Researchers unlock the potential of genetic glycoengineering to advance vaccines and therapeutics technology

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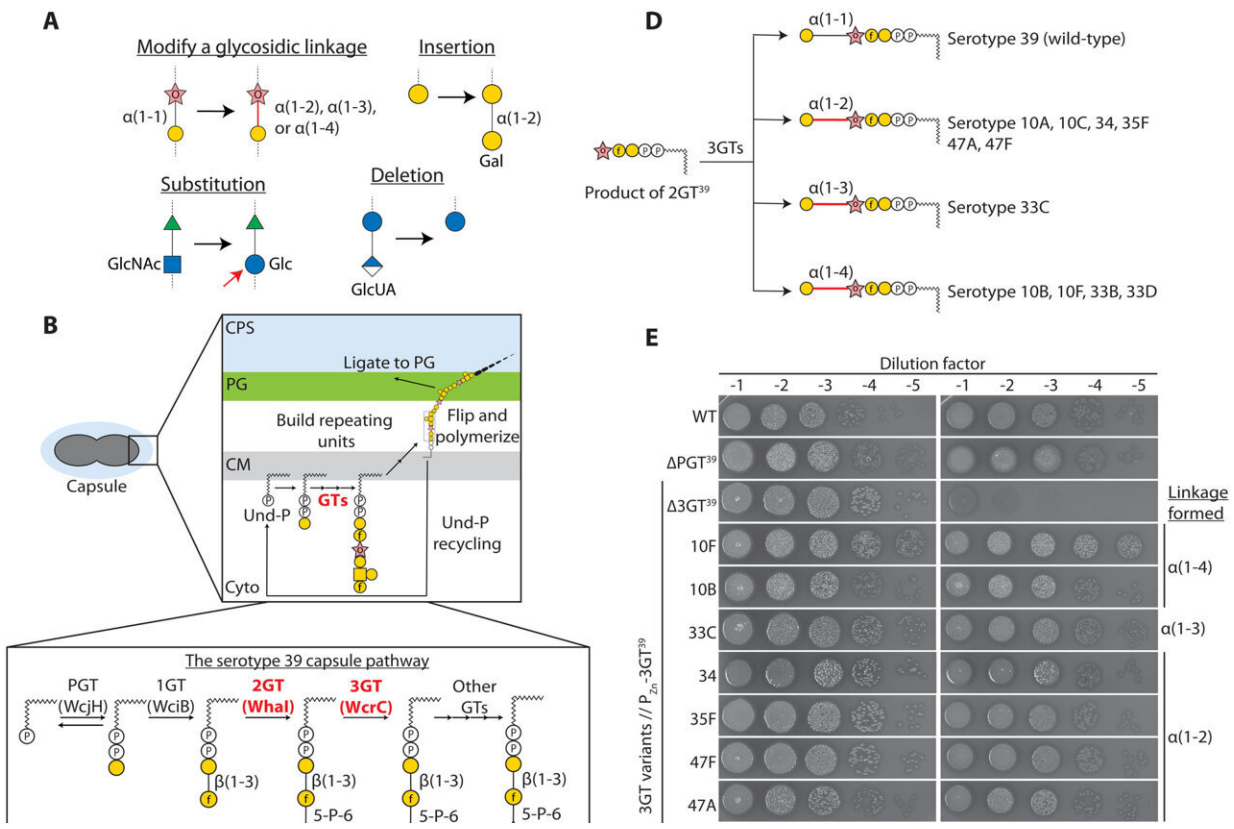

Genetic glycoengineering in bacteria.(**A**) Reprogramming the pneumococcal capsule pathway to produce structurally related glycans. Glycosidic linkages were modified (top left), and sugar residues were substituted (bottom left), inserted (top right), and deleted (bottom left). (**B**) Capsule synthesis pathway of serotype 39. The repeating units are assembled on a lipid carrier Und-P by the activities of GTs in the cytoplasm. The lipid-linked precursor is then flipped, polymerized,

and ligated to the cell wall peptidoglycan (PG). Und-P released is recycled for another round of synthesis. As Und-P is shared with the PG synthesis pathway, interruptions of the pathway result in the sequestration of Und-P and killing of the cell. For simplicity, GTs are annotated based on the steps they catalyze. The second (2GT; WhaI) and the third (3GT; WcrC) GTs are highlighted in red as they are the focus of this study. (**C**) 3GT in serotype 39 ($3GT^{39}$) is essential. Strains NUS1223 [P_{Zn}-$3GT^{39}$] and NUS1487 [$\Delta 3GT^{39}$ // P_{Zn}-$3GT^{39}$] were grown in BHI broth supplemented with Zn^{2+} overnight. Cells were collected by centrifugation, washed, and normalized. Cultures were then serially diluted and spotted on blood agar plates with or without Zn^{2+} supplements, followed by incubation overnight before imaging. (**D**) The product of $2GT^{39}$ can be modified by 3GTs of the indicated serotypes to introduce a change in the regiochemistry of the third glycosidic bond. (**E**) Strains NUS0253 [WT; isogenic serotype 39 capsule-switched mutant], NUS1365 [ΔPGT], and derivatives of strain NUS1303 [$\Delta 3GT^{39}$::P-SweetJanus P_{Zn}-$3GT^{39}$] that harbor the noncognate 3GT indicated were grown, serially diluted, and spotted on blood agar plates as described in (C) and imaged after overnight incubation at 37°C in 5% CO_2. (C and E) Representative images from three biological replicates. See also figs. S1 to S5. Credit: *Science Advances* (2023). DOI: 10.1126/sciadv.adi8157

A novel glycoengineering platform, created by the laboratory of Assistant Professor Chris Lok-To Sham from the Yong Loo Lin School of Medicine, National University of Singapore (NUS Medicine), is poised to revolutionize future production of vaccines and therapeutics to fight infectious diseases. Glycoengineering aims to manipulate sugars to produce useful carbohydrates. This innovative platform simplifies the customizing and production of sugar carbohydrates known as glycans that plays a crucial role in various therapeutic applications.

Sugar-adding enzymes called glycosyltransferases (GTs) produce glycans and control the structural diversity of glycans. The team found that the capsular polysaccharide (CPS), the sugar layer which encases many bacteria have extreme diversity, where its enzymes can be exploited to

build many customized glycans.

"These enzymes are like Legos. The more types of Lego bricks you have, the more unique types of glycans you can build," explained Asst Prof Chris Sham from the Department of Microbiology and Immunology at NUS Medicine.

Armed with this knowledge, Asst Prof Chris Sham and his graduate student Su Tong from the Department of Microbiology and Immunology, together with their team from the Infectious Diseases Translational Research Program at NUS Medicine, took advantage of the diverse pathways of the bacterial CPS and the ease of modifying its pathways to create this novel glycoengineering platform.

This platform provides increased versatility in modifying GTs, facilitating the engineering of newly-customized glycans. Customized glycans, essential for diverse therapeutic applications, requires a versatile platform capable of the insertion, deletion, substitution and general modification of glycan linkages.

The team found that by relaxing the specificity of the precursor transporters, they could broaden the range of residues entering the cytoplasm. This innovation enables the production of customized glycans with unprecedented flexibility.

"The process of customizing glycans, or glycoengineering, is made more challenging because it mostly relies on in-vitro approaches. These issues affect the efficient production of vaccines and other biological therapeutics. The new platform circumvents this challenge by demonstrating the possibility to genetically manipulate and engineer new glycans, giving rise to new knowledge about GTs, which ultimately signals an important advancement in glycoengineering," Su Tong said.

To date, the team has already celebrated significant achievements, including the successful synthesis of clinically relevant glycans such as the Galili antigen, blood group antigens and Lewis antigens. These glycans can contribute to positive outcomes in the areas of organ transplants and blood transfusion when antibody rejection occurs in situations where the patient's blood group is incompatible with the donor, resulting in severe inflammation and cell death.

Asst Prof Chris Sham is optimistic about the future development of this glycoengineering platform in creating more glycans for a broad variety of specific needs. "The current focus is to make glycans found in mammals, but in the future the team hopes to use this novel platform technology and adapt it to multiple bacterial species to generate more useful carbohydrates for other applications, such as countering immunological paralysis and graft rejection."

The findings are published in the journal *Science Advances*.

More information: Tong Su et al, Rewiring the pneumococcal capsule pathway for investigating glycosyltransferase specificity and genetic glycoengineering, *Science Advances* (2023). DOI: 10.1126/sciadv.adi8157

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