

# Ingestible probiotic capable of supplemental production of lactose-cleaving enzyme to combat symptoms of lactose intolerance

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Lactose Intolerance (LI) afflicts 70% of the global population. LI is caused by a deficiency in the natural production of lactase, the enzyme produced by intestinal epithelial cells that breaks lactose into its digestible components glucose and galactose. With no remedy, it is common for the afflicted to avoid dairy products, which may lead to lowered dietary calcium intake and loss of bone density. A synthetic biology approach to supplement the loss or lack of natural lactase production is described wherein an ingestible probiotic releases the enzyme  $\beta$ -galactosidase in the presence of dairy-related saccharides, specifically lactose. The release of  $\beta$ -galactosidase is facilitated by the expression of the *ydfD* gene, which codes for a novel lytic protein. An edited *lac* promoter (*lacUV5*) expresses this gene such that autolysis only occurs in the presence of lactose.  $\beta$ -galactosidase is produced in the period leading up to the stationary phase of the chassis by the *lacZ* gene, and is regulated by the *osmY* promoter. Additionally, the *dicB* gene, known to abolish *ydfD* induced lysis, is regulated by the *osmY* promoter, disallowing premature autolysis. These two devices ensure that the NGF-1 *E. coli* chassis produces supplemental lactose-cleaving enzyme and releases said enzyme in accordance with environmental cues to effectively combat the symptoms of LI.

**Keywords:** Lactose intolerance, probiotic, autolysis

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Watch a video introduction by the authors at <https://youtu.be/FoZ3q-MfjKY>

## Background

Lactose Intolerance is the inability to sufficiently break down lactose, a disaccharide composed of glucose and galactose. In lactase-persistent individuals, lactose is hydrolyzed by the enzyme lactase into D-glucose and D-galactose before it can be absorbed by the body. Especially in younger patients, LI may be caused by mutations to the LCT gene, a gene within intestinal epithelial cells in the human small intestine which produces lactase. These mutations affect the function of the lactase enzymes it produces. In the majority of cases, lactose intolerance is the decreasing expression of the LCT gene via a regulatory element found upstream in the MCM6 gene (Labrie, Buske, Oh, et al. 2016). Undigested lactose continues into the large intestine where it may ferment into short-chain fatty acids, H<sub>2</sub>, and methane. In the presence of some enteric bacteria, such as *Streptococcus lactis*, the malabsorbed lactose may ferment into lactic acid (Fassio, Facioni and Guagnini 2018). These processes cause abdominal discomfort or cramps, abdominal bloating, diarrhea, and gas.

LI is a chronic disorder with no cure and little recourse. Lactose intolerant people seeking treatment or relief options may encounter lactase enzyme supplements, but these are not ideal for most due to their inconvenience. Lactase tablets are meant to be taken with the first bite of any lactose containing meal and every 30 minutes after, requiring they be on-hand and in abundance. The lack of viable treatment options may lead to total avoidance of dairy products, which in turn drastically lowers dietary calcium intake. When the body doesn't get enough calcium it takes calcium from the bones, a process which makes bones weak and porous, and can lead to future conditions like osteoporosis. Other than this, infants who are born with mutations to the LCT gene can end up sick and malnourished, especially without access to lactose-free formula.

Probiotics are live microbes which have a positive effect on their host organism. Probiotics have been observed to have important roles in inhibiting the growth of pathogenic bacteria through various mechanisms, including modification of the gut microbiota and fortification of the intestinal epithelial barrier. Probiotics have also been found to exhibit antagonistic abilities with respect to antigens, outcompeting these antigens in adhering to the mucosa, aggregation and coaggregation, and bacteriocin production. One probiotic found in yogurt, *Streptococcus thermophilus*, has been observed to break down lactose and allow those with LI to digest yogurt with fewer or lesser symptoms (Markowiak and Śliżewska 2017). Probiotics must be able to survive gastrointestinal conditions.

The lactose operon (lac operon) is an operon found in *E. coli* among other species. It is responsible for the production of  $\beta$ -galactosidase, an enzyme which catalyzes the hydrolysis of the disaccharide lactose into the monosaccharides glucose and galactose. The gene encoding  $\beta$ -galactosidase is lacZ.  $\beta$ -galactosidase works by breaking the  $\beta$ -1,4-D-galactosidic linkages of lactose (Juers, Matthews and Huber 2012).

The intestinal epithelial cells that normally produce the human supply of lactose cleaving enzyme are found in the brush border of the small intestine, so it would be ideal for the probiotic supplement to colonize this region of the gastrointestinal tract.

## Systems level

The intent was to design an ingestible probiotic bacteria administered on a monthly time frame which could supplement the host with  $\beta$ -galactosidase in order to combat the effects of LI or lactose sensitivity by working to cleave undigested lactose into galactose and glucose before it becomes fermented in the large intestine. It was not complex to produce  $\beta$ -galactosidase, as the lacZ gene is part of the well documented lac operon.  $\beta$ -galactosidase is a large enzyme (1021 amino acids), and due to its size cannot be secreted through any known bacterial protein channels (Dwyer, Malinverni, Boyd, et al. 2014). For this reason cell lysis is necessary to disseminate it.

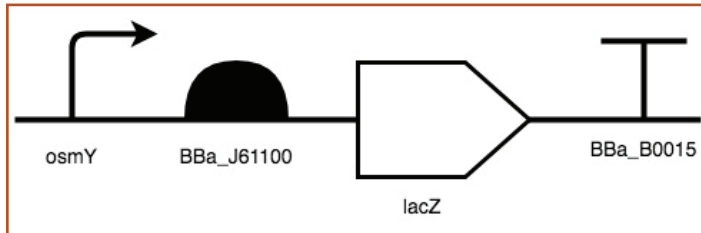
This autolysis occurs under the stimulus of lactose, so there must be a reserve of  $\beta$ -galactosidase within the cell prior to the consumption of lactose. The solution is to produce  $\beta$ -galactosidase during the lag and log phases of bacterial growth. Lysis before the stationary phase, when only a small amount of  $\beta$ -galactosidase will be available, would be ineffective so a lysis-preventative gene is expressed during this phase.

The probiotic is ingested and the NGF-1 *E. coli* chassis is given time to adjust to the gastrointestinal conditions and colonize the mucosa of the large intestine. After some time, the bacterial population will reach its stationary phase and be primed for  $\beta$ -galactosidase expulsion. At this point, the introduction of lactose will cause some percentage of the population to lyse, and the burst of  $\beta$ -galactosidase will work to break down any lactose into glucose and galactose before it can be fermented into methane and lactic acid by other enteric bacteria. This prevents the symptoms of LI.

The remaining population reenters the log phase, growing in size until entering the stationary phase and again priming for the next instance of lactose.

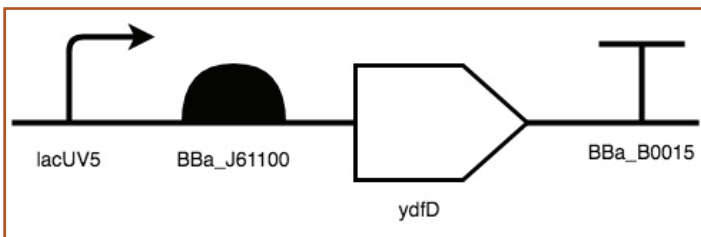
## Device level

Device 1 (Figure 1) produces an internal reserve of  $\beta$ -galactosidase leading up to the stationary phase. The *osmY* promoter ensures that the *lacZ* gene responsible for the production of  $\beta$ -galactosidase is active in the hyperosmotic conditions of the gastrointestinal tract but is cut off once the colony enters the stationary phase.



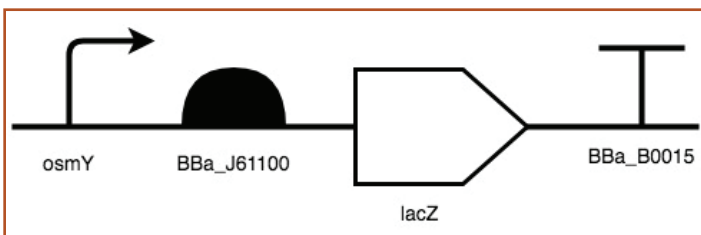
**Figure 1.** Depiction of assembled Device 1

Device 2 (Figure 2) uses the *lacUV5* promoter to over-express the *ydfD* gene in the presence of lactose, causing the bacteria to lyse thereby releasing the  $\beta$ -galactosidase.



**Figure 2.** Depiction of assembled Device 2

Device 3 (Figure 3) prevents premature autolysis by activating the *dicB* gene, which is known to prevent *ydfD* induced autolysis. The *osmY* promoter ensures that once the bacteria enter the stationary phase this function shuts off, priming the chassis to utilize the second device to the highest efficiency.



**Figure 3.** Depiction of assembled Device 3

The lambda red system is used to insert these devices directly into the chromosome of the chassis bacteria. Chromosomal integration ensures all three devices will

be passed on to the daughter cells, which is necessary for the overall design but difficult to achieve using plasmids as the sole expression vector. Because this is bacterial chromosomal integration, the lambda red genes will be expressed through a plasmid so that they can be removed from the cell after successful creation of recombinant clones. To insert the devices into the chromosome of the chassis, an insert site (a gene) is selected, and specialized primers are used to basically flank the devices with around 50 base pairs of said gene. Once the sequence is prepared for insertion the plasmids expressing the lambda red genes are induced. The device is introduced into the chassis via a controlled electric pulse which opens membranous pores (Datsenko and Wanner 2000).

## Parts level

### *lacUV5* promoter

*lacUV5* is derived from the *lac* promoter found in *E. coli*. The *lac* promoter is repressed transiently in the presence of glucose and also through catabolite repression. *lacUV5* is insensitive to these forms of repression in even extreme conditions while still maintaining a level of expression on par with the wild type promoter. These factors make *lacUV5* a strong promoter, and being a derivative of the *lac* promoter, still induced in the presence of lactose (Silverstone, Arditti and Magasanik 1970). A strong promoter sensitive to lactose is important for well-timed  $\beta$ -galactosidase expulsion.

### *osmY* promoter

The *osmY* promoter is found in *E. coli* and has been found to be stimulated under both hyperosmotic conditions and growth phase signals (Yim, Brems and Villarejo 1994). This promoter is ideal as it can be induced by both log phase signals and the hyperosmotic conditions of the intestines. Most importantly, the promoter is no longer induced once the colony enters its stationary phase.

### *lacZ* gene

The *lacZ* gene is found in *E. coli* in the well documented *lac* operon. *LacZ* codes for  $\beta$ -galactosidase, making it the most essential component of the project.

### *ydfD* gene

The *ydfD* gene is found in *E. coli* and codes for a lytic protein. *ydfD* induced lysis entails one to two cell ruptures and an observed 99.9% mortality rate within 2 hours (Masuda, Awano and Inouye 2016).

### *dicB* gene

The *dicB* gene is found in *E. coli* and primarily serves to inhibit stable Z ring formation, preventing cell division. It has also been shown to abolish *ydfD* induced cell lysis (Masuda, Awano and Inouye 2016). By expressing this gene during the lag and log phases premature cell lysis is prevented.

Expressing this gene during the log phase seems impossible without some editing, as prevention of cell division does not lend itself to population growth. The range of the *dicB* protein most important to Z ring interference has been mapped to the N terminus, so various primers could be used during PCR to edit out different lengths of the N region. These edited *dicB* genes will have to be tested until one is found to inhibit *ydfD* induced lysis without preventing cell division.

### Safety

Given that this design is intended for commercial release and ingestion, extensive measures must be taken to ensure no health risks are posed. No precaution is too far for consumer goods. Although the NGF-1 *E. coli* chassis successfully colonized mice intestines with no noted negative health effects (Ziesack, Karrenbelt, Bues, et al. 2018), it would be wise to utilize simulated human gastrointestinal conditions or even highly controlled human trials in the later stages of development.

### Discussions

The time frame this probiotic operates on is difficult to predict without testing. This testing will focus the time it takes for the colony to reach its stationary phase, at which point lactose can be consumed by the host. Persistence of the colony once introduced to lactose and the time this remaining population takes to enter its stationary phase must also be understood so that the host is aware of what amount and at what times it would be appropriate to consume dairy and expect the reduced symptoms this probiotic offers.

Due to the prevalence of LI, it is uncommon to think of this widespread condition as something that solicits treatment. Persons affected by LI, having no recourse save attempting to fill the void with the most bland and unworkable ice cream substitutes imaginable, may find a commercial probiotic that opens up their world to dairy highly exciting.

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