Monitoring and detection of ingredients and pathogens in ice cream facilities and materials using metagenomics

IcyTrace

THE PROJECT

The aim of the research project is to develop an innovative technological solution to prevent and detect food fraud. To ensure food safety and authenticity in terms of proper & correct labelling, preventing adulteration, and other economically motivated food frauds.

IcyTrace focuses on the following research areas
- ✓ Food **safety** and **authenticity**
- ✓ Food fraud prevention and
- ✓ Ensuring data integrity using blockchain technology

The expected results of the project will provide significant competitive advantages to the future products of the companies involved in the project. They are expected to allow entry into new, global markets and to bring innovation and research into publications at reputable international conferences and magazines.

THE TECHNOLOGY

Long-read sequencing has the potential to be a promising tool for food control and authenticity. In the present study, samples of ice cream and its raw materials were collected and sequenced through a portable Oxford Nanopore Technologies device. The produced reads were initially processed with the DIAMOND software for taxonomic identification, but only the reads aligned to eukaryotic organisms were selected for further confirmation with NCBI BLAST. Our analysis shows that long reads (700 bp – kb) that were aligned with low percent identity (<60%) even to the wrong eukaryotic species by DIAMOND software, were valuable for species identification.

RESULTS

Example of ingredient authentication: A commercial pre-mixed powder used as an ingredient in the preparation of several ice cream flavors was analyzed. Its specified ingredients include pea protein, sunflower lecithin, guar gum (carob gum), locust bean gum, sugar, and dextrose. DIAMOND reads aligned to *Camellia sinensis* species were assigned with high accuracy by NCBI BLAST to one of the listed ingredients, *Prosopis alba* (carob). Only closely related such as *Prosopis alba* were detected with low identity (<85%) by DIAMOND. Furthermore, DIAMOND identified reads as *Pistacia vera* (pistachio) with a low accuracy rate (38%), which was later aligned to *Fraxinus pennsylvanica* with 85-90% identity by NCBI BLAST.

Read ID	DIAMOND Hits			NCBI BLAST validation hits			Allergen
	Species	Identity (%)	Length (bp)	Species	Identity (%)	Length (bp)	
5c566867-48b9-4853-9e2d-d6b266280c6d	*Prosopis alba*	55,9	810	*Prosopis alba*	84,26	2306	No
b253d809-3d8f-47da-afa6-3b73c3af8f5e	*Phoenix dactylifera, Pistacia vera*	38,9 - 45,9	1022 - 1531	*Fraxinus pennsylvanica*	85,34 - 90,27	3902	No
f74e3445-5dae-46f8-afbf-e2dad59e00d7	*Camellia sinensis*	41,9	1393	*Ceratonia siliqua*	96,63	4928	No
a744c87e-30cf-4e7c-98d1-d45162c897e8	*Camellia sinensis, Telopea speciosissima*	39,6 - 39,7	1467	*Ceratonia siliqua*	96,33	6430	No
96399430-9251-4b7b-a092-5de7359c204b	*Lipotes vexillifer*	38,1	1970	*Cosmarium reniforme*	75,37	1486	No
17ab25b9-2fd5-42da-870a-961a725b84a8	*Pistacia vera*	38	1338	*Fraxinus pennsylvanica*	85,49 - 90,25	3736	No
2c491be6-82c9-4d4b-a34f-5493964a7ca1	*Phoenix dactylifera, Rosa chinensis*	34,7 - 41,6	1501 -1801	*Phoenix dactylifera*	74,89 - 76,85	5205	No
c9df26d2-41a3-4d90-8cd2-8fd8be7ba745	*Cicer arietinum, Populus trichocarpa*	36,3 - 38,3	1762 - 1792	*Ceratonia siliqua*	94,77	7080	No
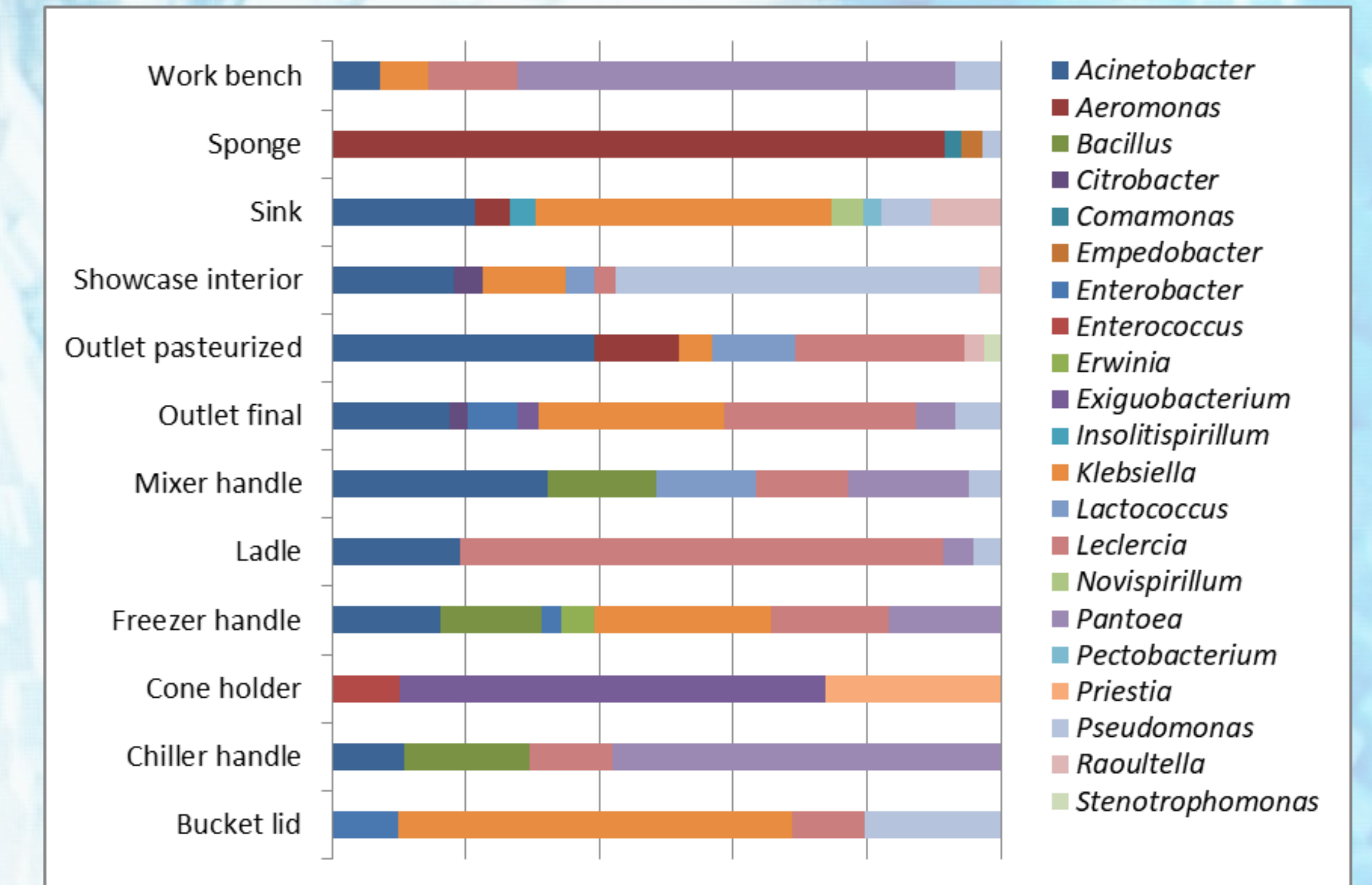
Food safety evaluation: At first, the food production area was mapped with a portable ATP device to assess its cleanliness. Based on the ATP results, 12 of the most problematic areas were chosen for sample collection and incubation in a growth medium. Subsequently, the full-length 16S rRNA gene was sequenced. A total of 234 bacterial genera were identified, with *Exiguobacterium*, *Klebsiella*, *Acinetobacter*, *Leclercia*, *Pantoea*, *Pseudomonas* and *Aeromonas* being the most prevalent. Fig 1 shows the distribution of the most abundant species across samples. Fig 2 highlights the number of species per sample.
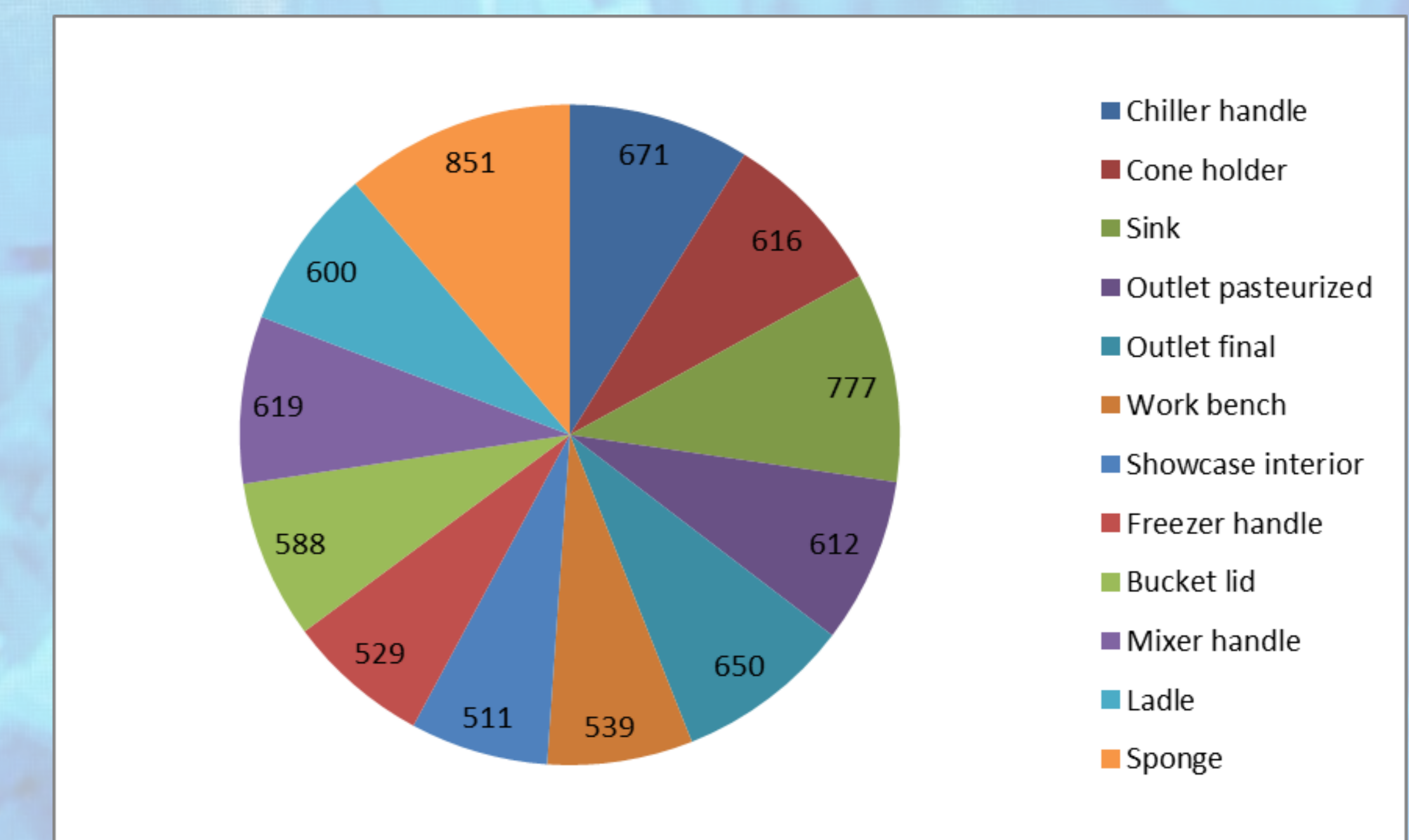
Related publications:
Tsirigoti E, Valasiadis D, Rihani V, Tragaki V, Alexandridou A, Vassiliou D, Chasapi A, Karapiperis C. Quasimetagenomics approach for biosafety monitoring in an ice cream production facility. Journal of Bioinformatics and Systems Biology 6 (2023)
Valasiadis D, Rihani V, Tsirigoti E, Alexandridou A, Vassiliou D, Tragaki V, Karapiperis C, Chasapi A. Identification of ice cream ingredients through long-read sequencing. Journal of Bioinformatics and Systems Biology 6 (2023)