Resistance to Industrial Sanitizers due to the Presence of HorA

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http://beerstreetjournal.com/wp-content/uploads/beer-head-800x450.jpg

Brewing Industry

- In 2011, craft brewing in Texas had an economic impact of $608 million (Texas Craft, 2012).
- Hops preserve beer due to iso-α-acids (Sakamoto, 2001).
- Cleaners prevent spoilage from hop-resistant organisms.

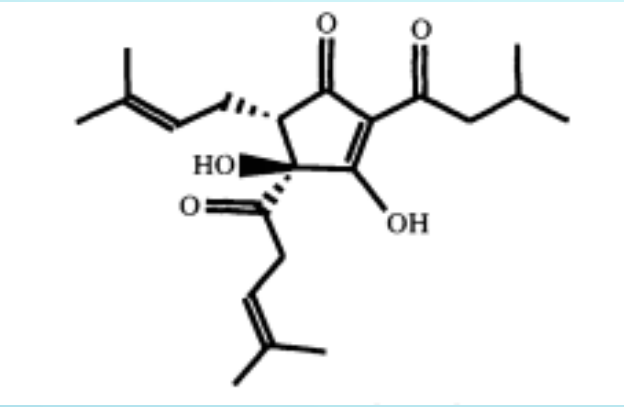


Figure 1: Chemical Structure of Trans-isohumulone (Simpson, 1993)

Hop-Resistance

- *Lactobacillus brevis* BSO 464 contains a hop-resistance gene *horA* on plasmid pLb464-1 (Bergsveinson, 2014).
- HorA pumps iso-α-acids out of the cell (Sakamoto, 2001).
- HorA shows similarity to other ATP-Binding Cassette efflux pumps with wide specificities (Sakamoto, 2001).
- Hypothesis: Organisms that express *horA* are more resistant to industrial sanitizers than those that don't.

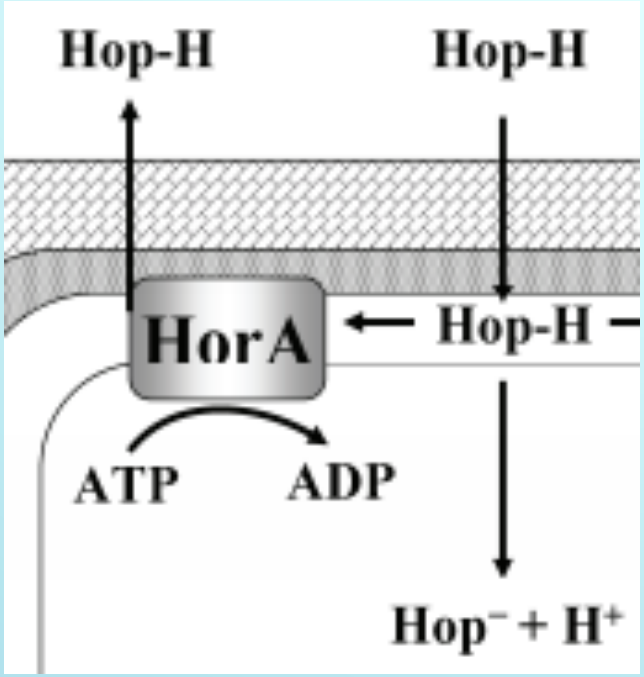
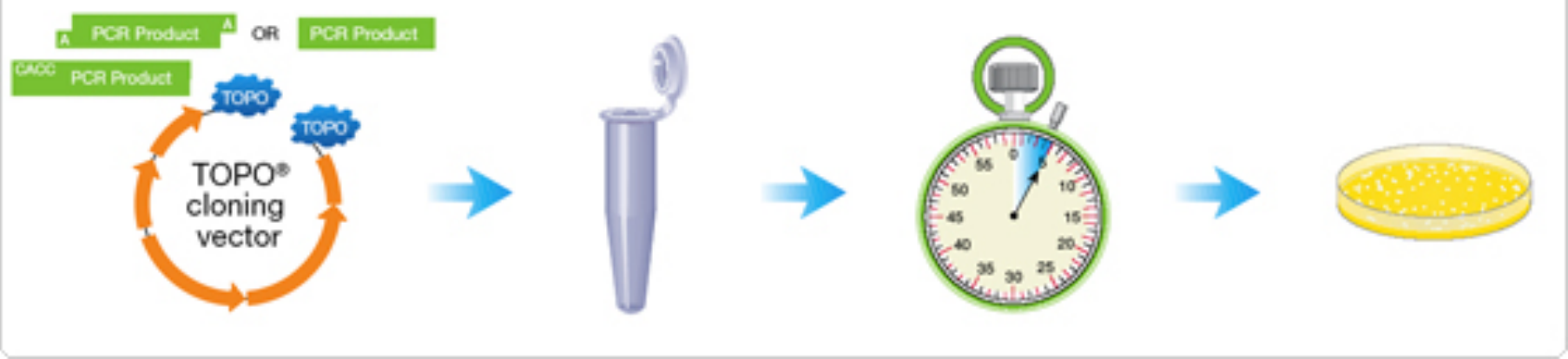


Figure 2: HorA Uses ATP to Extrude Hop Compounds (Suzuki, 2006)

Cloning *horA* into *E. coli*



Perform PCR of *horA*

Add PCR to TOPO vector

Incubate 5 min

Transform competent *E. coli*

Figure 3: TOPO TA Cloning Kit Process
http://www.b2b.invitrogen.com/etc/medialib/en/images/mainbody/data/diagram.Par.33726.Image.560.172.1.TOPO-Cloning-Protocol-jpg.gif

Dose-Response curve

1. Minimum Inhibitory Concentration assay on cloned *E. coli* for sanitizing compounds:
   - Hexalone hop extract, isopropanol, sodium percarbonate, bleach, peracetic acid, ethanol, iodine
   - Serial dilution of 2 orders of magnitude in triplicate.
2. Plate Minimum Inhibitory Concentration assays to determine Colony Forming Units.
3. Generate Dose-Response Curves from CFU's
   - Compare means of IC50 values

Troubleshooting PCR

- Difficulty isolating plasmid
  - 8 plasmids ranging from 2.3kb to 85kb
  - Probably low copy number
  - 5 are novel and uncharacterized
- Road blocks
  - Obtaining a useful DNA extraction
  - Have tried usual suspect PCR issues – primers, annealing temperatures, polymerases

References

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