Big Ideas Contest: Engineering Probiotics for Vitamin A Deficiency

I. Problem Statement

Curd or “dahi” as it is known in South Asia, is a traditional yogurt and a pivotal component of the diet of the region. The age-old tradition of making and eating this yogurt is ingrained in South Asian culture and provides an important nutritional probiotic that supports healthy digestion in a community that is largely strictly vegetarian. Today, this traditional yogurt is an essential part of every meal for a vast majority of the population. The traditional method of preparation entails a sustainable production using previously prepared yogurt, and the method and science has been passed on from generation to generation allowing autonomous production by the population. This uniquely positions yogurt to be a key candidate to attend to nutrient deficiencies in the South Asian population through modification and engineering.

According to an estimate by the World Health Organization (WHO), micronutrient deficiencies affect over 2 billion people around the globe [1]. These micronutrient deficiencies, also known as hidden hunger, are one of the three main contributors to malnutrition and present a massive threat to global health, especially for children and pregnant women. Our solution specifically targets South Asia, which has one of highest rates of VAD in the world [11]. About 44–50% of South Asian preschool children have severe VAD while 85% of South Asian children have xerophthalmia (dry conjunctiva and cornea associated with VAD) [5]. With this hidden hunger, children may face stunted physical and mental development, vulnerability to disease, and blindness [3]. Even lactating mothers with VAD pass on reduced vitamin A levels to their infants through breast milk, affecting infant development and growth [5].

Vitamin A is a fat-soluble vitamin whose preformed versions, like retinol, can be consumed through certain fish, organ meat, dairy, and eggs. Alternatively, the provitamin form appears as carotenoids, like beta-carotene, commonly found in leafy greens and orange and yellow vegetables [9]. Vitamin A is integral to vision health, cell growth and division, bone remodeling, reproduction, immunity, and maintaining healthy endothelial cells [10]. However, inadequate dietary intake and diversity, poor bioavailability, and sociocultural limitations can lead to VAD and its associated health complications. The Copenhagen Consensus Center found that supplementing vitamin A to children under five years old can reduce the risk of mortality by 23% [4]. This improvement in mortality is why the Lancet Journal cited vitamin A supplementation as one of the leading potential treatments for micronutrient deficiencies.

However, at a $1 cost per child, only 38% of children in South Asia can realistically receive adequate vitamin A to treat VAD, and more investment into the program is required for a reduction in cost [2]. Due to delivery issues, a more easily integrated treatment for addressing malnutrition should be developed. We propose a yogurt-based, engineered probiotic powder that can synthesize the provitamin beta-carotene in the gut, providing a method for low-dose, continuous delivery of vitamin A to micronutrient deficient South Asian populations.

II. Landscape Analysis

Current strategies to mediate vitamin A deficiency (VAD) in South Asia include dietary diversification, vitamin supplementation, and food fortification [5]. Dietary diversification requires a substantial amount of resources spent on educational intervention as well as changes to the traditional diet. Although this diversification is able to simultaneously address multiple deficiencies, it relies on individuals going out of their way to grow and prepare the new foods which could conflict with religious dietary restrictions and traditions [5].

Vitamin supplementation is regarded as the most effective and widely practiced strategy addressing VAD in low-income countries. Currently, children in South Asia are supposed to receive a high-dose injection of vitamin A every 6 months, but a lack of strict regulation results in many children not receiving the shots. Additionally, if they do receive the shot, such a high dose of vitamin A at once has shown to cause headaches, nausea, or vomiting. Another prevalent issue with supplementation is government support, as previous instances of distributing vitamin A to regions in South Asia have shown [5]. Due to the requirement of a daily intake of vitamin A, vigorous government intervention is needed to motivate people to take the supplements daily.

The process of nutritionally enhancing staple crops such as vegetable oils, wheat, and sugar (a process known as biofortification), is becoming another viable strategy towards addressing VAD and is well-established in several regions. Pakistan, India, and Bangladesh all have policies requiring vegetable oils to be vitamin A fortified, a cost-effective and safe method, as there is no risk associated with high intakes of properly fortified oil. However, the success of a fortification program also relies heavily on appropriate surveillance and monitoring of the fortification and distribution process, which has been poorly managed in Pakistan, for example, where only few of the edible oil processors fortify the oil in accordance with the law. The effectiveness of the fortified oil has also been found to depend on the type and quality of the original oil and its stability in storage conditions, which further complicates the fortifying process and usage [6]. An additional issue that comes with the biofortification of beta-carotene specifically, is that the antioxidant is also a yellow-orange pigment, and thus not all beta-carotene-fortified 'staple foods' are able to be seamlessly incorporated into the diet. Altering the color of a traditional food through genetic engineering has shown both cultural and religious implications, one example being the attempted implementation of Golden Rice and the resistance it has received by some groups in South Asia [7].

III. Proposed Innovation

We have identified multiple critical issues with current treatments for vitamin A deficiency in South Asia, including irregular delivery of treatment and incompatibility with way of life. In developing our product, we will focus on three main goals: **1) reducing government intervention, 2) integration into daily life, and 3) decreasing the need for high-doses**.

To meet the increased demand for vitamin A in South Asian populations and to counter the issues with current methods, we propose a low-cost, powdered yogurt starter culture engineered with a Live Biotherapeutic Product (LBP) (a product containing live organisms engineered to provide a therapeutic benefit) that can continuously synthesize beta-carotene in the gut. Our starter culture will include *Saccharomyces boulardii,* a well-known probiotic yeast, engineered to secrete beta-carotene and last a long time in the gut .

In **reducing the need for government intervention**, our product has the potential to last for much longer than the current interval of 4-6 months. New batches of yogurt can be made from previous cultures, allowing families to rely on a single spoonful of powdered starter and milk to keep cultures alive for generations. Our probiotic would act to supplement families' cultures, integrating vitamin A-producing microorganisms into food that they eat on a daily basis. Cultures typically need to be recultured once a week, and as such our provided powder would only need about 52 servings to provide an entire family with one years' worth supply of vitamin A. Ultimately, our product would reduce the frequency of government involvement in vitamin A supplementation and decrease the demand for workers trained to administer injections.

The **tradition of making yogurt and cultivating new batches weekly is deeply ingrained in South Asian culture**. Families typically make yogurt at home – an already well-established and economical process capable of producing large batches. We envision our product to be used either as a substitute for, or in addition to existing cultures, maintaining the same taste and texture, with the added nutritional benefit. As many South Asian cultures already have traditional ways of raising yogurt, the starter powder will provide them with a platform to turn something they use daily into a useful vitamin A boosting agent [8].

To **combat the side effects of taking extremely large doses of vitamin A** at once, our engineered probiotic will instead continuously synthesize vitamin A precursors as shown possible by previous research [12, 13, 14]. By providing beta-carotene, the body only converts those provitamins into vitamin A as it is needed, reducing the risks associated with high-doses of vitamin A. Use of an engineered probiotic largely tackles the issues encountered with irregular delivery of treatment as well. Rather than taking daily pills or visiting someone trained to administer vitamin A injections, families will be able to have a constant source of vitamin A production within their own bodies and homes.

Our first year of implementation will be focused on R&D of our engineered probiotic, expanding on past research and working to culture *S. boulardii* as a probiotic yogurt powder. We will also work on increasing the typical amount of beta-carotene that can be produced by *S. boulardii* in order to amplify our titer in addition to increasing the expression of adhesins (the protein that helps microorganisms attach to surfaces) to extend the probiotic lifetime in the gut. While we already have potential lab space in Dr. Gian Garriga's lab at Berkeley, our more immediate challenges will be to gain access to the correct equipment and strains we need to begin experimentation. We will work to acquire these items with local professor and funding from our club. Longer term challenges involve implementing the product in South Asia. Because we are expecting to distribute our product yearly to South Asian families, we will need to get in touch with the vulnerable communities we pilot the product in and understand any other cultural or governmental roadblocks to implementation within the next few years.

The hidden hunger prevalent across South Asia presents a dire global health issue that needs sustainable, integrable solutions as soon as possible. Our proposed product is culturally conscious, easily accessible, and could provide South Asian families with an effective and reliable method for combating vitamin A deficiency, placing the power back in the hands of the people it affects most.

IV. Team Bios

Kylie Akiyama - Team Lead, Bioengineering Undergraduate
Kylie has spent multiple years working in a synthetic biology lab, learning how we can engineer organisms to synthesize new products. As team lead and teacher of a student-run synbio course at Berkeley, she has experience with team management and scientific communication.

Aaron Feldman - Chemical Engineering, Undergraduate
Aaron has experience in the fundamentals of modeling flow systems like the secretion of chemicals by probiotic organisms in the gut and has learned how to model these systems in MATLAB. He has been part of ChemE Car, so he has experience building a finished product (a car that runs using chemical reactions) with a team, and can transfer these skills into this project.

Katya Panchenko - Molecular and Cell Biology/Computer Science, Undergraduate
Katya has taken coursework on general biology as well as courses on the microbiome, integrative human biology, bioengineering, and CRISPR. She has also had experience with human-centered product design for social good.

Ananya Chawla - **Molecular and Cell Biology/Computer Science, Undergraduate**
Ananya is a Junior studying molecular and cellular biology with an emphasis in neurobiology and minoring in CS. She has technical experience extracting and analyzing DNA at the Gillespie lab where she works on studying adaptive radiation in spiders on the Hawaiian Islands.

Nikhit Kambdur - Molecular and Cell Biology/Data Science, Undergraduate
Nikhit has academic and industry experience with structural biology, bioengineering, enzyme function and characterization, cytokines, and CAR T-cell therapy. He also has experience engineering microorganisms in the context of the food industry, such as his work engineering *L. lactis* to express chitinases to prevent cheese spoilage.

Sophia Jia - Molecular and Cell Biology/Data Science, Undergraduate
Sophia is studying MCB with an emphasis on immunology and minoring in data science. She previously conducted research on the possible production of *Angelica Akesikei* on Diabetes Type 1 treatment and has experience in ELISA assays, flow-cytometry, and drafting proposals.

Isabelle Pummill - Bioengineering, Undergraduate
Isabelle has experience working in clinical laboratories where she helped culture and identify microbes. Additionally, she has conducted research which observed bladder overactivity in patients to develop less invasive methods of characterizing and diagnosing the condition.

Kieran Brown - Microbial Biology/Aerospace Engineering, Undergraduate
Kieran is a senior interested in space biology and unique applications of microbes. After working with Amgen, SSL, UC Space Health Program, and both the Biological & Physical Sciences and Human Research Programs at NASA, he will join a lab at NASA Ames after graduation.

Logan Krause - Bioengineering and Material Science Engineering, Undergraduate
Logan is studying Bioengineering and Material Science Engineering at UC Berkeley.

Deniz Dumusoglu - Advisor, Chemical Engineering Postdoctoral Researcher
Deniz is a postdoctoral researcher at North Carolina State University and is a key advisor of this proposal. Deniz completed her graduate work under Nathan Crook at NC State, where she worked to establish probiotic yeast as an in-gut biomanufacturing platform. She published a proof-of-concept paper showing the capacity for *S. boulardii* to produce beta-carotene in the gut of mouse models and provides scientific guidance to the team.

Additional members: If selected, we would recruit students more experienced in marketing and finance in order to help manage the business aspect of this challenge. We would also consider adding students studying global or public health who are more familiar with implementation strategies in vulnerable regions. Recruitment would occur along with our club's timeline beginning in the Spring semester.

V. Citations and References

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