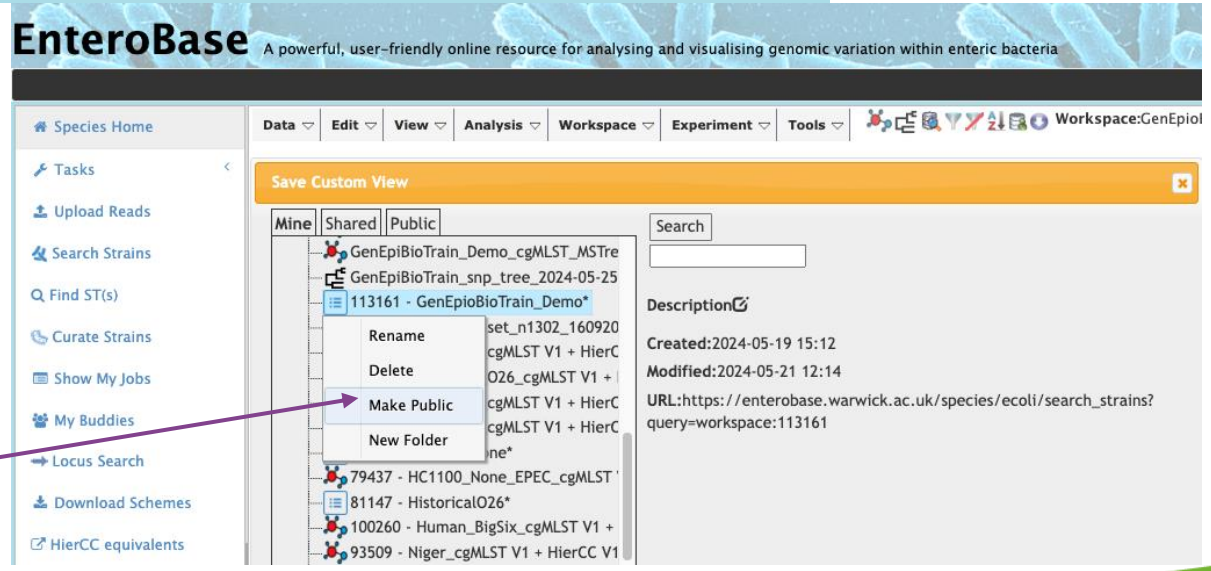
Data sharing

- **Workspaces**, **trees** and **custom views**, even when **private**/unreleased, can be shared with trusted user using the "**buddies**" system from EnteroBase.

- Depending on the rights given to the user, they can **visualize** and/or **edit** information, including **strain metadata**.

- It is also possible to make workspaces and tree **public to all EnteroBase users** by right-clicking on a given project in the "Workspace > Load" menu.





In summary

- EnteroBase is a free web-based tool for visualization and analysis of genomes from different bacterial pathogens;
- It includes a back-end pipeline of quality control and genome assembling, as well as a large set of analysis tools;
- It hosts and curates the cgMLST schemes of different foodborne pathogens like *E. coli*, *Shigella* and *Salmonella*;
- The HierCC schemes provides a uniform nomenclature for cgMLST analysis, facilitating the exchange of information for epidemiological purposes;
- Data can be shared and exported in a simple way with trusted users as well as made publicly.

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- Zhou Z, Alikhan NF, Sergeant MJ, et al. GrapeTree: visualization of core genomic relationships among 100,000 bacterial pathogens. Genome Res. 2018;28(9):1395-1404. doi:10.1101/gr.232397.117

- Zhou Z, Charlesworth J, Achtman M. HierCC: a multi-level clustering scheme for population assignments based on core genome MLST. Bioinformatics. 2021;37(20):3645-3646. doi:10.1093/bioinformatics/btab234

- https://enterobase.readthedocs.io/en/latest/index.html

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