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WGS CHARACTERIZATION OF *ENTEROCOCCUS FAECALIS* H1041 ISOLATED FROM THE TRADITIONAL BULGARIAN GREEN CHEESE

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ABSTRACT

In this study, *Enterococcus faecalis* H1041 which was isolated from the traditional Bulgarian green cheese was characterized in depth by a whole-genome sequencing performed on the Illumina HiSeq platform using 2 x 150 bases paired-end reads mode. The bioinformatic analysis performed only with free online tools revealed that the strain resembles other cheese-born *E. faecalis* strains by its antibiotics' resistances and virulence genotype, thus its presence within the green cheese should not be considered as a health risk. Only two antibiotic resistance genetic determinants were found, but they both carry mutations, and probably are not functional. Furthermore, 18 virulence factor genetic determinants were found, but only 6 of them possessed 100% identities with the canonical sequences, and being more or less intrinsic for the *Enterococcus* genus. None of them were found on contigs that contain sequences with known plasmid origin of replication. Enterolysin A gene was also detected which most probably plays role in ripening process of the Bulgarian green cheese.

Keywords: *Enterococcus faecalis*; whole-genome sequencing (WGS); Bulgarian green cheese isolate

INTRODUCTION

The members of the *Enterococcus* genus have an ambiguous nature. Many of them are part of the mammalian and human gastrointestinal microflora, thus having the potential to be opportunistic pathogens causing nosocomial infections (McBride et al., 2007), while others play an important role in the dairy, meat, and other food industries, participating in the fermentation processes and contributing to the organoleptic properties of the foods (Ogier and Serror, 2008). They also could have a protective role in food ripening by inhibiting pathogen species as reported (Álvarez et al., 2020). This implies that in every case a new *Enterococcus* strain is isolated from a food source, before declaring it safe for use, a complete characterization is obligatory. This characterization includes exact taxonomic identification, antibiotic resistances description, and presence of virulence factors (Ogier and Serror, 2008).

The Bulgarian green cheese is the only kind of cheese in Bulgaria which ripening is marked out by noble molds. This cheese which is considered as a part of the national cultural heritage is produced artisanally from raw ewe's or goat's milk only in the village Tcherni Vit (Teteven municipality). As no starter culture is added, it relies only on environmental microbiota, most probably depending on the specific climatic conditions in the village, which explains the geographic limitations of its production.

In this study, we used the Illumina HiSeq NGS platform generating 2 x 150 bases paired-end reads to characterize an *E. faecalis* isolate from Bulgarian green cheese, which was revealed to be a fast, cost- and labor-effective method for the assessment of newly isolated food-borne bacterial strains.

MATERIALS AND METHODS

Strains isolation

50 mg from the core part of the Bulgarian green cheese were taken off in sterile conditions, and homogenized within 1 ml of sterile peptone water (PW). 10-fold serial dilutions in PW were made up to 10^{-4}, then 100 µl of the 10^{-3} and 10^{-4} dilutions were plated on Petri dishes containing the selective medium D-coccosel (Biomériuex). After incubation for 36-48 hours at 30 °C, colonies with black halos appeared which were picked up and inoculated in 3 ml BHI liquid broth, and incubated at 30 °C for 24 hours. After that period ten isolates were plated on a BHI 1.5% agar with a sterile loop by the agar streaking method, and incubated at 30 °C for 24 hours. The cultures were monitored microscopically for contamination. Stocks in skim milk were prepared from each isolate which were kept at -70 °C.

Species identification

A polyphase approach including other biochemical and physiological tests was used for the characterization of the isolates. They included the ability to grow in liquid medium at low and high temperatures, as well in the presence of 6.5% NaCl (Leclerc et al., 1996). For each of these experiments, a single colony of the isolates was inoculated in 3 ml BHI broth and incubated for a week at 10 °C, overnight at 45 °C and 72 hours at 30 °C in BHI broth supplemented with 6.5% NaCl. Total DNA was isolated by "Gram Plus & Yeast Genomic DNA Purification Kit", cat. № 3585 (EURx), according to the manufacturer's instructions. The DNA concentrations were determined on a Quantus fluorimeter (Promega), while the quality was checked by 0.8% agarose gel electrophoresis. The isolates were further subjected to genus- and species-specific PCRs. All primers are listed in Table 1. *Enterococcus* sp. genus-specific PCR was performed accordingly Ke et al. (Ke et al., 1999). PCR amplification with *E. faecalis* specific primers EDA1_F1/EDA1_R1 was carried out as described by Peykov et al. (Peykov et al., 2012), while PCR amplification with *E. durans* specific primers DU1/DU2 as described by Jackson et al. (Jackson et al., 2004). The specific reactions' conditions are listed in Table 2. After the completion of the PCR reactions, 5 µl of the PCR products were analyzed by electrophoresis on a 1.6% agarose gel in a TBE buffer system, followed by staining with GelRed™ (Sigma-Aldrich). To check their clonal relatedness, all the isolates were subjected to RAPD analyses as previously described (Gyurova et al., 2021).

Table 1 PCR primers used in this study.

Target	Primer pairs	Sequence (5′–3′)	Product size (bp)	Annealing temperature (°C)	Source
Genus-specific primers					
tuf gene encoding EF-Tu	Ent1 Ent2	5'-TACTGACAAACCATTCATGATG-3' 5'-AACTTCGTCACCAACGCGAAC-3'	112 bp	55 ºC	*(Ke et al., 1999)*
Species-specific primers					
eda gene encoding KDPG aldolase	EDA1_F1 EDA1_R1	5'-GGGGACAGTTTTGGATGCTA-3' 5'-TCCATATAGGCTTGGGCAAC-3'	404 bp	51 °C	*(Peykov et al., 2012)*
soda gene, encoding superoxide dismutase	DU1 DU2	5'-CCTACTGATATTAAGACAGCG-3' 5'-TAATCCTAAGATAGGTGTTTG-3'	295 bp	55 °C	*(Jackson et al. 2004)*
16S ribosomal RNA gene	27F 1492R	5'-AGAGTTTGATCMTGGCTCAG-3' 5'-ACCTTGTTACGACTT-3'	1465 bp	52 °C	*(Lane, 1991)*
RAPD primers					
n/a	E1	5'-TCACGCTGCA-3'	n/a	n/a	*(Barbier et al. 1996)*
n/a	L2	5'-ATGTAACGCC-3'	n/a	n/a	*(Fitzsimons et al. 1999)*

Table 2 PCR reactions conditions used in this study

	***Enterococcus* genus-specific PCR**	***E. faecalis* species-specific PCR**	***E. durans* species-specific PCR**
Primers pair	Ent1/ Ent2	EDA1_F1/ EDA1_R1	DU1/ DU2
Cycles			
Denaturation (at 94 °C)	15 sec.	30 sec.	30 sec.
Annealing (at the corresponding t°)	15 sec. at 55 °C	60 sec. at 51 °C	60 sec. at 55 °C
Synthesis (at 72 °C)	30 sec.	45 sec.	60 sec.

All PCR reactions were performed in a total volume of 20 µl in a KCl buffer system containing 1,5 mM $MgCl_2$, 0,4 U of Taq polymerase, dNTPs at a final concentration of 0,2 mM, each primer at a final concentration of 0,2 µM and 10 ng of template DNA. The initial denaturation in all reactions was performed at 94 °C for 5 min., while the final extension lasted for 3 min. at 72 °C. In all reactions, 35 amplification cycles were performed.

Whole genome sequencing and data analyses

Whole Genome Sequencing of *E. faecalis* H1041 was performed on the Illumina HiSeq platform, via 2×150 bp paired-end sequencing (Novogene, UK). The library construction was performed by Novogene. Sequencing data were pre-processed and assembled using the Shovill assembly pipeline for Illumina paired-end reads that is integrated into the Galaxy web-based platform (Afgan et al., 2018; Seemann, 2017). Next, the resulting assembly was evaluated using QUAST (Gurevich et al., 2013). MLST typing was performed on the MLST server (Larsen et al., 2012). In addition, the fasta-file with the draft genome sequence was uploaded to ResFinderFG 1.0 (https://cge.cbs.dtu.dk/services/ResFinderFG/) and VirulenceFinder 2.0 (https://cge.cbs.dtu.dk/services/VirulenceFinder/) servers to test for the presence of antibiotic resistance determinants and virulence factors in this isolate (Joensen et al., 2014; Zankari et al., 2012). The scan for plasmid origins of replication within the assembled contigs was performed by the PlasmidFinder 2.1. online tool (https://cge.cbs.dtu.dk/services/PlasmidFinder/) (Carattoli and Hasman, 2020). Putative bacteriocin open reading frames (ORF), immunity genes and transporters sequences were identified from the draft genome sequences through BAGEL4 (http://bagel4.molgenrug.nl/index.php) using default settings (de Jong et al., 2010). The exact locations of the putative bacteriocin ORFs were determined using the tblastn algorithm (Camacho et al., 2009).

RESULTS AND DISCUSSION

The ability of the isolates to grow on D-coccosel medium producing black halos around the colonies is indicative that they belong to the *Enterococcus* genus because of the ability to hydrolyze esculin (Facklam and Moody, 1970). Their genus affiliation was further confirmed by other classical approaches as the abilities to grow at low and high temperatures, as well as in the presence of 6.5% NaCl (Leclerc et al., 1996). Next, DNA-based approaches were used for confirmation of the results of the physiological tests. They included genus- and species-specific PCRs performed with genus-specific primers (Ke et al., 1999), *E. durans* specific primers (Jackson et al., 2004), and *E. faecalis* specific primers (Peykov et al., 2012). Positive results were obtained only with the genus-specific PCR as well as with the PCR performed with the *E. faecalis* specific primers, meaning that the isolates belonged to this species. When the clonal relatedness of the isolates was studied with RAPD analyzes with primers L2 and E1, lowest similarity coefficients of 91,4% and 96,2% respectively were obtained. As the generally accepted thresholds for clonal relatedness are 70%-75% (Grundmann et al., 1997; Webster et al., 1996), this means that all the 10 isolates belong to a single strain which was named H1041.

To assess the role of *E. faecalis* H1041 for the Bulgarian green cheese ripening, as well as to consider its application in the food industry, we chose to sequence its entire genome on the Illumina HiSeq platform because the information obtained would permit in one single experiment to type exactly the strain, to evaluate its potential to be pathogenic, as well as to check it for the presence of antibiotics resistances genes, bacteriocins genetic determinants, etc. The detailed statistics for the quality of sequencing data are shown in Table 3, which indicates that the sequencing is of good quality, allowing to proceed with the draft genome assembly. It was performed by the Shovill assembly pipeline for Illumina paired-end reads on the Galaxy web-based platform (Afgan et al., 2018; Seemann, 2017). The processing resulted in 91 contigs, from which 28 were larger than 1000 bp, the largest one being 455 750 bp. The total length of the genome was 2 944 653 with a GC content of 37.4%. These values are in agreement with those for *E. faecalis* with publically available genomes on NCBI's GenBank server. The data was uploaded to the GenBank server for public availability (accession number PRJNA682033 and ID: 682033).

Table 3 Statistics for the quality of sequencing data

Sample	Library Flowcell Lane	Raw reads	Raw data (Gb)	Effective reads (%)	Error rate (%)	Q20(%)	Q30(%)	GC(%)
H1041	FDSW202493774-1r_HCLFNDSXY_L4	5130998	1.6	99.91	0.03	97.99	93.60	38.27
	FDSW202493774-1r_HF75CDSXY_L1	5791302		99.92	0.02	98.70	95.31	38.22

Table 3 Statistics for the quality of sequencing data

Sample	Library Flowcell Lane	Raw reads	Raw data (Gb)	Effective reads (%)	Error rate (%)	Q20(%)	Q30(%)	GC(%)

Legend: Library Flowcell Lane: Library ID Flowcell ID lane ID; Raw reads: the total amount of reads of raw data; Raw data: the total length of the reads, calculated in gigabases (Gb); Effective reads: percentage of the clean reads; Error rate: base error rate; Q20 and Q30: percentages of the base counts with Phred values of more than 20 and 30, respectively; GC: percentage of the G & C base counts.

The MLST analysis was performed on the MLST server (Larsen et al., 2012), and it attributed *E. faecalis* H1041 to the sequence type 220, showing 100% sequence identity with this type's allele variants: *aroE_9*, *gdh_27*, *gki_12*, *gyd_6*, *pstS_23*, *xpt_10*, and *yqiL_7*. *E. faecalis* ST 220 isolates were reported most often to be from an animal origin (Olsen et al., 2012; Si et al., 2015) which can explain the presence of our strain in a dairy product that was prepared from raw milk.

First, the *E. faecalis* H1041 was scanned for the presence of antibiotics resistance genetic determinants by the ResFinderFG 1.0 tool. The results are presented in Table 4 and Fig. 1. Tetracycline and trimethoprim resistance genetic determinants were found on contigs 16 and 1 respectively. However, the similarity to the reference sequences was not complete due to 17 and 4 nucleotide substitutions found in *TetM* and dihydrofolate reductase genes. Despite not knowing the effect of these point mutations, their high number, as well the fact that the BLAST analysis we ran with the canonical sequences, didn't show variants within the GenBank's database which were similar to ours, allow speculations that these genes are probably not functional because they have never been reported till now.

Table 4 ResFinderFG 1.0 results

Hit name	Identity	Query/HSP	Contig	Position in contig	Drug treatment	NCBI accession number
spanning Tet(M) and variants	99.02	1935/1935	Contig 16	12364..14298	Tetracycline	KU543782.1
dihydrofolate reductase	99.19	495/495	Contig 1	89702..90196	Trimethoprim	KF627019.1

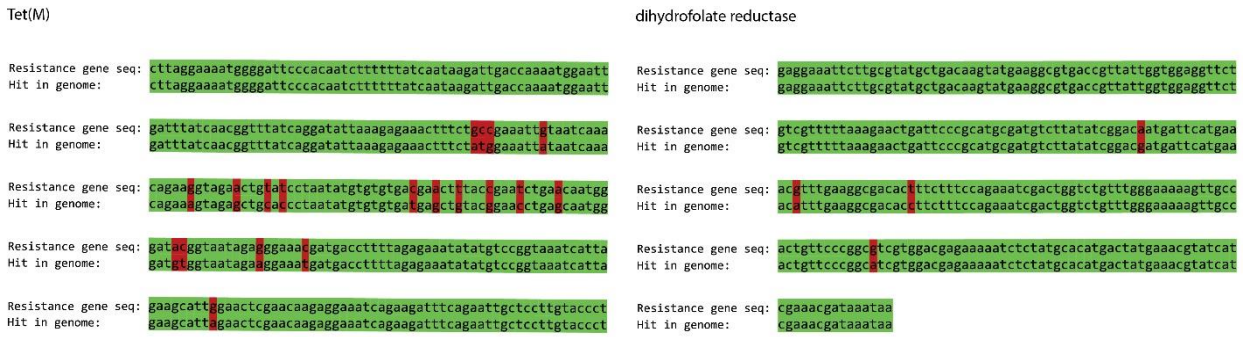

Figure 1 Partial alignment of the tet(M) (left panel) and the dhfr genes (right) panel of E. faecalis H1041 (up), carrying mutations, and the canonical sequences (down). The nucleotide substitutions are highlighted in red.

Next, we uploaded the assembled genome to the VirulenceFinder 2.0 server. In total, 18 virulence factors were reported (Table 5). However, 100% nucleotide identity to the corresponding reference sequences was reported for only 6 of them - *ElrA*, *SrtA*, *efaAfs*, *fsrB*, *gelE*, and *tpx*. *ElrA* encodes an internalin-like protein that prevents the adhesion to macrophages (Nunez et al., 2018). StrA is a sortase enzyme that is essential for biofilm formation (Das et al., 2018) together with the *fsrB* (Gaspar et al., 2009). *tpx* encodes a thiol peroxidase determining oxidative stress resistance (Elghaieb et al., 2019). *efaAfs* encodes an adhesin protein, while *gelE* – a gelatinase enzyme (Zhong et al., 2021), both believed to be more or less intrinsic for the genus (Fiore et al., 2019). The presence of these intact virulence factors within the genome of a strain isolated from a dairy product could raise some well-motivated concerns. However, in the scientific literature, many reports exist where food-derived enterococci carry many of these genes, and some of the others listed in Table 5, in more or less similar combinations – in fermented meats (Barbosa et al., 2010), ready-to-eat salads (Campos et al., 2013), and retail meat (Elghaieb et al., 2019; Golob et al., 2019). Considering all these facts the virulence genetic profile of *E. faecalis* H1041 is not an exception for a traditional raw milk dairy product, and does not obligatory imply to be a health concern as it was the case of some other artisanal cheeses (Câmara et al., 2020).

Table 5 VirulenceFinder 2.0 results

Virulence factor	Identity	Query / Template length	Contig	Position in contig	Protein function	NCBI accession number
ElrA	**100**	**2172 / 2172**	**Contig 10**	**3499..5670**		**CP003726.1**
SrtA	**100**	**735 / 735**	**Contig 8**	**58242..58976**		**CP003726.1**
ace	98.07	2025 / 2025	Contig 2	248603..250627	collagen adhesin precursor	AE016830.1
cCF10	99.76	828 / 828	Contig 12	97546..98373		CP002491.1
cCF10	99.76	828 / 828	Contig 12	97546..98373		CP002621.1
cOB1	99.88	819 / 819	Contig 3	128672..129490		CP002491.1
cad	99.78	930 / 930	Contig 9	4676..5605		CP002621.1
camE	99.4	501 / 501	Contig 14	88451..88951	sex pheromone cAM373 precursor	AF435437.1
camE	99.4	501 / 501	Contig 14	88451..88951	sex pheromone cAM373 precursor	AF435438.1
camE	99.4	501 / 501	Contig 14	88451..88951	sex pheromone cAM373 precursor	AF435439.1
ebpA	99.7	3312 / 3312	Contig 2	260557..263868		295112306
ebpB	99.86	1431 / 1431	Contig 2	259123..260553		CP002491.1
efaAfs	**100**	**927 / 927**	**Contig 5**	**45134..46060**		**CP003726.1**
fsrB	**100**	**729 / 729**	**Contig 17**	**11545..12273**	**biofilm formation**	**CP003726.1**
gelE	**100**	**1530 / 1530**	**Contig 17**	**13851..15380**		**CP003726.1**
hylA	99.94	3266 / 3266	Contig 8	82724..85989		CP003726.1
hylB	99.4	3015 / 3015	Contig 4	19754..22768		CP002621.1
tpx	**100**	**510 / 510**	**Contig 15**	**40489..40998**		**CP003726.1**

One of the characteristics of pathogenic enterococcal strains is that they contain plasmids harboring virulence factors genes, as well as antibiotics resistances genes. In the case of *E. faecalis* H1041 only one hit was obtained with the PlasmidFinder tool: the origin of replication *rep9b* of a RepA_N-type plasmid (GenBank accession No. CP002494) found on Contig 16 with 100% sequence identity. This type of plasmids is broadly distributed among Gram-positive bacteria but they have a narrow host range and the intergeneric transfers are rare because of the reported co-evolution with the host (Weaver et al., 2009). None of the genetic determinants of the virulence factors detected were positioned on Contig 16, and only the mutations carrying the copy of the tetracycline resistance gene was on the same contig (Fig. 1, Table 4). The probably not functioning copy of the *Tet(M)*, the lack of plasmid-associated virulence factors genes, and the strongly limited rates of the plasmid's transfers indicate that the presence of this plasmid within *E. faecalis* H1041 should not be regarded as a pathogenicity trait.

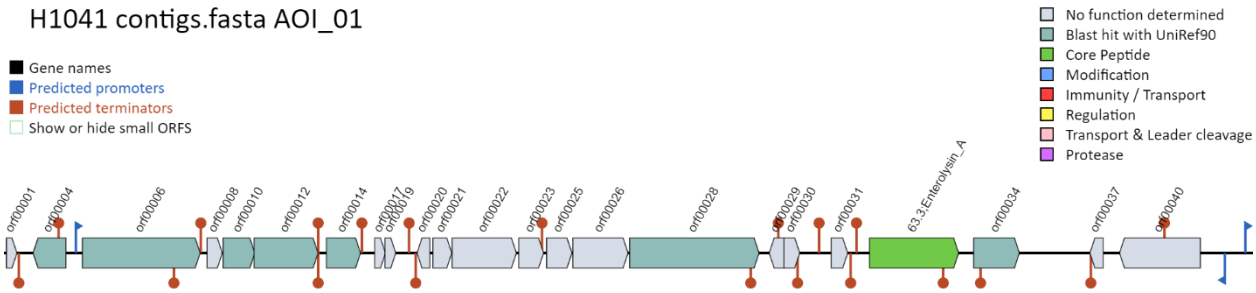

Figure 2 Organization of the genetic cluster containing the enterolysin A gene.

Finally, the assembled contigs were scanned for the presence of bacteriocin encoding genetic determinants using the BAGEL4 online tool. Only one genetic cluster was found – that of enterolysin A, positioned on contig 5 (Fig. 2). Enterolysin A is a well-characterized bacteriocin (a metalloendopeptidase) that is typical for many *E. faecalis* strains, and that is known to inhibit the growth of closely related species and genera such as other enterococci, pediococci, lactobacilli, and lactococci by a bacteriolytic mode of action (Nilsen et al., 2003). The presence of enterolysin A has nothing to do with the presence or absence of pathogenicity characteristics, but rather with the cheese ripening process – the autolysis of the lactic acid bacteria of the starter cultures is important for the development of the flavor and to the debittering occurring during the cheese ripening (Crow et al., 1995; Hickey et al., 2004). So, in this regard, *E. faecalis* H1041 is a typical dairy strain.

CONCLUSION

Pathogenic Enterococci are not highly virulent, and till now enterococcal infections have never been linked to cheese consumption (Dapkevicius et al., 2021). Cheese- and other food-borne enterococci usually harbor few virulence factors and antibiotic resistance genes, a fact that does not mean that they are obligatory pathogens, every case requiring an individual study (Deng et al., 2021). In this regard, *E. faecalis* H1041 is a typical dairy representative of the species, which contributes to the development of the organoleptic properties of the Bulgarian green cheese. It should not be considered as a health risk, especially because of the lack of vancomycins' resistances, as well as because of the presence of numerous mutations within the two antibiotics resistances genes found. Finally, the WGS technology which was chosen to characterize *E. faecalis* H1041 revealed to be a cost efficient and fast method for in depth characterization and assessment of the pathogenicity potential of newly isolated enterococcal strains.

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REFERENCES

Afgan, E., Baker, D., Batut, B., van den Beek, M., Bouvier, D., Čech, M., Chilton, J., Clements, D., Coraor, N., Grüning, B.A., Guerler, A., Hillman-Jackson, J., Hiltemann, S., Jalili, V., Rasche, H., Soranzo, N., Goecks, J., Taylor, J., Nekrutenko, A., Blankenberg, D., 2018. The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2018 update. Nucleic Acids Research 46, W537-W544. https://doi.org/10.1093/nar/gky379

Álvarez, M., Rodríguez, A., Peromingo, B., Núñez, F., Rodríguez, M., 2020. Enterococcus faecium: a promising protective culture to control growth of ochratoxigenic moulds and mycotoxin production in dry-fermented sausages. Mycotoxin Research 36, 137-145. https://doi.org/10.1007/s12550-019-00376-6

Barbosa, J., Gibbs, P.A., Teixeira, P., 2010. Virulence factors among enterococci isolated from traditional fermented meat products produced in the North of Portugal. Food Control 21, 651-656. https://doi.org/10.1016/j.ijfoodmicro.2015.10.016

Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., Madden, T.L., 2009. BLAST+: architecture and applications. BMC Bioinformatics 10, 421. https://doi.org/10.1186/1471-2105-10-421

Câmara, S.P.A., Dapkevicius, A., Silva, C.C.G., Malcata, F.X., L. N. Enes Dapkevicius, M., 2020. Artisanal Pico cheese as reservoir of Enterococcus species possessing virulence and antibiotic resistance properties: implications for food safety. Food Biotechnology 34, 25-41. https://doi.org/10.1080/08905436.2019.1710844

Campos, J., Mourão, J., Pestana, N., Peixe, L., Novais, C., Antunes, P., 2013. Microbiological quality of ready-to-eat salads: an underestimated vehicle of bacteria and clinically relevant antibiotic resistance genes. Int J Food Microbiol 166, 464-470. https://doi.org/10.1016/j.ijfoodmicro.2013.08.005

Carattoli, A., Hasman, H., 2020. PlasmidFinder and In Silico pMLST: Identification and Typing of Plasmid Replicons in Whole-Genome Sequencing (WGS), in: de la Cruz, F. (Ed.), Horizontal Gene Transfer: Methods and Protocols. Springer US, New York, NY, pp. 285-294.

Crow, V.L., Coolbear, T., Gopal, P.K., Martley, F.G., McKay, L.L., Riepe, H., 1995. The role of autolysis of lactic acid bacteria in the ripening of cheese. International Dairy Journal 5, 855-875. https://doi.org/10.1007/978-1-4939-9877-7_20

Dapkevicius, M.d.L.E., Sgardioli, B., Câmara, S.P.A., Poeta, P., Malcata, F.X., 2021. Current Trends of Enterococci in Dairy Products: A Comprehensive Review of Their Multiple Roles. Foods 10, 821. https://doi.org/10.3390/foods10040821

Das, S., Srivastava, V.K., Parray, Z.A., Jyoti, A., Islam, A., Kaushik, S., 2018. Identification of potential inhibitors of sortase A: Binding studies, in-silico docking and protein-protein interaction studies of sortase A from Enterococcus faecalis. International Journal of Biological Macromolecules 120, 1906-1916. https://doi.org/10.1016/j.ijbiomac.2018.09.174

de Jong, A., van Heel, A.J., Kok, J., Kuipers, O.P., 2010. BAGEL2: mining for bacteriocins in genomic data. Nucleic Acids Research 38, W647-W651. https://doi.org/10.1093/nar/gkq365

Deng, F., Chen, Y., Zhou, X., Xiao, H., Sun, T., Deng, Y., Wen, J., 2021. New Insights into the Virulence Traits and Antibiotic Resistance of Enterococci Isolated from Diverse Probiotic Products. Microorganisms 9, 726. https://doi.org/10.3390/microorganisms9040726

Elghaieb, H., Tedim, A.P., Abbassi, M.S., Novais, C., Duarte, B., Hassen, A., Peixe, L., Freitas, A.R., 2019. From farm to fork: identical clones and Tn6674-like elements in linezolid-resistant Enterococcus faecalis from food-producing animals and retail meat. Journal of Antimicrobial Chemotherapy 75, 30-35. https://doi.org/10.1093/jac/dkz419

Facklam, R.R., Moody, M.D., 1970. Presumptive Identification of Group D Streptococci: the Bile-Esculin Test. Applied Microbiology 20, 245-250. https://doi.org/10.1128/am.20.2.245-250.1970

Fiore, E., Tyne, D.V., Gilmore, M.S., Fischetti, V.A., Novick, R.P., Ferretti, J.J., Portnoy, D.A., Braunstein, M., Rood, J.I., 2019. Pathogenicity of Enterococci. Microbiology Spectrum 7, 7.4.9. https://doi.org/10.1128/microbiolspec.GPP3-0053-2018

Gaspar, F., Teixeira, N., Rigottier-Gois, L., Marujo, P., Nielsen-LeRoux, C., Crespo, M.T.B., Lopes, M.d.F.S., Serror, P., 2009. Virulence of Enterococcus faecalis dairy strains in an insect model: the role of fsrB and gelE. Microbiology (Reading) 155, 3564-3571. https://doi.org/10.1099/mic.0.030775-0

Golob, M., Pate, M., Kušar, D., Dermota, U., Avberšek, J., Papić, B., Zdovc, I., 2019. Antimicrobial Resistance and Virulence Genes in Enterococcus faecium and Enterococcus faecalis from Humans and Retail Red Meat. BioMed Research International 2019, 2815279. https://doi.org/10.1155/2019/2815279

Grundmann, H.J., Towner, K.J., Dijkshoorn, L., Gerner-Smidt, P., Maher, M., Seifert, H., Vaneechoutte, M., 1997. Multicenter study using standardized protocols and reagents for evaluation of reproducibility of PCR-based fingerprinting of Acinetobacter spp. Journal of clinical microbiology 35, 3071-3077. https://doi.org/10.1128/jcm.35.12.3071-3077.1997

Gurevich, A., Saveliev, V., Vyahhi, N., Tesler, G., 2013. QUAST: quality assessment tool for genome assemblies. Bioinformatics 29, 1072-1075. https://doi.org/10.1093/bioinformatics/btt086
Gyurova, A., Vladimirova, A., Peykov, S., Dimitrov, M., Strateva, T., Dimov, S.G., 2021. Characterization of Enterococcus durans EDD2, a strain from beehives with inhibitory activity against Paenibacillus larvae. Journal of Apicultural Research, 1-14. https://doi.org/10.1080/00218839.2021.1936915
Hickey, R.M., Ross, R.P., Hill, C., 2004. Controlled Autolysis and Enzyme Release in a Recombinant Lactococcal Strain Expressing the Metalloendopeptidase Enterolysin A. 70, 1744-1748. https://doi.org/10.1128/AEM.70.3.1744-1748.2004
Jackson, C.R., Fedorka-Cray, P.J., Barrett, J.B., 2004. Use of a genus- and species-specific multiplex PCR for identification of enterococci. Journal of clinical microbiology 42, 3558-3565. https://doi.org/10.1128/JCM.42.8.3558-3565.2004
Joensen, K.G., Scheutz, F., Lund, O., Hasman, H., Kaas, R.S., Nielsen, E.M., Aarestrup, F.M., Carroll, K.C., 2014. Real-Time Whole-Genome Sequencing for Routine Typing, Surveillance, and Outbreak Detection of Verotoxigenic Escherichia coli. 52, 1501-1510. https://doi.org/10.1128/JCM.03617-13
Ke, D., Picard, F.J., Martineau, F., Ménard, C., Roy, P.H., Ouellette, M., Bergeron, M.G., 1999. Development of a PCR assay for rapid detection of enterococci. Journal of clinical microbiology 37, 3497-3503. https://doi.org/10.1128/JCM.37.11.3497-3503.1999
Larsen, M.V., Cosentino, S., Rasmussen, S., Friis, C., Hasman, H., Marvig, R.L., Jelsbak, L., Sicheritz-Pontén, T., Ussery, D.W., Aarestrup, F.M., Lund, O., 2012. Multilocus sequence typing of total-genome-sequenced bacteria. Journal of clinical microbiology 50, 1355-1361. https://doi.org/10.1128/JCM.06094-11
Leclerc, H., Devriese, L.A., Mossel, D.A.A., 1996. Taxonomical changes in intestinal (faecal) enterococci and streptococci: consequences on their use as indicators of faecal contamination in drinking water. Journal of Applied Bacteriology 81, 459-466. https://doi.org/10.1111/j.1365-2672.1996.tb03533.x
McBride, S.M., Fischetti, V.A., LeBlanc, D.J., Moellering, R.C., Jr., Gilmore, M.S., 2007. Genetic Diversity among Enterococcus faecalis. PLOS ONE 2, e582. https://doi.org/10.1371/journal.pone.0000582
Nilsen, T., Nes, I.F., Holo, H., 2003. Enterolysin A, a Cell Wall-Degrading Bacteriocin from Enterococcus faecalis LMG 2333. Applied and environmental microbiology 69, 2975-2984. https://doi.org/10.1128/AEM.69.5.2975-2984.2003
Nunez, N., Derré-Bobillot, A., Gaubert, S., Herry, J.-M., Deschamps, J., Wei, Y., Baranek, T., Si-Tahar, M., Briandet, R., Serror, P., Archambaud, C., 2018. Exploration of the role of the virulence factor ElrA during Enterococcus faecalis cell infection. Scientific Reports 8, 1749. https://doi.org/10.1038/s41598-018-20206-6
Ogier, J.-C., Serror, P., 2008. Safety assessment of dairy microorganisms: The Enterococcus genus. International Journal of Food Microbiology 126, 291-301. https://doi.org/10.1016/j.ijfoodmicro.2007.08.017
Olsen, R.H., Schønheyder, H.C., Christensen, H., Bisgaard, M., 2012. Enterococcus faecalis of Human and Poultry Origin Share Virulence Genes Supporting the Zoonotic Potential of E. faecalis. Zoonoses and Public Health 59, 256-263.
https://doi.org/10.1111/j.1863-2378.2011.01442.x
Peykov, S.Z., Aleksandrova, V.D., Dimov, S.G., 2012. Rapid identification of Enterococcus faecalis by species-specific primers based on the genes involved in the Entner-Doudoroff pathway. Mol Biol Rep 39, 7025-7030. https://doi.org/10.1007/s11033-012-1533-z
Seemann, T., 2017. Shovill: Faster SPAdes assembly of Illumina reads
Si, H., Zhang, W.-J., Chu, S., Wang, X.-M., Dai, L., Hua, X., Dong, Z., Schwarz, S., Liu, S., 2015. Novel plasmid-borne multidrug resistance gene cluster including lsa(E) from a linezolid-resistant Enterococcus faecium isolate of swine origin. Antimicrobial agents and chemotherapy 59, 7113-7116. https://doi.org/10.1128/AAC.01394-15
Weaver, K.E., Kwong, S.M., Firth, N., Francia, M.V., 2009. The RepA_N replicons of Gram-positive bacteria: a family of broadly distributed but narrow host range plasmids. Plasmid 61, 94-109. https://doi.org/10.1016/j.plasmid.2008.11.004
Webster, C.A., Towner, K.J., Humphreys, H., Ehrenstein, B., Hartung, D., Grundmann, H., 1996. Comparison of rapid automated laser fluorescence analysis of DNA fingerprints with four other computer-assisted approaches for studying relationships between Acinetobacter baumannii isolates. Journal of Medical Microbiology 44, 185-194. https://doi.org/10.1099/00222615-44-3-185
Zankari, E., Hasman, H., Cosentino, S., Vestergaard, M., Rasmussen, S., Lund, O., Aarestrup, F.M., Larsen, M.V., 2012. Identification of acquired antimicrobial resistance genes. Journal of Antimicrobial Chemotherapy 67, 2640-2644. https://doi.org/10.1093/jac/dks261
Zhong, R., Zhou, Z., Liu, H., Zhong, Z., Peng, G., 2021. Antimicrobial resistance and virulence factor gene profiles of Enterococcus spp. isolated from giant panda oral cavities. Journal of Veterinary Research 65, 147-154. https://doi.org/10.2478/jvetres-2021-0030