

Abstract

The multidrug efflux pump HorA confers resistance to hops in certain beer-spoilage organisms. Because of HorA's similarity to other multidrug efflux pumps, HorA might also confer resistance to industrial sanitizers. The model organism *E. coli* DH5 α 's lack of susceptibility in a disk diffusion assay suggests a need to increase compound volumes. Ubiquitous growth in a minimum inhibitory concentration assay suggests the iso- α -acids were structurally altered when extracted. An alignment of *horA* sequences determined that the gene is highly conserved. Thus, the negative PCR results on several cultures suggests a possible need to adjust the PCR protocol.

Background

- Iso- α -acids in hops preserve beer by disrupting the proton motive force (Sakamoto, 2001).
- Sanitizers prevent spoilage from hop-resistant organisms (Palmer 1999).
- *Lactobacillus brevis* strain BSO 464 contains a hop-resistance gene *horA* on the plasmid pLb464 (Bergsveinson, 2012).
- HorA pumps iso- α -acids out of the cell and shows similarity to other ABC multidrug efflux pumps (Sakamoto, 2001).
- Hypothesis: Organisms expressing *horA* are more resistant to industrial sanitizers than those without.

Disk Diffusion Assay

- *E. coli* DH5 α was chosen as the model organism.
- *E. coli* DH5 α were tested for susceptibility to: Betadine, 10% Bleach, 70% EtOH, H₂O₂, 100% Isopropanol, and iso- α -acids (5 IBUs, 10 IBUs, and 20 IBUs) on LB agar.
- *E. coli* DH5 α did not demonstrate susceptibility to Betadine, 10% Bleach, 70% EtOH, 100% Isopropanol, or iso- α -acids.

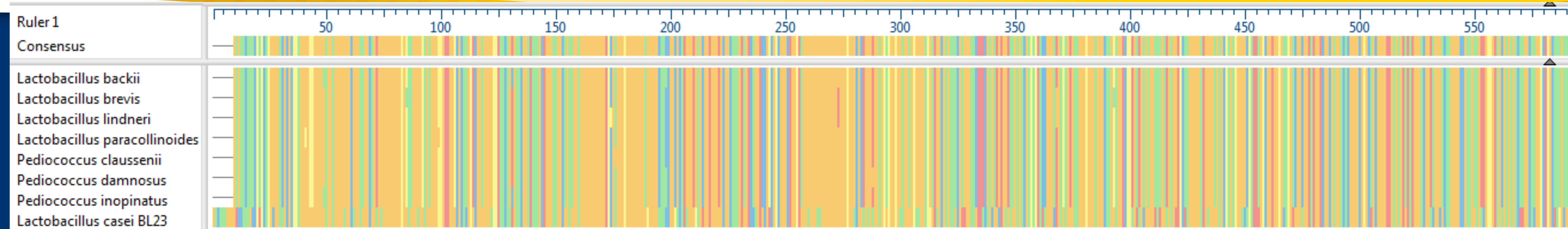


Figure 1: Clustal Omega Alignment of HorA protein sequence in Lactobacillus and Pediococcus spp.

Disk Diffusion Assay cont.

- Diffused growth around H₂O₂ made an accurate zone of clearing measurement not possible.

Minimum Inhibitory Concentration

- Iso- α -acids were diluted from 2⁻¹ to 2⁻¹² in LB Broth and inoculated with *E. coli* DH5 α to find the minimum inhibitory concentration of iso- α -acids.
- *E. coli* DH5 α grew ubiquitously across the serial dilution.

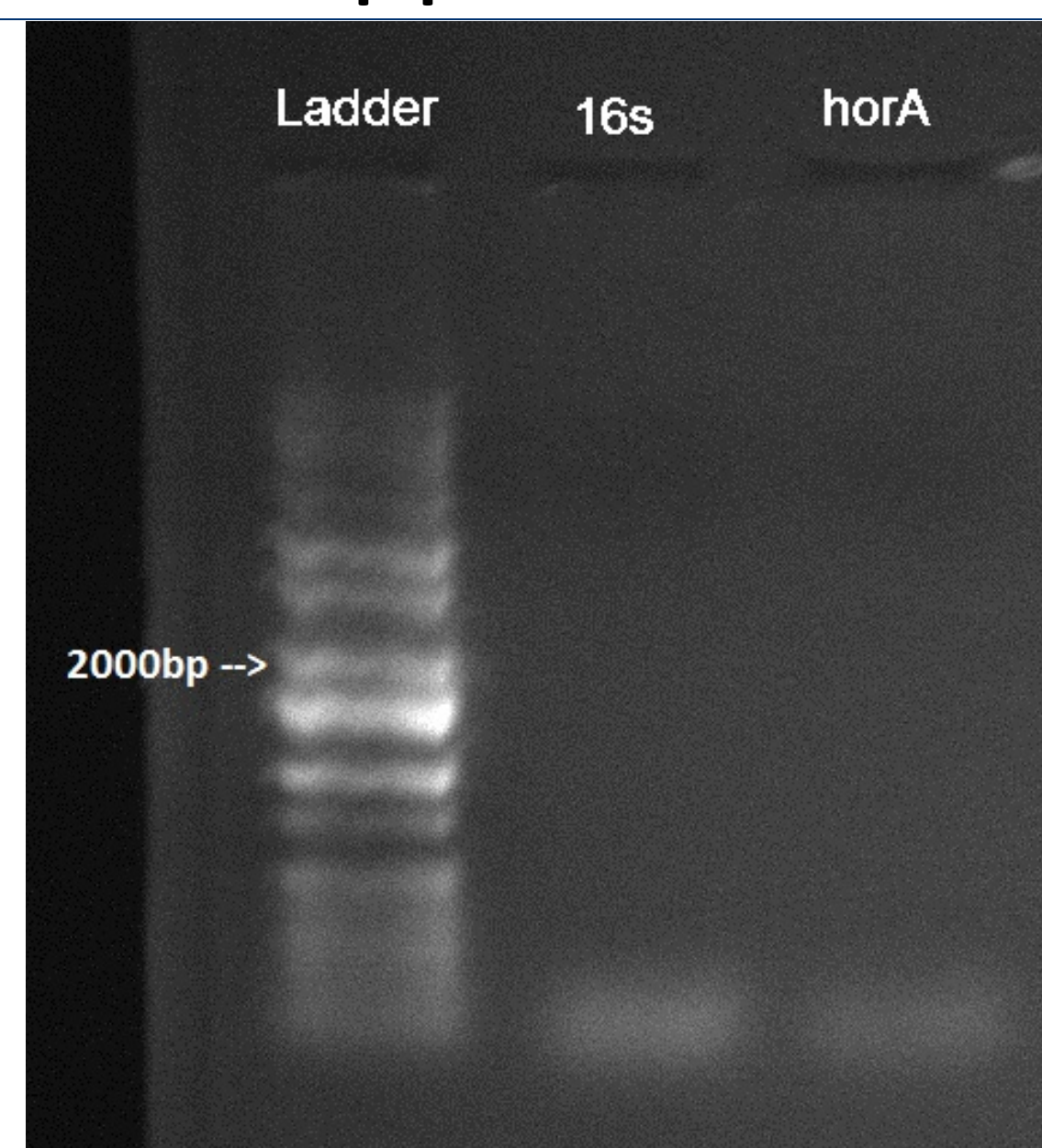
HorA Alignment and Culture Methods

- Due to limited access to *L. brevis* BSO 464, HorA amino acid sequences of lactic acid bacteria were aligned using Clustal Omega to study conservation.
- Lactic acid bacteria were cultured from a Wyeast Lactobacillus and Pediococcus samples and a Jester King sour beer.
- Samples were cultured in MRS broth and 85% Lager 15% 2X MRS broth.
- MRS+Lager broth cultures were then plated on MRS agar.

PCR of Culture Samples

- To test for the presence of *horA*, PCR was performed on cultures from:
 - Wyeast Pediococcus MRS broth
 - Jester King MRS broth
 - 3 colonies from Jester King MRS agar
 - 16s amplified as positive control
 - Gel electrophoresis performed.
- Primers are expected to amplify 2158bp.

- **Figure 2:** Amplification of 16s and *horA* from Jester King MRS broth sample had no band at the expected amplicon length of 2158bp.



Conclusions

- Disk volumes might be too low on diffusion assay.
- Iso- α -acids might be chemically altered.
- *horA* is highly conserved in lactic acid bacteria.
- Either no sample contained *horA*, or the PCR primers or temperatures need to be adjusted.

Future Directions

- Culture *L. brevis* BSO 464.
- Clone *horA* into *E. coli* with TOPO TA Kit.
- Determine survivorship with increasing concentrations of sanitizers with cloned cells.
- Construct Dose Response Curves, t-test IC50 values

References

- Bergsveinson J, Pittet V, Ziola B. 2012. RT-qPCR analysis of putative beer-spoilage gene expression during growth of *Lactobacillus brevis* BSO 464 and *Pediococcus clausenii* ATCC BAA-344(T) in beer. Appl Microbiol Biotechnol 96(2):461-70.
- Palmer J. 1999. How to Brew: Everything You Need to Know to Brew Beer Right the First Time [Internet]. First Edition. Boulder(CO):Brewers Publications; [cited 2014 Nov 20] Available from: <http://www.realbeer.com/jjpalmer/sitemap.html>
- Sakamoto K, Margolles A, van Veen HW, Konings WN. 2001. Hop resistance in the beer spoilage bacterium *Lactobacillus brevis* is mediated by the ATP-binding cassette multidrug transporter HorA. J Bacteriol 183(18):5371-5.