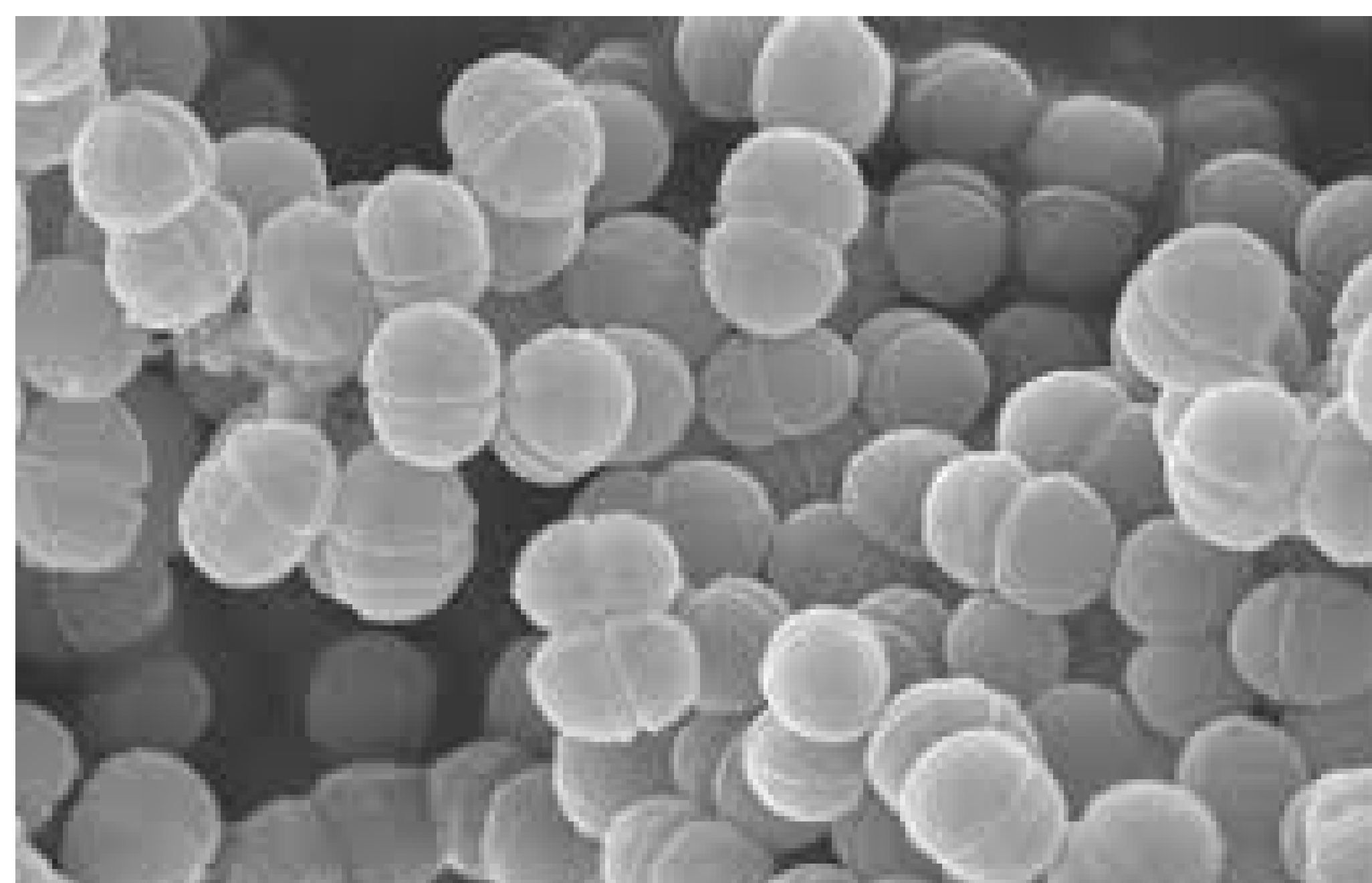


Pediococcus spp.

Pediococcus is a genus of gram-positive, facultatively anaerobic, lactic acid producing bacteria¹. Like other lactic acid genera such as *Lactobacillus*, *Pediococcus* is homofermentive and acid tolerant but can be distinguished through its tetrad structure.



Purpose of the research

As bioethanol continues to grow as an alternative fuel, more demand will be placed on methods of limiting bacterial contamination to maximize ethanol production.

Therefore, we will develop methods to study *Pediococcus* persistence in ethanol fermentations in order to inform decisions on how to control it.

Materials and Preparation

All *Pediococcus* strains have been obtained from Ferm Solutions.

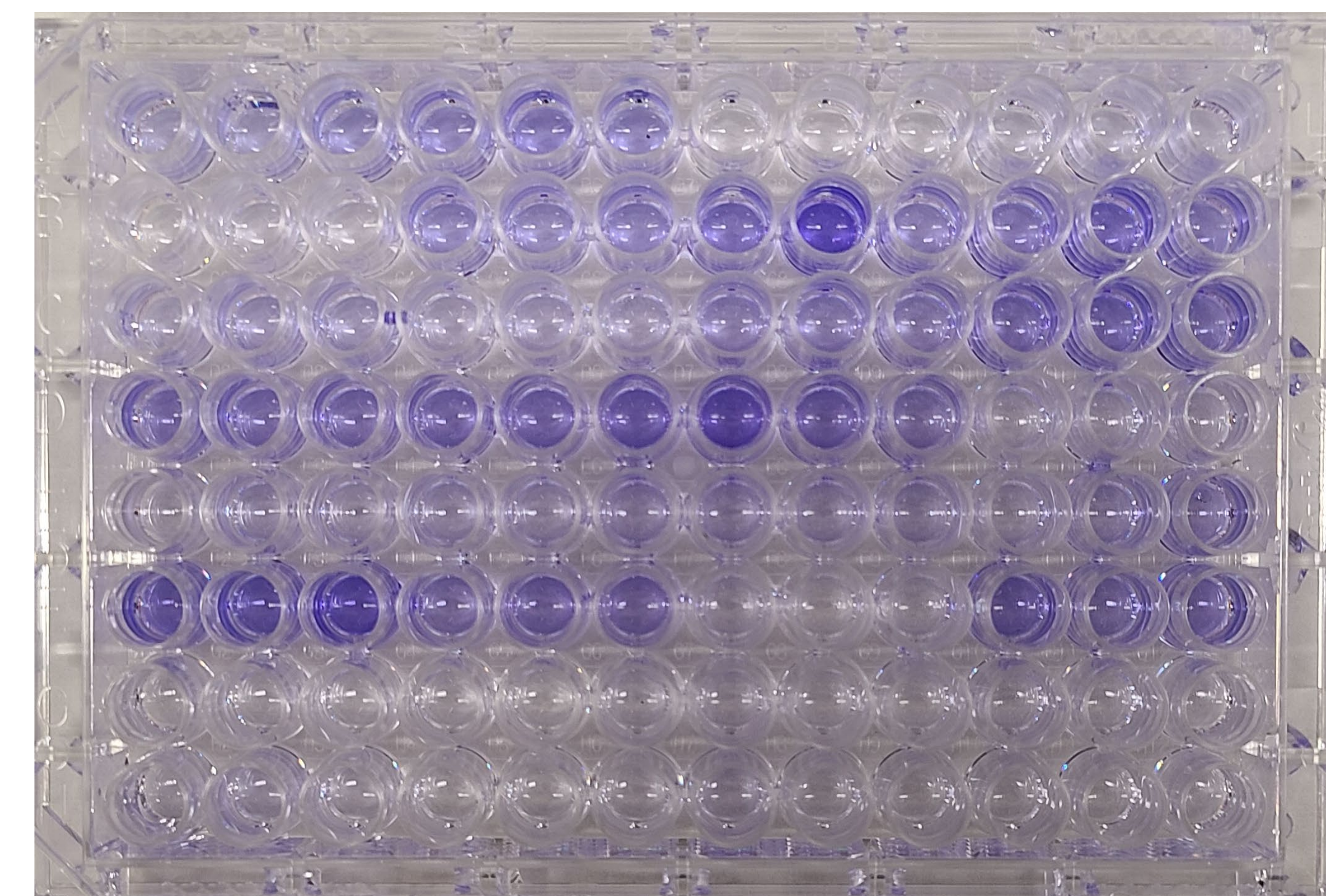
All strains were cultured on MRS agar at 28°C for 24h and had their DNA extracted with Sigma-Aldrich GenElute Bacterial Genomic DNA kit. The DNA was then amplified with 16S primers, sequenced, and uploaded to NCBI Blast to confirm the strains as *Pediococcus*.

All strains belonged to three species within *Pediococcus*:

Species	Strain designation
<i>P. loli</i>	CFR2298
<i>P. acidilactici</i>	5560
<i>P. acidilactici</i>	8613
<i>P. acidilactici</i>	FMAC22
<i>P. acidilactici</i>	LAB4
<i>P. acidilactici</i>	PMC65
<i>P. pentosaceus</i>	ML101
<i>P. pentosaceus</i>	NBRC 101987
<i>P. pentosaceus</i>	4335
<i>P. pentosaceus</i>	6108
<i>P. pentosaceus</i>	6327
<i>P. pentosaceus</i>	6340
<i>P. pentosaceus</i>	6461
<i>P. pentosaceus</i>	6464
<i>P. pentosaceus</i>	6643
<i>P. pentosaceus</i>	HBUAS 53375
<i>P. pentosaceus</i>	HBUAS 7-2
<i>P. pentosaceus</i>	JT3
<i>P. pentosaceus</i>	KFG2
<i>P. pentosaceus</i>	LAB2
<i>P. pentosaceus</i>	NWAFU 5034
<i>P. pentosaceus</i>	OPQ 3
<i>P. pentosaceus</i>	PP
<i>P. pentosaceus</i>	WG 2

*Strain designations are taken from NCBI BLAST

Inducing and measuring biofilms



Pediococcus strains are plated in 96 well plates at a concentration of 10⁸ CFU/mL with 200 μL of MRS broth. The plate is then incubated at 28 °C for 48h. After incubation, planktonic cells are washed away with PBS and biofilms stained with crystal violet. The crystal violet is then dissolved with acetic acid and absorbance taken at 595nm with the bottom two rows as negative controls.

Issues in ethanol production and solving contamination

Pediococcus is a bacterial contaminant in ethanol fermentors. This is because the bacteria:

- Consumes available hexoses
- Produces lactic acid as a product
- Forms biofilms

The former two traits inhibit the yeast's ability to convert sugars into ethanol while the latter increases the probability of future contamination.

Despite the threat that *Pediococcus* poses to ethanol production, current literature focuses on characterization of the genus in-vitro rather than on its persistence within the fermentor.

Pediococcus resilience through biofilm poses a threat to ethanol production due to the potential of systemic contamination, leading to a continuous decrease in ethanol production efficiency

Upcoming work

Now with a confirmed method of generating biofilms and a library of *Pediococcus* strains, we can progress with generating data relevant to the ethanol fermentation industry. Future experiments entail:

- Testing the resiliency of biofilms against cleaners
- Comparing biofilm load to fermentation outcome
- Measuring biofilm stability during fermentation

Acknowledgments and references

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