Development of a Synthetic Metabolic Pathway for Sugar Bioalcohol Production in Thermophilic Microorganisms

**Background:**
- The production of ethanol from sugars by microorganisms has been achieved to date by only two types of metabolic pathway and typically only at moderate temperatures.
- Production of higher alcohols requires the engineering of an alcohol-specific pathway into microorganisms to produce one alcohol at a time.

**Approach:**
- A fundamentally different synthetic pathway for bioalcohol production at 70°C was constructed by inserting a single gene, that encodes a bacterial alcohol dehydrogenase (AdhA), into a model thermophilic archaeon (*Pyrococcus furiosus*).

**Outcomes:**
- The engineered thermophile converted glucose to ethanol via acetate and acetaldehyde, catalyzed by the host-encoded aldehyde ferredoxin oxidoreductase (AOR) and heterologously-expressed AdhA, in an energy-conserving, redox-balanced pathway.
- The AOR/AdhA pathway also converted exogenously-added carboxylic acids to the corresponding alcohol (e.g., propanol, isobutanol, 1-pentanol, isoamyl alcohol, 1-hexanol or phenylethanol), using glucose and/or hydrogen gas as the source of reducing power.
- By heterologous co-expression of a carbon monoxide dehydrogenase, carbon monoxide could be used as a reductant for converting carboxylic acids to alcohols.

**Significance:**
- The AOR/AdhA pathway is a new energy-conserving, redox-balanced route for sugar to ethanol conversion that could potentially be used in any CBP microorganism.
- Carbon monoxide and hydrogen gas (syngas) can be used as the driving force for bioalcohol production from a range of aliphatic and aromatic aldehydes, and is a potentially game-changing strategy for syngas fermentation.