

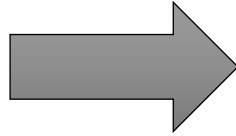
Genomic and metagenomic approaches for the characterization of bacteria and microbial communities of ecological niches relevant for the human health

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PhD Workshop, 4th December 2020

Next Generation Sequencing (NGS)



Crucial for genomics and metagenomics:

- Big data
- Short time
- Tools replacing in vitro techniques

Thesis objectives:

Application of genomic and metagenomic techniques to food-matrices and human gut microbiota:

- Comparative genomics of *Leuconostoc carnosum*
- Metagenomic investigation of β -glucuronidases (GUS) in the human gut microbiome
- 16S rRNA metagenomic profiling: mucin degraders in gut microbiota and the metagenome of *Hermetia illucens* larvae

Comparative genomics of *Leuconostoc carnosum*

Introduction and objectives

Leuconostoc carnosum:

- Lactic acid bacterium;
- Meat products colonizer;
- Associated to spoilage;
- Also proposed as bioprotective starter due to production of bacteriocins effective against *Listeria monocytogenes*

12 *Leuconostoc carnosum* strains previously taxonomically characterized through 16S rRNA sequencing.

Isolated from cooked ham packaged in modified atmosphere and fresh sausage, produced by European producers from different countries



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Microbiota of sliced cooked ham packaged in modified atmosphere throughout the shelf life:
Microbiota of sliced cooked ham in MAP

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Bacterial community of industrial raw sausage packaged in modified atmosphere throughout the shelf life

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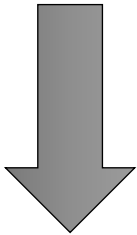
Objectives:

- Study the genetic variability of *L. carnosum* through WGS technology, including 5 publicly available genomes
- Reconstruct metabolic capabilities

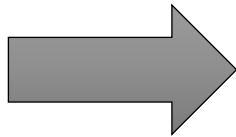
Workflow

Sequencing and *in silico* analysis

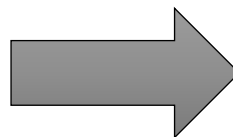
DNA
extraction



Sequencing



Assembly



- Identification
- Plasmid prediction
- Annotation
- Phylogeny
- Pangenome
- Average Nucleotide Identity (ANI)
- digital-DNA/DNA hybridization (dDDH)
- *In silico* bacteriocins identification
- Metabolic reconstruction
- Research of phages and CRISPR/Cas
- Antibiotic resistance

Results

Strains	Source	Genome size (bp)	No. of contigs	N50	L50	Coverage	G+C (%)	No. of CDS	No. of tRNA	Plasmids
WC0318	Cooked ham	1,745,630	15	1142374	1	638	37.2	1739	51	pFRA18, pALB18
WC0319	Cooked ham	1,700,071	11	1137580	1	521	37.1	1679	49	pELI19
WC0320	Cooked ham	1,812,114	18	1109738	1	561	37.1	1853	51	pFRA20, pALB20, pICCOLO
WC0321	Cooked ham	1,804,293	40	1106035	1	532	37.1	1854	51	pFRA21, pGLO21, pALB21
WC0322	Cooked ham	1,853,239	16	306690	2	515	37.0	1898	50	pALB22, pLQ22
WC0323	Cooked ham	1,773,698	23	256026	3	560	37.2	1802	51	pFRA23, pGLO23
WC0324	Cooked ham	1,765,760	13	1137276	1	460	37.1	1768	49	pELI24
WC0325	Cooked ham	1,830,248	27	229291	3	502	37.2	1881	51	pLQ25, pICCOLO
WC0326	Sausage	1,770,048	22	412621	2	574	37.2	1767	49	pSTE
WC0327	Sausage	1,769,683	20	412621	2	568	37.2	1768	49	pSTE
WC0328	Sausage	1,815,949	36	239739	3	538	37.1	1826	51	pALAN28, pUFO, pELI28
WC0329	Cooked ham	1,650,966	14	256123	3	455	37.2	1644	51	pFRO29
JB16	Kimchi	1,645,096	5	1645096	1	-	37.2	1686	64	pKLC1 (21) pKLC2 (34) pKLC3 (45) pKLC4 (39)
CBA3620	Kimchi	1,590,008	3	1590008	1	-	37.4	1635	66	unnamed1 (69) unnamed2 (67)
MFPC16A2803	Packaged beef	1,786,865	50	242386	3	-	37	1846	32	pMFPC16A2803B (26) pMFPC16A28E (3)
MFPA29A1405	Packaged beef	1,634,774	22	165377	4	-	37.3	1660	46	pMFPA29A1405B (24) pMFPA2A1405C (14)
DSM 5576	Packaged beef	1,820,660	21	147749	4	-	37	1740	47	-

	WC0318	WC0319	WC0320	WC0321	WC0322	WC0323	WC0324	WC0325	WC0326	WC0327	WC0328	WC0329	JB16	CBA3620	MFPC16A2803	MFPA29A1405	DSM 5576
WC0318		99.0	99.8	99.7	99.6	99.7	99.0	99.7	99.7	99.7	99.1	99.7	99.1	99.1	99.8	99.8	99.6
WC0319	91.7		98.9	99.0	99.0	99.0	100	99.0	98.9	98.9	99.2	99.0	99.2	99.2	99.0	99.0	98.9
WC0320	98.2	90.8		99.7	99.7	99.8	98.9	99.7	99.8	99.8	99.0	99.8	99.1	99.0	99.8	99.8	99.7
WC0321	97.7	91.6	96.8		99.8	100	99.0	99.8	99.8	99.8	99.1	99.9	99.1	99.2	99.9	100	99.8
WC0322	97.5	91.7	95.5	97.7		99.8	99.0	99.8	99.7	99.7	99.0	99.9	99.1	99.1	99.8	99.9	99.8
WC0323	98.3	91.5	97.4	99.6	97.9		99.0	99.8	99.8	99.8	99.1	99.9	99.1	99.2	99.9	100	99.9
WC0324	91.7	99.7	92.2	90.7	91.4	91.4		99.0	98.9	98.9	99.1	99.0	99.2	99.2	99.0	99.0	98.9
WC0325	97.4	92.1	97.1	97.2	96.9	97.6	91.7		99.7	99.7	99.1	99.9	99.2	99.1	99.9	99.9	99.8
WC0326	98.0	91.0	98.0	98.1	98.2	98.2	91.0	97.6		100.0	99.1	99.8	99.1	99.1	99.9	99.8	99.7
WC0327	98.0	91.0	98.0	98.1	98.2	98.2	91.0	97.7	100		99.1	99.8	99.1	99.1	99.9	99.8	99.7
WC0328	92.6	93.4	90.5	91.8	90.6	92.3	92.9	91.3	92.3	92.3		99.1	100	99.8	99.2	99.2	99.1
WC0329	98.3	91.7	98.3	99.6	99.4	99.6	91.8	99.2	98.4	98.4	93.3		99.2	99.2	99.9	100	99.9
JB16	93.2	94.2	92.9	93.4	92.1	93.4	94.2	93.4	92.7	92.7	99.7	93.8		99.9	99.2	99.2	99.1
CBA3620	92.9	93.8	92.2	93.8	92.5	93.8	93.8	93.4	92.7	92.7	98.4	93.8	99.1		99.1	99.2	99.1
MFPC16A2803	98.8	92.1	98.1	99.1	97.4	98.7	91.9	98.9	99.2	99.2	92.6	99.5	93.6	93.4		99.9	99.9
MFPA29A1405	98.5	92.2	98.7	99.7	99.3	99.6	92.2	99.0	98.9	98.9	93.6	100	93.7	93.8	100		99.9
DSM 5576T	96.7	91.3	96.7	98.2	97.2	98.7	91.5	98.6	97.1	97.1	92.1	99.1	91.9	93.5	99.6	99.4	

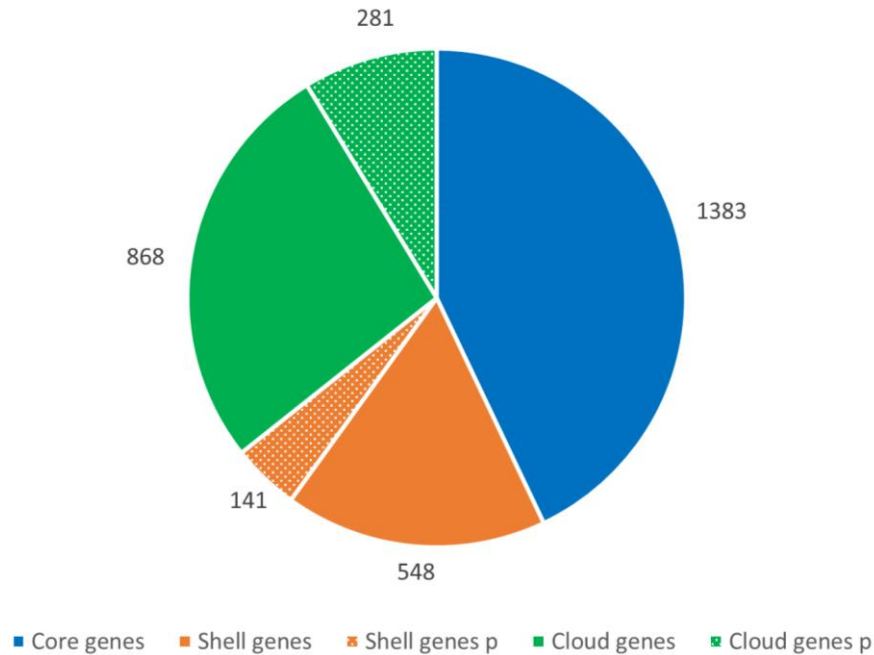
ANI

dDDH

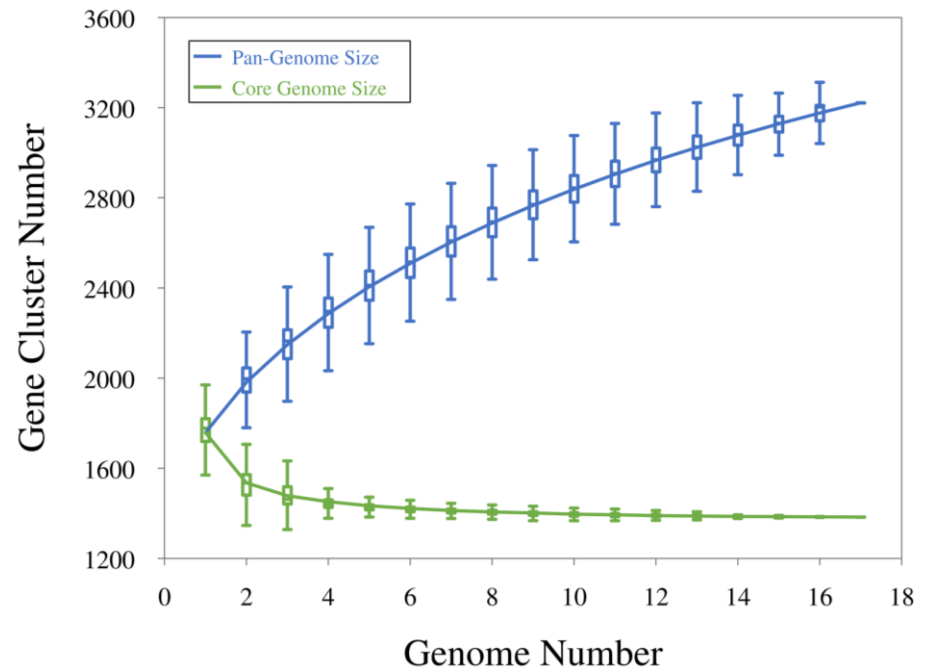
dDDH > 90.5% (species demarcation 70%)

ANI > 98.9% (species demarcation 95%)

Leuconostoc carnosum pangenome

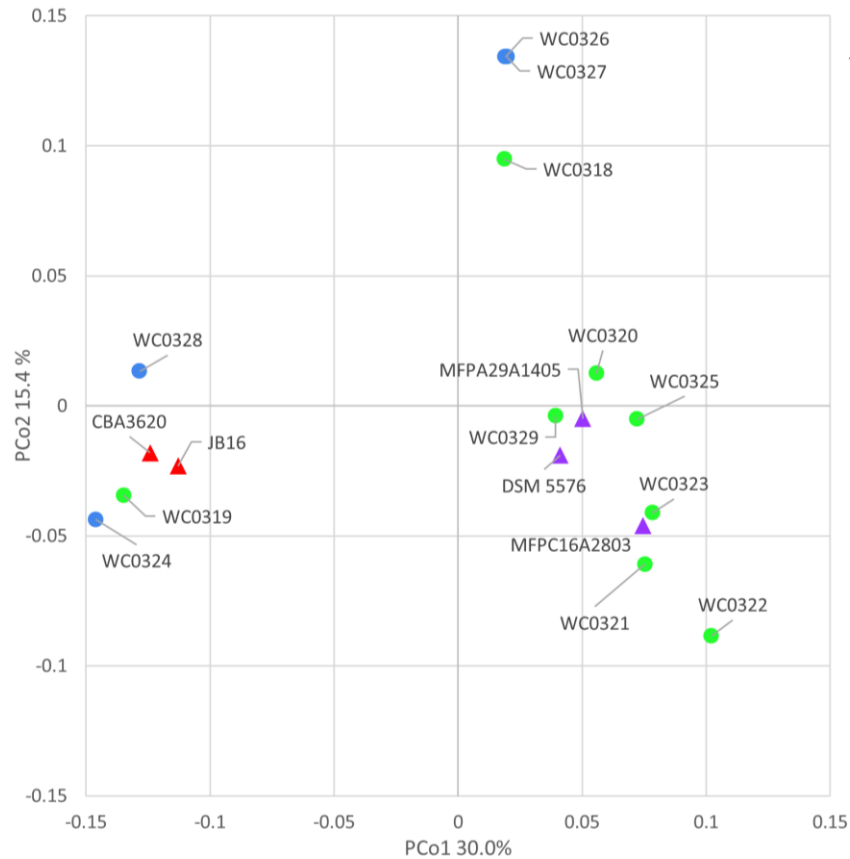


Total genes: 3221
Core genes: 1383
Shell genes: 689 (141 in plasmids)
Cloud genes: 868 (281 in plasmids)

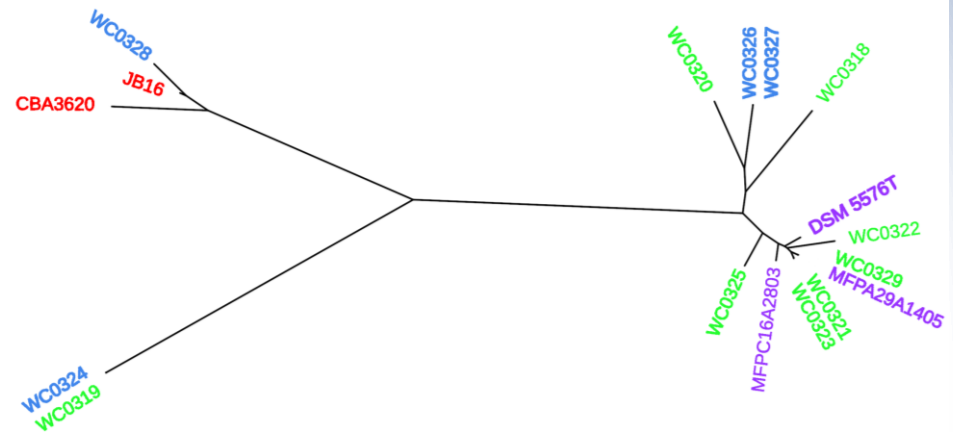


Open pangenome
Heaps' Law $\gamma = 0.35$ ($\gamma > 0$)

PCoA based on Jaccard distance due to presence/absence of genes



Tree scale: 0.01



Low phylogenetic distance
3 major clades



This work



Public genomes

Isolation source:

Sausages

Cooked ham

Packaged beef

Kimchi

Plasmids

20 different plasmids

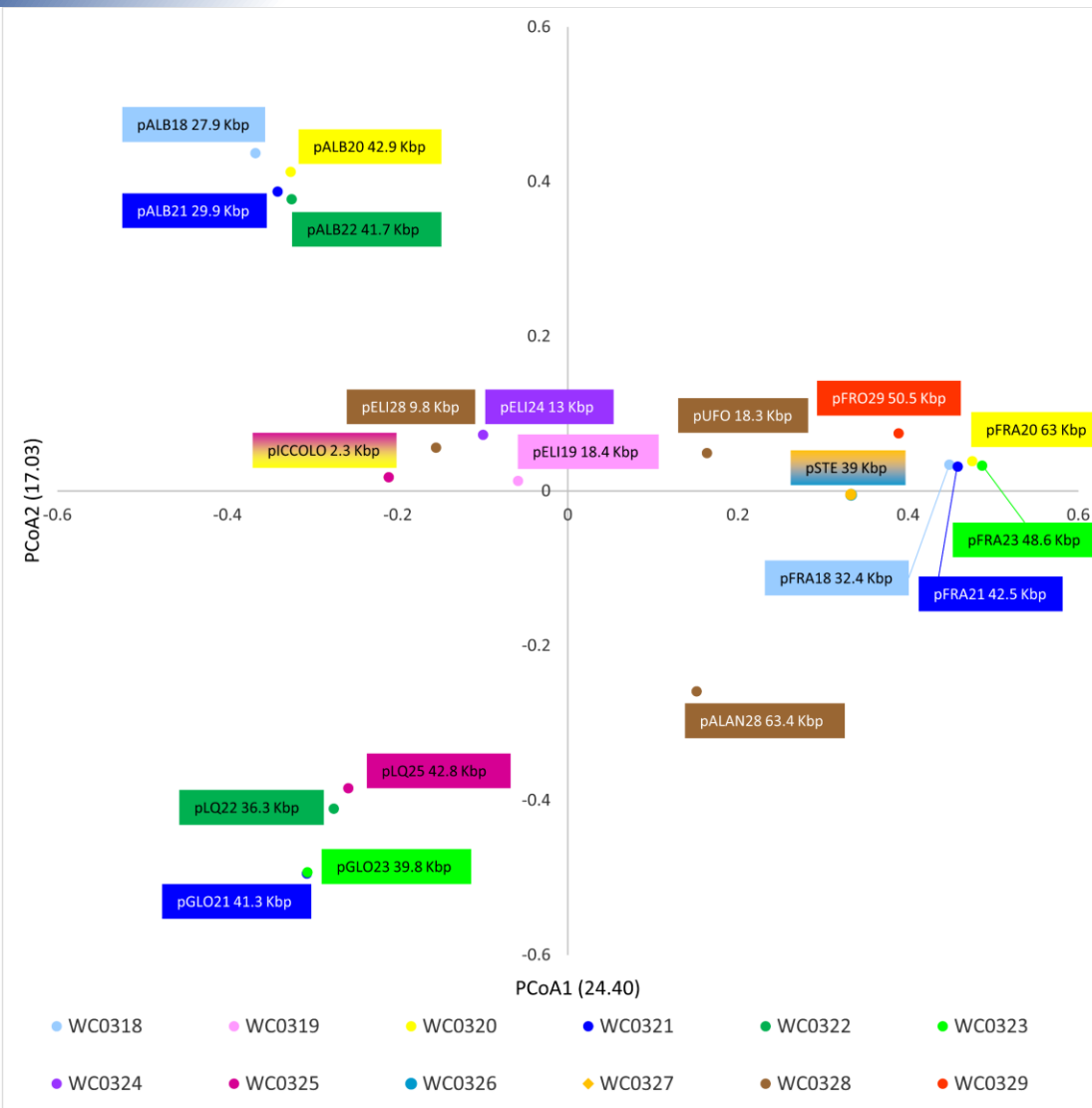
Length: 2351 – 63454 bp

CDS: 3 – 72

Relevant features:

- Toxin-antitoxin systems (pSTE; pLQ25; pGLO)
- Stress response and arsenic and heavy metal detoxification genes
- Bacteriocin (pFRA21; pFRA23; pFRO29; pALAN28)

Identified Leucocin-B, effective against *Listeria monocytogenes*



PCoA based on Jaccard distance due to presence/absence of genes in plasmids

Reconstruction of metabolic features

Core features:

- Pentose phosphate and phosphoketolase pathway genes
- Genes for acetoin and 2-3 propandiol from pyruvate
- 23 peptidase genes (with 2 more genes in accessory genome)
- Unable to synthesize any biogenic amine
- Biosynthesis of menaquinone
- Aminoacids biosynthesis:
 - From pre-formed serine: glycine and cysteine
 - From pre-formed glutamate: glutamine, arginine, and proline
 - From pre-formed aspartate: asparagine, threonine, and methionine
 - Complete pathways for branched aminoacids
 - Tryptophan synthesis (except WC0318, WC0319, WC0324)

Other relevant features:

- Vancomycin-resistant (due to D-Ala-D-Ala ligase)
- Type II CRISPR-Cas locus identified in WC0319 and WC0324
- Leucocin-B (WC0321, WC0323, WC0329), mesentericins (WC0328) on plasmids; putative lactococcin in almost all the strains (10/12)

Conclusions

L. carnosum: a compact group of bacteria, with open pangenome

From 1 to 3 plasmids per strain

Autotrophy for several aminoacids and other compounds (i.e. nucleotides, vitamins, cofactors) related to nitrogen-rich environment

Potential bacteriocin synthesis for application as bioprotective starter

**THANK YOU FOR
YOUR ATTENTION!**

