Draft Genome Sequence of Pseudomonas carnis Strain 23-145, Causing Blue Discolorations on Rabbit Carcasses

Biggel, Michael ; Lienhard, Julia ; Stephan, Roger

DOI: https://doi.org/10.1128/mra.00136-23

AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

Draft Genome Sequence of *Pseudomonas carnis* Strain 23-145, Causing Blue Discolorations on Rabbit Carcasses

Michael Biggel,[a] Julia Lienhard,[b] Roger Stephan[a]

[a]Institute for Food Safety and Hygiene, Vetsuisse Faculty, University of Zürich, Zürich, Switzerland
[b]Section of Veterinary Bacteriology, Institute for Food Safety and Hygiene, Vetsuisse Faculty, University of Zürich, Zürich, Switzerland

ABSTRACT Here, we report the genome sequence of *Pseudomonas carnis* strain 23-145, which was recovered from a rabbit carcass with blue discolorations. The strain harbored two *trpABCDF* loci involved in tryptophan biosynthesis, which is characteristic of blue-pigment-producing *Pseudomonas* strains.

Some species of the *Pseudomonas fluorenscens* group can spoil food by producing pigments that lead to color changes (1–3). The aim of this work was to identify the microbiological cause of blue discolorations found on the carcass of a fattening rabbit 10 days after slaughter in an abattoir in Switzerland in January 2023 (Fig. 1). For the microbiological examination, the surface of the discolored meat was disinfected with ethanol. Swab samples from cut meat were then spread on various agar plates (Columbia blood agar, Gassner agar, Schaedler agar, and Columbia nalidixic acid agar [CNA]; Thermo Fisher Scientific). The plates were incubated at 37°C aerobically and anaerobically for up to 2 days. Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (Bruker Daltronics) was used for preliminary species identification of grown colonies. A low-grade mixed flora consisting of *Carnobacterium divergens*, *Staphylococcus saprophyticus*, and two morphologically distinct *Pseudomonas* species was found. MALDI-TOF MS typed one *Pseudomonas* sp. isolate (23-145) as a representative of the *Pseudomonas fluorescens* group and the second as either *Pseudomonas lundensis* or *Pseudomonas taetrolens*.

To determine which of the two *Pseudomonas* isolates was responsible for the observed color changes, we spread the isolates on distinct surfaces of a fresh rabbit carcass and stored the carcass at 4°C. After 4 days, the surface area inoculated with isolate 23-145 showed an incipient color change, which increased massively in the following days. The area inoculated with the second isolate (*Pseudomonas lundensis* or *Pseudomonas taetrolens*) did not show visible changes over the same period.

Genomic DNA of isolate 23-145 was extracted using the DNeasy blood and tissue kit (Qiagen) from subcultures obtained from single colonies that had been grown for 24 h at 37°C on sheep blood agar. Libraries were prepared using the Nextera DNA Flex library preparation kit (Illumina) and sequenced on the Illumina MiniSeq platform (2 × 150 bp). Read trimming and quality control were performed with fastp v0.23.2 (4). A draft assembly was generated from 347 Mbp of read data (2,332,296 reads [coverage, 52×]) using SPAdes v3.14.1 (5) implemented in Shovill v1.1.0 (https://github.com/tseemann/shovill). The genome was annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) v6.4 (6). *In silico* taxonomic classification was performed using ribosomal multilocus sequence typing (rMLST) (7) (https://pubmlst.org). Default parameters were used for all software unless otherwise stated.

Isolate 23-145 was identified by rMLST as *Pseudomonas carnis*, a member of the *P. fluorenscens* group. The 6.3-Mbp draft assembly consisted of 186 contigs (N_{50}, 81.6 kb; GC content, 59.95%) and contained two sets of the tryptophan biosynthesis genes *trpABCDF*. The presence of multiple *trpABCDF* homologues has recently been linked to pigment (indigo

Editor Simon Roux, DOE Joint Genome Institute

Address correspondence to Roger Stephan, roger.stephan@uzh.ch.

The authors declare no conflict of interest.

Received 28 February 2023
Accepted 15 March 2023
Published 5 April 2023

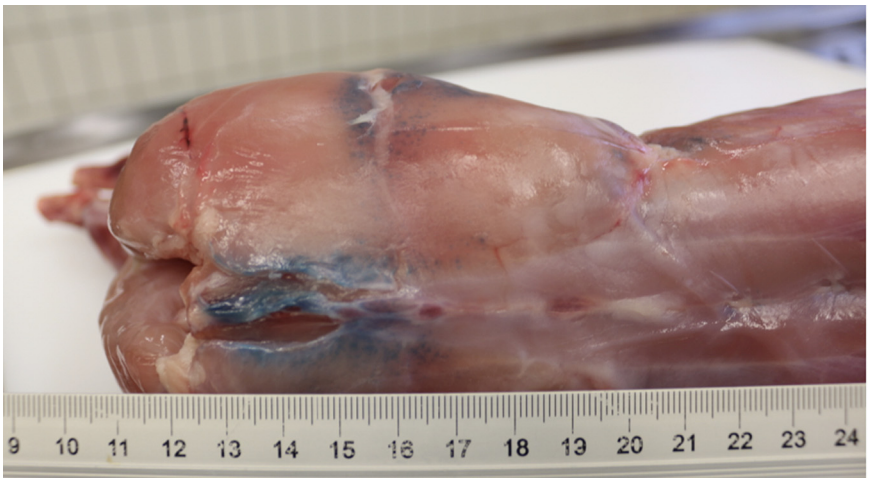

FIG 1 Blue discolorations on a rabbit carcass 10 days after slaughter.

derivate) production and discoloration of food products (8, 9). Our data provide further evidence that the accessory tryptophan biosynthesis genes could be used as diagnostic targets for the identification of pigment-producing *Pseudomonas* strains.

Data availability. The draft assembly is available at NCBI GenBank under assembly accession number GCA_028863905.1. The BioProject and SRA accession numbers are PRJNA935533 and SRR23495213, respectively.

ACKNOWLEDGMENT

We thank Nicole Cernela for her technical support with Illumina sequencing.

REFERENCES

1. Circella E, Schiavone A, Barrasso R, Camarda A, Pugliese N, Bozzo G. 2020. *Pseudomonas azotoformans* belonging to *Pseudomonas fluorescens* group as causative agent of blue coloration in carcasses of slaughterhouse rabbits. Animals 10:256. https://doi.org/10.3390/ani10020256.
2. Gennari M, Dragotto F. 1992. A study of the incidence of different fluorescent *Pseudomonas* species and biovars in the microflora of fresh and spoiled meat and fish, raw milk, cheese, soil and water. J Appl Bacteriol 72:281–288. https://doi.org/10.1111/j.1365-2672.1992.tb01836.x.
3. Martin NH, Murphy SC, Ralyea RD, Wiedmann M, Boor KJ. 2011. When cheese gets the blues: *Pseudomonas fluorescens* as the causative agent of cheese spoilage. J Dairy Sci 94:3176–3183. https://doi.org/10.3168/jds.2011-4312.
4. Chen S, Zhou Y, Chen Y, Gu J. 2018. fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 34:i884–i890. https://doi.org/10.1093/bioinformatics/bty560.
5. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb.2012.0021.
6. Haft DH, DiCuccio M, Badretdin A, Brover V, Chetvernin V, O'Neill K, Li W, Chitsaz F, Derbyshire MK, Gonzales NR, Gwadz M, Lu F, Marchler GH, Song JS, Thanki N, Yamashita RA, Zheng C, Thibaud-Nissen F, Geer LY, Marchler-Bauer A, Pruitt KD. 2018. RefSeq: an update on prokaryotic genome annotation and curation. Nucleic Acids Res 46:D851–D860. https://doi.org/10.1093/nar/gkx1068.
7. Jolley KA, Bliss CM, Bennett JS, Bratcher HB, Brehony C, Colles FM, Wimalarathna H, Harrison OB, Sheppard SK, Cody AJ, Maiden MCJ. 2012. Ribosomal multilocus sequence typing: universal characterization of bacteria from domain to strain. Microbiology (Reading) 158:1005–1015. https://doi.org/10.1099/mic.0.055459-0.
8. Andreani NA, Carraro L, Zhang L, Vos M, Cardazzo B. 2019. Transposon mutagenesis in *Pseudomonas fluorescens* reveals genes involved in blue pigment production and antioxidant protection. Food Microbiol 82:497–503. https://doi.org/10.1016/j.fm.2019.03.028.
9. Reichler SJ, Martin NH, Evanowski RL, Kovac J, Wiedmann M, Orsi RH. 2019. A century of gray: a genomic locus found in 2 distinct *Pseudomonas* spp. is associated with historical and contemporary color defects in dairy products worldwide. J Dairy Sci 102:5979–6000. https://doi.org/10.3168/jds.2018-16192.