**Efficiently Producing 2,3-Butanediol Via Genetic Modifications for Industrial Applications**

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**2,3-butanediol (BDO) is a chemical that is effective in the production of insecticides, plastic coatings, intermediates in pharmaceuticals, and its (2R,3R)-BDO isomer has various potential applications by the US Department of Energy. Despite the various industries that the chemical could be used in, methods of producing 2,3-BDO and its isomers are too expensive for manufacturing, making production inefficient. This problem has led scientists to explore more economical methods of production for industrial use. Currently, genetic modification is the leading solution to solving this problem. Microbiologists can now take isolated colonies of *Bacillus licheniformis*, a bacterium that is known to naturally produce 2-3-BDO, and genetically modify them to produce 2,3-BDO faster than non-mutant strains. However, both strains produce (2R,3R)-BDO and (2R,3S)-BDO isomers in a 1:1 ratio, resulting in recent experimentation for isomer selectivity. Mutant strains of *B. lichenformis* can undergo gene deletion in order to guarantee stereospecific isomers produced during fermentation. This revolutionary method of modifying bacteria shows the potential that genetic modification has on bringing an effective chemical back to the market in an economical and efficient way.**

**Introduction**

2-3-BDO is a chemical that has historically shown effectiveness when used in the production of various products but is not currently being produced commercially because of the price competitiveness of newer-economical chemicals [1]. Due to the rapid depletion of fossil fuels, biofuels are now more important to produce and utilize than ever before. The stereospecific isomer (2R,3R)-BDO is considered a platform chemical with great potential by the US Department of Energy as they say it could be a drop-in-fuel additive because it burns at higher temperatures compared to other liquid fuels [2,4]. This means 2,3-butanediol has the potential to be an influential biofuel if the cost of producing it were to go down. Current methods of producing the chemical and separating it into its isomers are highly expensive and complex, using gas routes in butane from crack gases [1]. These methods are also insufficient for industrial production because chemically synthesizing it, rather than naturally producing it, restricts its function as a drop-in-fuel additive [6]. This has led to the focus on how to not only maximize the natural production of 2,3-BDO but also separate it into its isomers. Many microorganisms are known to naturally produce 2,3-butanediol, most of which are gram-negative bacteria [3]. However, most gram-negative bacteria are recognized as biosafety level two organisms, making them unsafe for industrial use [1]. This problem has led to the recent progress of using GRAS (generally recognized as safe) strains of the few gram-positive bacteria that produce 2-3-BDO in order to safely produce the chemical. Species that fit that criteria include, but are not limited to, are *Paenibacillus polymyxa*, *Bacillus licheniformis*, and *B. amyloliquefaciens* [1,5]. Of them, *Bacillus licheniformis* is favorable for experimentation because not only are the gene-editing tools readily available for the strain, but its fermentation conditions are of those favorable for industrial use [5]. Being that *B. licheniformis* is the optimal candidate for 2,3-BDO industrial production, it has been the main focus of how genetic engineering can economically increase the natural fermentation of the chemical.

**Recent Progress**

The challenge to maximizing 2,3-BDO production in *B. licheniformis* is that the bacteria exhibits mucoid features, which reduce the production of 2,3-BDO. Studies have shown that non-mucoid strains of *B. licheniformis* are able to produce more *bacillus* plasmids during fermentation than strains with mucoid features present [1]. This is important because the number of plasmids produced during fermentation directly correlates with the amount of 2,3-BDO produced [4]. This means that a non-mucoid strain will produce more of the chemical during fermentation. A non-mucoid strain of *B. licheniformis* can be produced via rounds of UV-random mutagenesis, the process of mutating bacterial colonies by exposing them to UV light for a certain amount of time [1]. Due to performing UV-random mutagenesis, the mutated bacterial colonies have a death rate of 99% and the surviving colonies are critically examined by fermentation studies and electroporation in order to select the non-mucoid strains. One study performed an experiment to calculate and compare the total amounts of 2,3-BDO produced during glucose fermentation of the bacteria. They took 2 cultures of the same size, one being a non-mucoid strain of *B. licheniformis* that had been selected through UV-random mutagenesis, and the other being a naturally mucoid-present parent strain. Both of the cultures were subject to glucose fermentation and it was concluded that the non-mucoid strain produced 35g/L of 2,3-BDO in only 19 hours while the mucoid strain produced the same 35g/L in 32 hours [1]. This means that the non-mucoid strain was able to ferment the chemical of interest roughly 59% faster than the mucoid-present parent strain. These results suggest that this process of genetic engineering could be a pioneering method in naturally optimizing 2,3-BDO production. However, (2R,3S)-BDO and (2R,3R)-BDO isomers were measured at a 1:1 ratio from both cultures, so the question for stereospecificity, in regard to the interest of biofuels [5], remains unanswered. The study helps support the suggestion that genetic modification can optimize the production of 2,3-butanediol in an efficient manner, but does not address a solution to stereospecific production.

**Discussion**

Being that the gene-editing toolbox is readily available for *B. Licheniformis* [1,4], further genetic modifications can be made to the microorganism in experimentation in order to selectively produce 2,3-BDO isomers. Once the genome of *B. Licheniformis* has been prepared for genetic engineering, the gene *budC,* which encodes for the stereospecific (2R,3S)-butanediol isomer, can be selected as a deletion target to selectively confirm the stereospecific production of (2R, 3R)-butanediol [1]. The hindrance to producing one isomer will lead to an increase in production of the other isomer. Once the *budC* gene is removed from the genome, batch fermentations of a non-mucoid Δ *budC* strain of *B. licheniformis* and a non-mucoid parent strain that still has the *budC* gene can be completed in glucose. These fermentations can be studied and measured to show that the non-mucoid Δ *budC* strain produces the (2R,3R)-BDO isomer at approximately a 9:1 ratio compared to the 1:1 ratio of the strain with the gene. [1]. This suggests a way to control the ratio of 2,3-BDO production. Despite being selectively stereospecific in the production of (2R,3R) -BDO, the non-mucoid Δ *budC* strain does not produce as much total 2,3-BDO. The same study as mentioned before found that although the Δ *budC* strain produced 105g/L in 70 hours and the *budC* strain produced 123 g/L in the same time, the Δ *budC* strain still produced 56% more of the (2R,3R)-BDO isomer than the *budC* present strain [1]. This method of selectively producing stereospecific 2,3-BDO isomers can be revolutionary for the biofuel industry but must be studied more in order to maximize production. The results of these studies show that it is possible to control the ratios of which isomers are produced, but does not suggest a method of pure separation. The results show a 9:1 ratio of the (2R,3R)-BDO isomers being produced, meaning that the total 2,3-BDO produced still contains roughly 10% of the (2R, 3S)-BDO isomer. Future studies should exploit efficient methods of naturally separating these isomers as it could have a monumental impact on the biofuel industry. There is also a need for more studies to be performed in the field that can support ways of genetically modifying organisms in order to produce the (3R,2R)-BDO isomer in larger quantities. These studies will be important because current studies are not able to produce the stereospecific isomer in as large of quantities as overall 2,3-BDO production. These tests should include non-mucoid control subjects, following the detailed experiments, in order to evaluate and compare the effectiveness of other genetic modifications on fermentation in bacteria. Furthermore, *B. licheniformis* is not the only gram-positive microorganism that is able to produce 2,3-BDO [5], so future studies should be performed with other GRAS bacteria that naturally produce the chemical in order to find the most economical and efficient way to produce it for industrial use. Other microorganisms may require alternative methods of genetic modification to produce large amounts of 2,3-BDO to satisfy industrial needs. These alternative methods may prove to be more efficient, which is why future studies are needed. The methods of optimizing natural 2,3-BDO production via genetic engineering are promising and have given rise to research focusing on stereospecific selectiveness in biological functions.

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