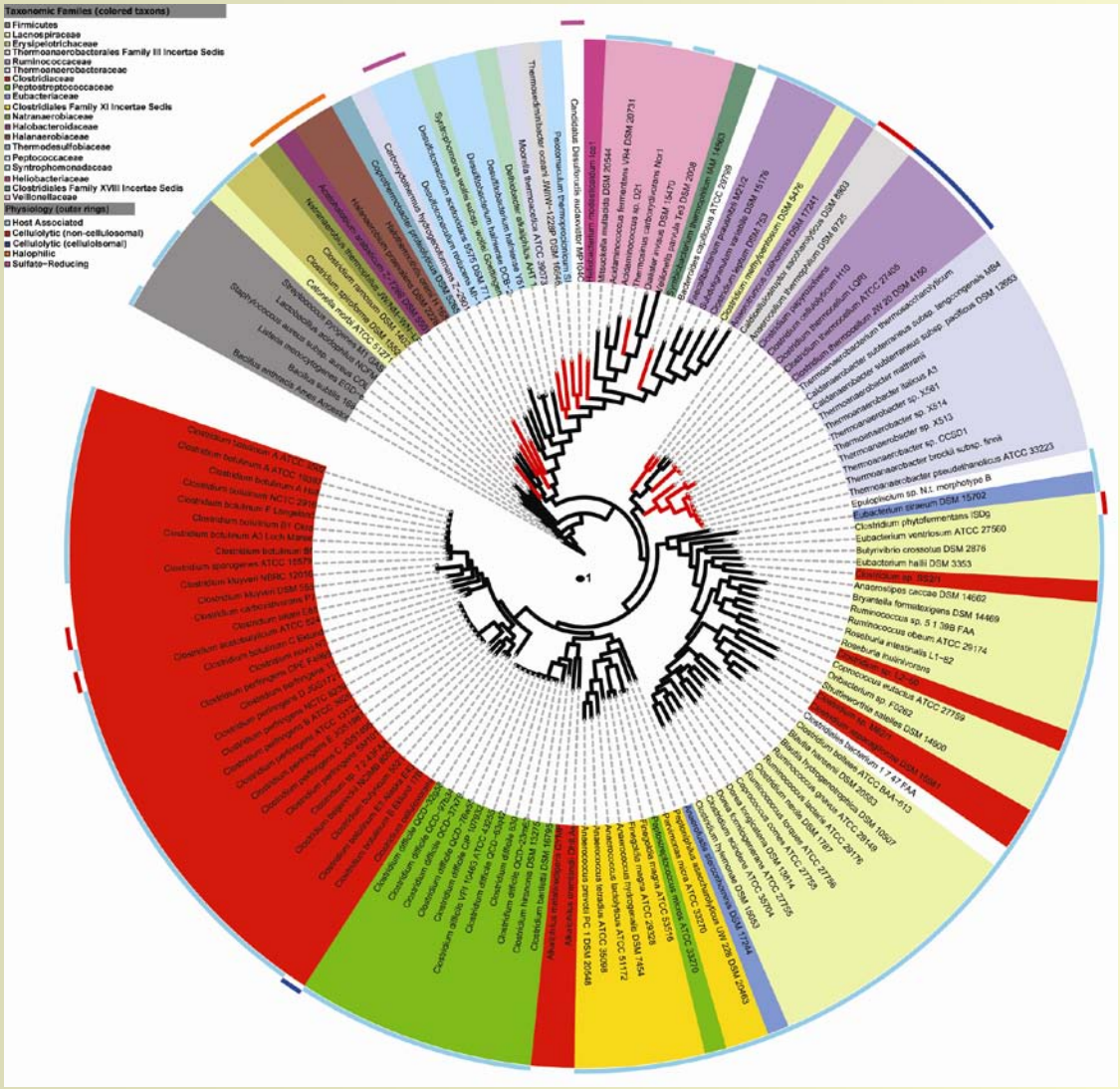
Aim 2.1 Analysis of *Clostridia and Thermoanaerobacter core genomes*



- **More *Clostridia* and *Thermoanaerobacter* genomes to update the phylogenetic tree**
- **RNA-seq and proteomic analyses to characterize cellulosomes from *Clostridia***
- ***Further analysis of Thermoanaerobacter with T. italicus***

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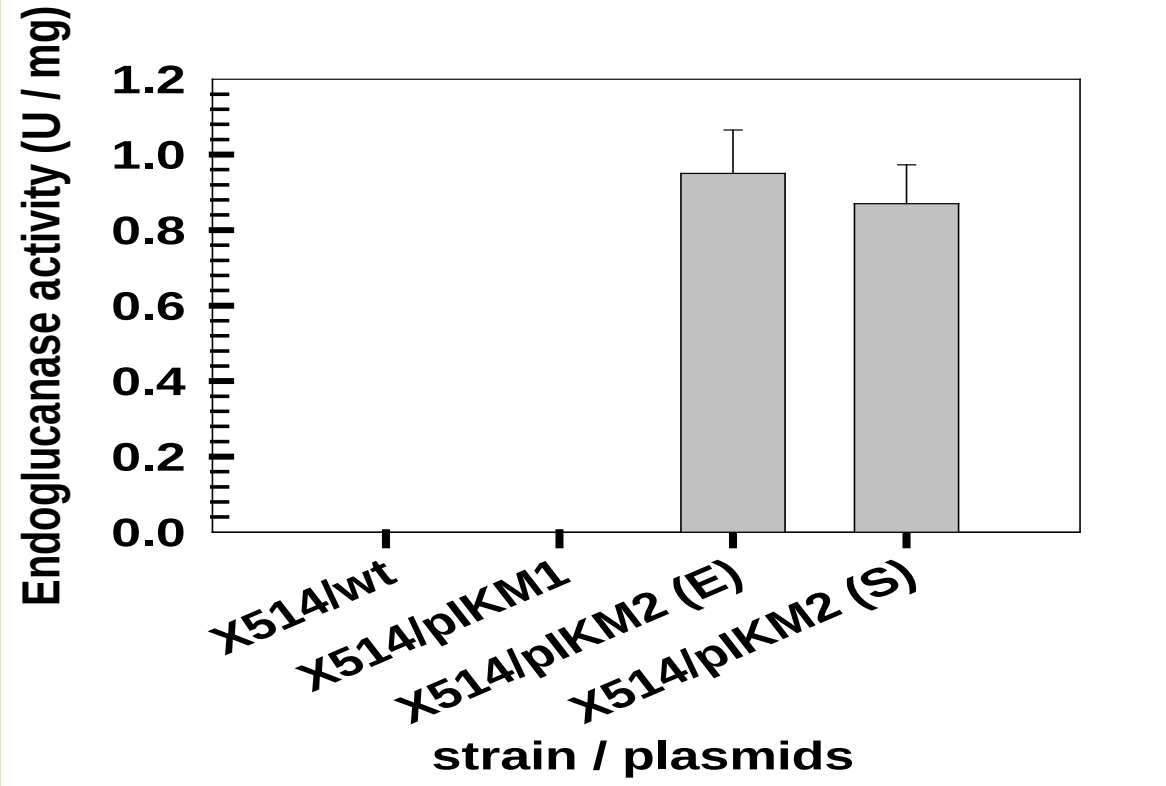
Aim 2.2 Understanding gene function, regulation and interaction network of co-culture and monocultures of *Clostridia* and *Thermoanaerobacter*

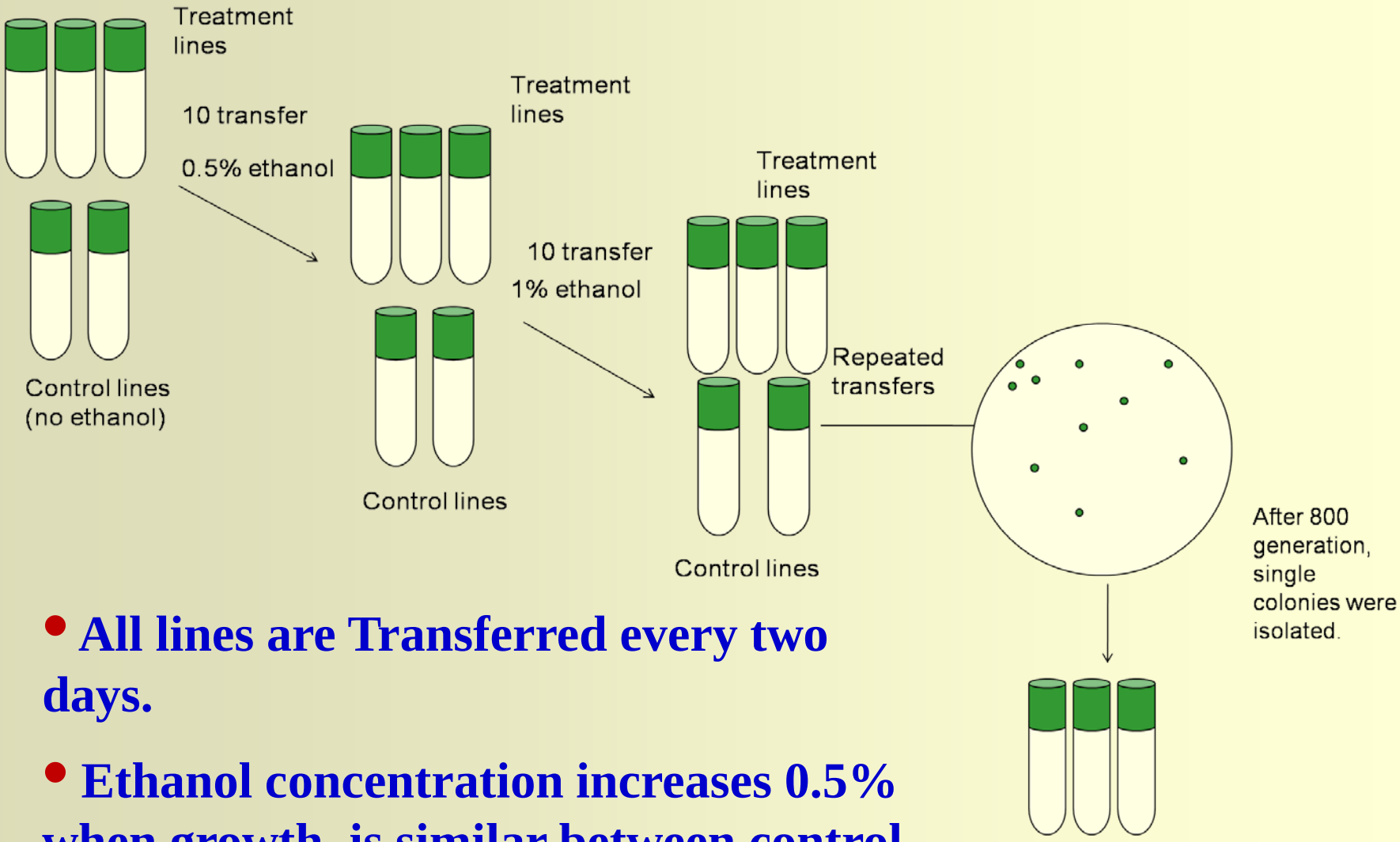
- **Successful transformation of *Thermoanaerobacter* sp. X514 by sonoporation and electroporation**
- **Long-term experimental evolution: ethanol adaptation for *Clostridium thermocellum* LQRI and *Thermoanaerobacter sp.* x514**

Endoglucanase activity of *Thermoanaerobter sp.* X514 transfomants



The X514 was transformed by electroporation (E) and sonoporation (S), respectively. Wild-type X514 and pIKM1 transformants were used as negative control.

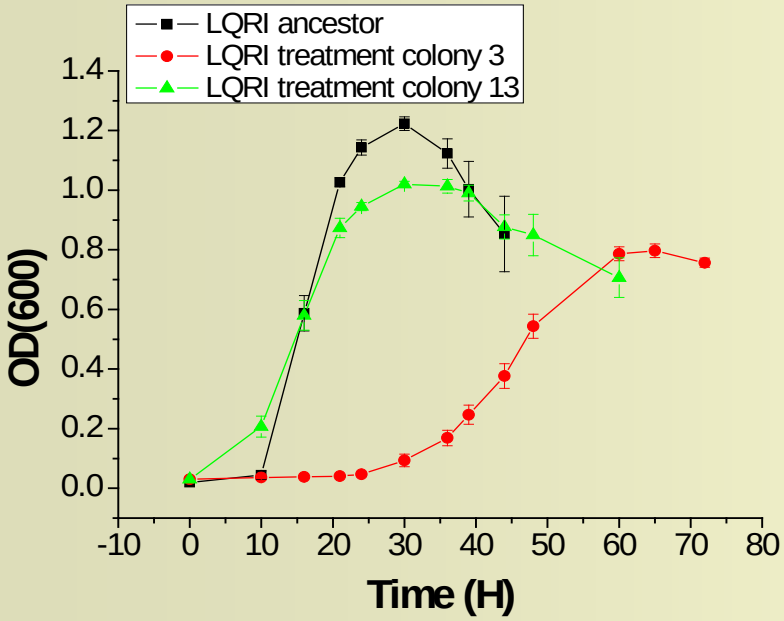
Experimental design



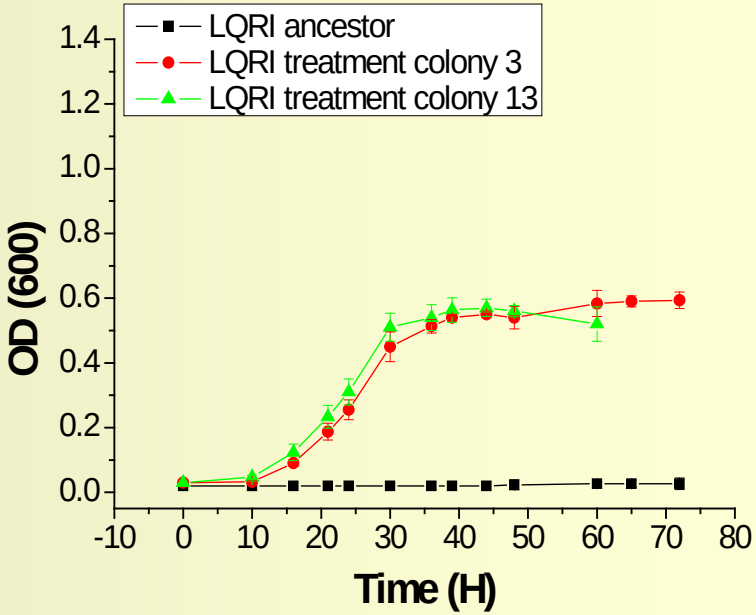
- **All lines are Transferred every two days.**
- **Ethanol concentration increases 0.5% when growth is similar between control lines and treatment lines.**

Growth of parent and evolved strains

A. No ethanol added



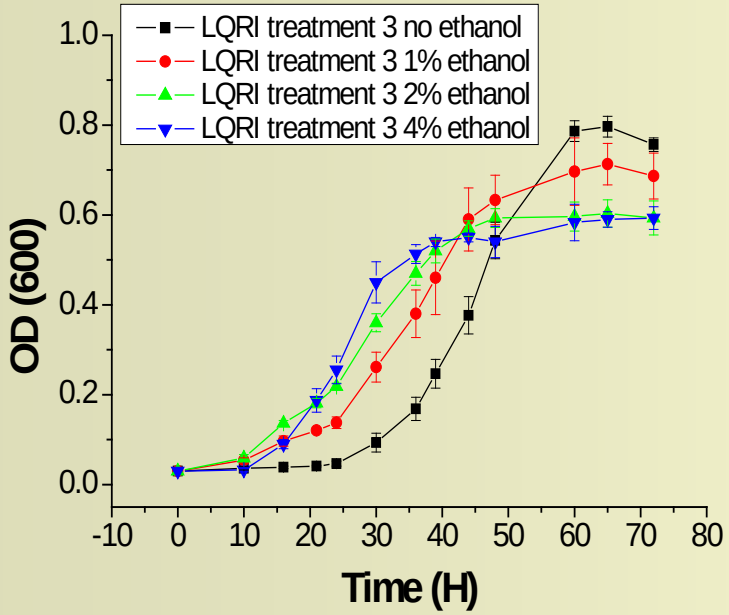
B. 4% ethanol



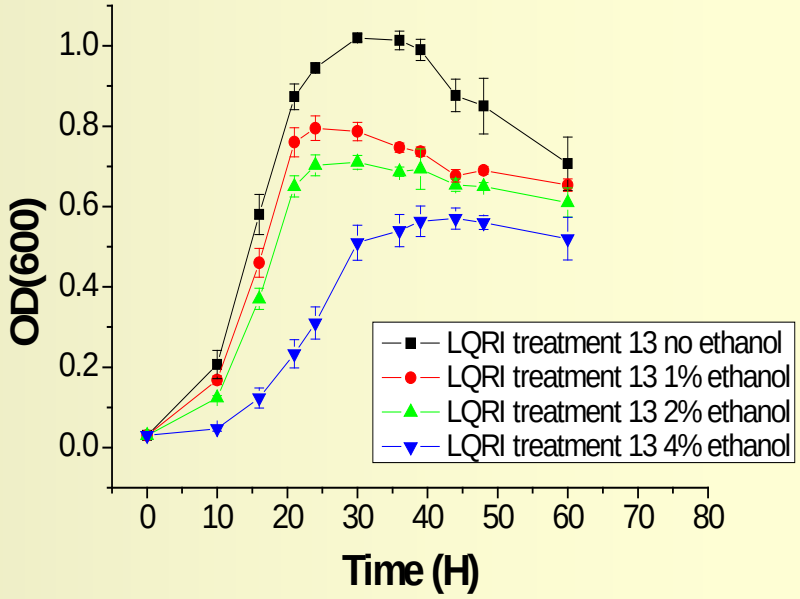
Ethanol –evolved strain grew much better at 4% ethanol but worse without ethanol added.

Growth of ethanol-evolved strains at different concentrations of ethanol

A. Colony 3

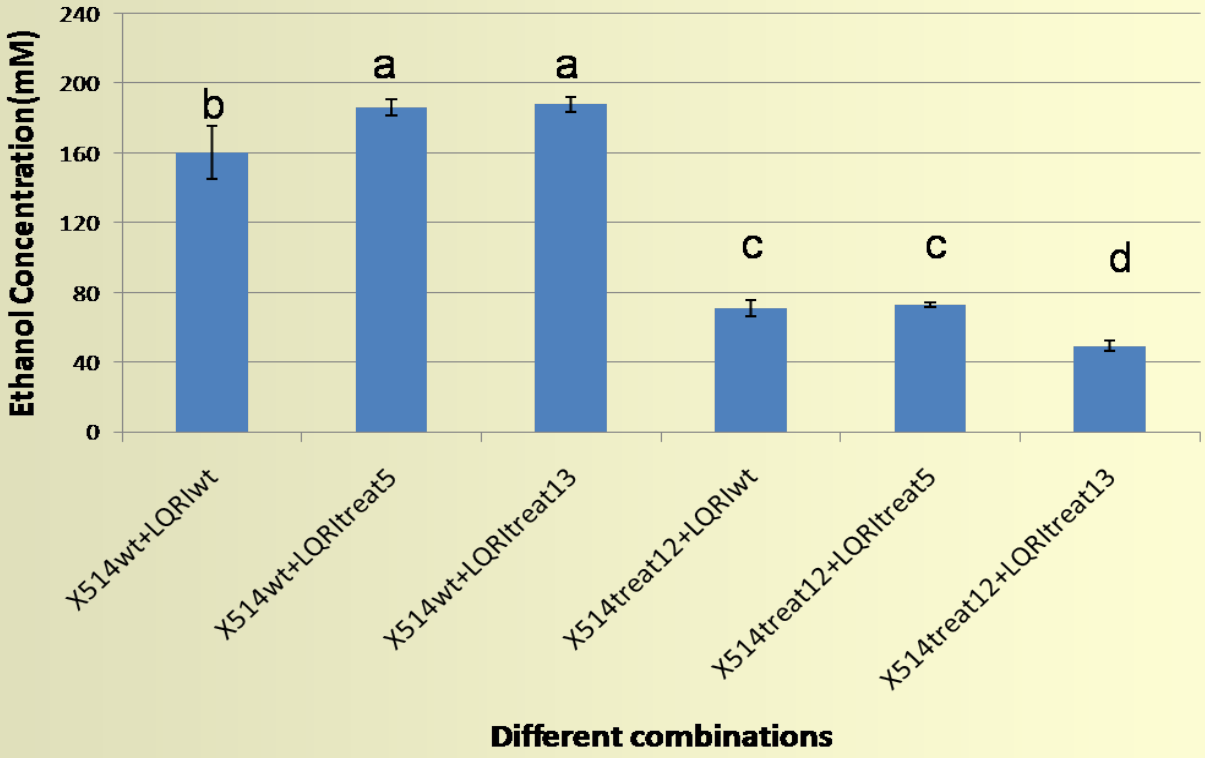


B. Colony 13



A. Colony 3 showed a shorter lag phase and faster growth rate with ethanol than without ethanol., suggesting that it may have already adapted to high ethanol conditions; B. Colony 13 showed different growth features.

Ethanol production of co-cultures with evolved strains



LQRI ethanol-evolved strains were able to produce more ethanol than the parent strain when co-cultured with parent X514.