TAS_Taipei 2018

SAY NO TO GLOW

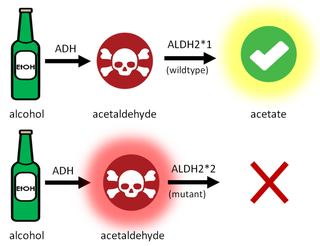
Reducing the carcinogenic effects of ALDH2 deficiency

Abstract & Introduction

Turning red after consuming alcohol may seem like a mere social inconvenience. Yet, this flushing response is caused by an accumulation of acetaldehyde, a carcinogenic intermediate of alcohol metabolism. Acetaldehyde is broken down into harmless acetate by aldehyde dehydrogenase 2 (ALDH2). ALDH2 deficiency, the result of a point mutation in the ALDH2 gene, produces a much less efficient ALDH2 enzyme, leading to an accumulation of acetaldehyde and the subsequent flushing response.[1] While about 8% of the global population is ALDH2 deficient, in our home, Taiwan, approximately 47% of the population carries this genetic mutation--the highest percentage in the world![2] Studies show that ALDH2 deficiency greatly increases the risk of developing esophageal and head and neck cancer.[3-10] Thus, our project aims to produce recombinant ALDH2 to decrease levels of acetaldehyde in the upper digestive tract region. We envision delivery of ALDH2 as a purified protein or in consumer-friendly probiotics.

What is ALDH2 Deficiency?

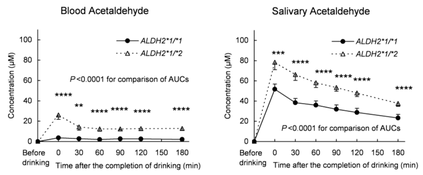
More commonly known as Asian Glow, ALDH2 Deficiency is a genetic condition that leads to the production of a less efficient ALDH2. This deficiency interferes with the metabolism of alcohol and causes toxic acetaldehyde to build up.



540 million people worldwide are deficient.[12]

47% of the Taiwanese population is ALDH2 deficient.[2]

Health Concerns



Acetaldehyde is a **Group 1 Carcinogen**[13]

High salivary acetaldehyde compared to blood[14]

2 to 8 times more likely to develop Head and Neck Cancers (oral, pharyngeal, etc.)[7-10]

2 to 12 times more likely to develop Esophageal Cancer[3-7]

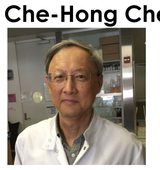
GOAL

Our goal is to **reduce salivary acetaldehyde levels** and the resulting increased cancer risks of ALDH2 deficiency by delivering functional ALDH2 in a candy that will remain in the mouth.

Human Practices

RESEARCH

Dr. Che-Hong Chen



Stanford ALDH2 Researcher

TAIES

Taiwan Alcohol Intolerance Education Society

Public Opinion

Survey

RAISING AWARENESS

Common Misconceptions

Healthy Liver
Fast Metabolism
High Blood Pressure

Education

Bandage Tests

Media

TheNewsLens 關鍵評論

今日報 TODAY NEWS www.todaynews.com.tw

POLICY CHANGE

Dr. Cheng-Hua Lee
Deputy Director General of National Health Insurance

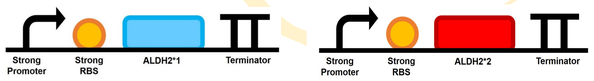
Proposal: NHI should cover free ALDH2 deficiency tests and oral cancer screenings.

Alcohol Warning Labels
Ministry of Health & Welfare
Ministry of Finance

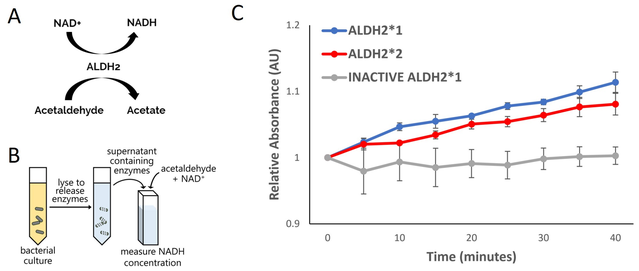
Proposal: Like tobacco labels, alcohol warning labels should always warn of health risks associated with alcohol consumption and be larger in size.

ALDH2*1 Lowers Acetaldehyde Levels

Expression of Recombinant ALDH2



Top: Figure 2-1. **Constitutive expression of ALDH2*1 and ALDH2*2.**



Top: Figure 2-2. **Testing ALDH2*1 and ALDH2*2 activity by measuring NADH production.**

NADH production was similar between ALDH2*1 and ALDH2*2-*E. coli*, so we isolated ALDH2 from other enzymes in the cell to directly test the effects of ALDH2.

Purification of HIS-ALDH2

Left: Figure 2-3. **Expression of HIS-tagged ALDH2*1 & ALDH2*2.**

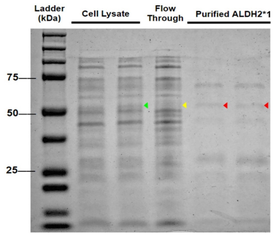


Figure 2-4. **Successful purification of ALDH2*1 (56 kDa).**

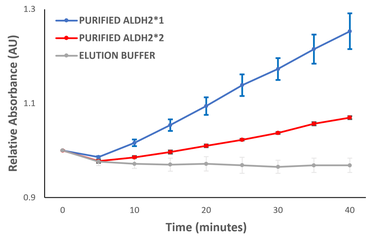


Figure 2-5. **Purified ALDH2*1 metabolizes acetaldehyde significantly faster than ALDH2*2.**

Regulation of ALDH2*1 Expression

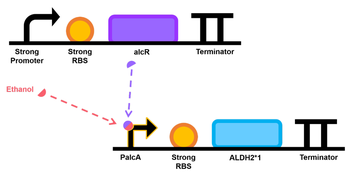


Figure 2-6. **In the presence of ethanol, PalcA will be activated to express ALDH2*1.**

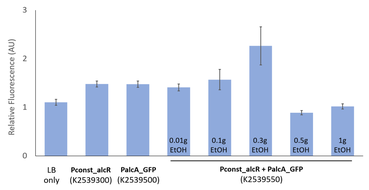
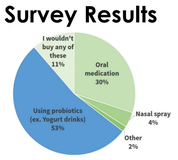


Figure 2-7. **GFP was used as a reporter to test PalcA activation at different EtOH concentrations.** Pconst is a constitutive promoter.

PalcA can be induced at 0.3g EtOH per 3 mL of culture, but is *very* inefficient. Thus, **this ethanol-regulated system is not applicable for our goal of regulating ALDH2*1 expression.**

PRODUCT DESIGN

Survey Results



Method of application?
A combined 83% preferred either probiotics or oral medication

Yakult

Probiotics in Commercial Products

Dr. Ying Chieh Tsai

Founder of Taiwan Association of Lactic Bacteria

References and Acknowledgements

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Product Design: ALDH2*1 Probiotic Candy

Probiotic *E. coli* Nissle 1917 (*EcN*)

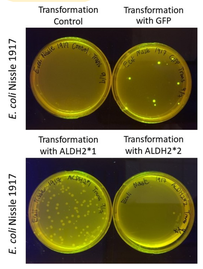


Figure 3-1. **Successful transformation of GFP, ALDH2*1, and ALDH2*2 into *EcN*.**

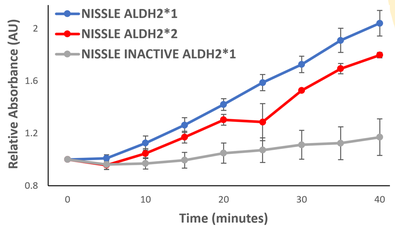


Figure 3-2. **ALDH2*1-*EcN* metabolizes acetaldehyde significantly faster than ALDH2*2-*EcN* in artificial saliva at 37°C.**

Proof of Concept: Making Probiotic *EcN* Candy

Determining Temperature Threshold

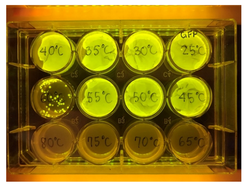


Figure 3-3. ***EcN* should be added to the candy at temperatures below 60°C.**

Production of Candy

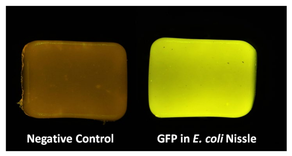


Figure 3-4. **We added *EcN* during the cooling phase of candy-making.**[15] GFP-*EcN* was used here to show that proteins remain functional!

Product Testing: ALDH2*1-*EcN* Candy

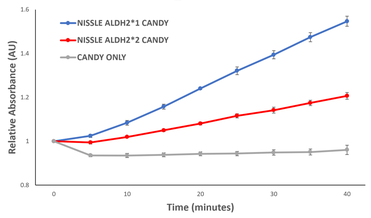


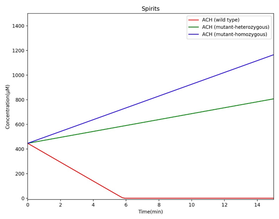
Figure 3-5. **ALDH2*1-*EcN* from the candy works!** After making and dissolving our probiotic candy, we show that the released ALDH2*1-*EcN* metabolizes acetaldehyde significantly faster than ALDH2*2-*EcN* in artificial saliva at 37°C.

Information for Manufacturers and Consumers

GOALS

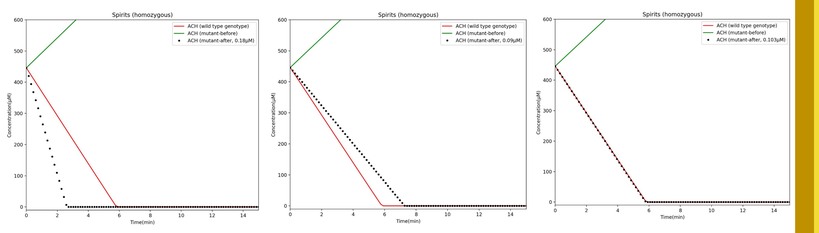
1. How much ALDH2 should be put into each probiotic candy?
2. How much ALDH2 should be consumed?

[Substrate 1 (Ethanol)] —Enzyme 1 (ADH)→ [Substrate 2 (Acetaldehyde)] —Enzyme 2 (ALDH2)→ [Product (Acetate)]



Left: Figure 3-6. **Oral [Acetaldehyde] changes after alcohol consumption for different ALDH2 genotypes.** Based on literature values of oral [Ethanol], oral [Acetaldehyde], ADH reaction rates, and experimental values for our ALDH2*1 reaction rates, we created a software to model sequential enzymatic reactions.

Below: Figure 3-7. **We adjusted [Acetaldehyde] until homozygous mutants process acetaldehyde at wild type ALDH2 levels.** Here, an additional 0.103 μM ALDH2 matches wild type activity.



2.67 mg of ALDH2*1-*EcN* should be put in one candy, releasing 0.103 μM ALDH2*1/min. Regardless of the alcohol type, **each candy allows a deficient consumer to match normal wild type ALDH2 activity**.

MARKETING PLAN

For our envisioned ALDH2 probiotic candy

02 Market Analysis
Competitors generally aim to reduce flushing.

Our product directly reduces cancer risks by supplying ALDH2 enzyme (which will also reduce flushing).

05 Challenges & Solutions
Examination of current probiotic, GMO, and health food regulations in order to responsibly put forth our product.