INTRO
At the Real Vegan Cheese Project, we are engineering the synthesis of bovine and human casein milk proteins by baker’s yeast. Saccharomyces cerevisiae, enabling cow-less cheese production. Animal agriculture contributes to animal suffering and a variety of environmental problems, including global warming, water pollution, and deforestation. A vegan cheese that more closely mimics cow cheeses could help many more people switch to a plant-based diet.

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We hope to produce cheeses synthetically that are more ethical, sustainable, and, by excluding lactose or allergenic bovine caseins, potentially healthier than traditional dairy cheeses.

BACKGROUND
The four main proteins in cow’s milk cheese are alpha-s1, alpha-s2, beta, and kappa casein (humans do not have alpha-s2). The hydrophobic caseins aggregate into casein micelles, with the hydrophilic tail of k-casein pointing outwards. During cheese making, this hydrophilic k-casein tail is cleaved off by chymosin (rennet), making the micelles coagulate into casein curds. We will also study the Fam20C kinase that phosphorylates caseins in milk [Taglialabracci et al., 2012], thought to be important for formation of casein micelles.

METHODS
Part design: 11 gene constructs
Clone into pD1214 shuttle vector
Transform into E. coli
Plasmid DNA extraction
Sequence gene inserts
Transform into S. cerevisiae
Assemble & optimize protein expression
Protein purification

Milestone 1: Part design.
We started with published sequences for cow and bovine caseins as protein sequences are common human allergens. For the protein kinase, we used sequence from human Fam20C. No animals needed! First, we codon-optimized gene sequences for expression in yeast using IDT’s codon optimization tool. We then added a native yeast secretion signal (alpha-factor) preceding each. The alpha-factor peptide is cleaved off the protein during secretion, by the Kex2 transmembrane protease. Since Kex2 protease recognition sites (KR and RR) also occur in the native sequence of three of our study proteins, we also created “Kex-” constructs in which extraneous Kex2 sites were changed to biochemically similar amino acids (KR -> KK).

METHODS

Milestone 2: Clone, transform plasmids to Escherichia coli, & sequence confirm.
We cloned composite DNA constructs into the DNA 2.0 Electro pD1214 plasmid backbone using a kit that simultaneously digests at the external SapI sites and ligation. This plasmid can be selected in E. coli using its Ampcillin-resistance gene. It also contains a strong constitutive TEF promoter for gene expression in yeast and a URA3 marker for selection in Ura deficient yeast strains. After transformation into E. coli, we extracted plasmid DNA and submitted for sequence confirmation.

Milestone 3: Transform plasmids to yeast.
Our yeast strain is Saccharomyces cerevisiae, UV-Sensitive Strain, G418-1C/U, alpha, rad1 rad18 phr1 ura3 mutant in excision repair. It is Ura deficient, allowing for selection of our desired plasmid transformants on URA negative media. Other yeasts such as Pichia may eventually be used for large-scale industrial production.

Milestone 4: Verify protein expression and secretion in yeast and purify proteins from yeast growth medium
We will verify protein expression in yeast culture supernatant proteins using SDS PAGE gel electrophoresis, then explore protein purification methods. We should be able to use the caseins’ propensity to become insoluble at specific pH and calcium concentrations to purify the proteins from the supernatant.

RESULTS

Milestone 1: Part design. Done!
Milestone 2: Clone, transform plasmids to E. coli, & sequence confirm. 10 done; 100% sequence alignment. No successful transformation of human kappa casein (Kex-) into E. coli yet.
Milestone 3: Transform plasmids to yeast.
Currently optimizing our yeast expression system.

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