Gut bacteria provide key to making universal blood

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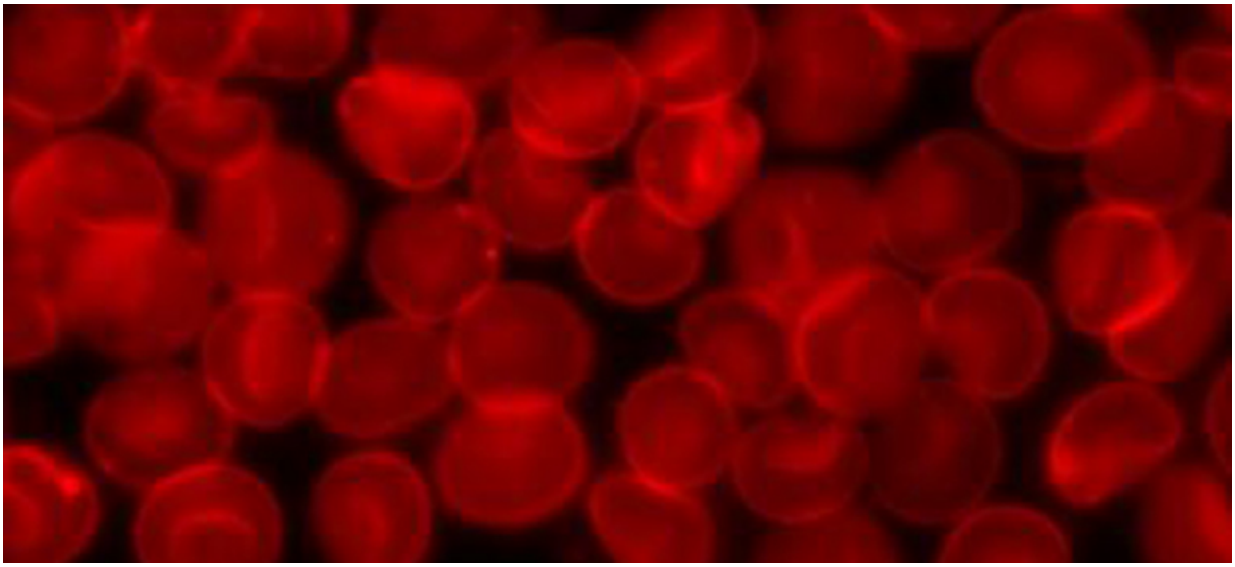

Credit: Wikimedia Commons

In January, raging storms caused medical emergencies along the U.S. East Coast, prompting the Red Cross to issue an urgent call for blood donations. The nation's blood supply was especially in need of O-type blood that can be universally administered in an emergency. Now, scientists say they have identified enzymes—from the human gut—that can turn type A and B blood into O, as much as 30 times more efficiently than previously studied enzymes.

The researchers will present their results today at the 256th National Meeting & Exposition of the American Chemical Society (ACS).

"We have been particularly interested in enzymes that allow us to remove the A or B antigens from red blood cells," Stephen Withers, Ph.D., says. "If you can remove those antigens, which are just simple sugars, then you can convert A or B to O blood." He says scientists have pursued the idea of adjusting donated blood to a common type for a while, but they have yet to find efficient, selective enzymes that are also safe and economical.

To assess potential enzyme candidates more quickly, Withers collaborated with a colleague at his institution, the University of British Columbia (UBC), who uses metagenomics to study ecology. "With metagenomics, you take all of the organisms from an environment and extract the sum total DNA of those organisms all mixed up together," Withers explains. Casting such a wide net allows Withers' team to sample the genes of millions of microorganisms without the need for individual cultures. The researchers then use *E. coli* to select for DNA containing genes that code for enzymes that can cleave sugar residues. So instead of using metagenomics as a means of learning about microbial ecology, Withers uses it to discover new biocatalysts. "This is a way of getting that genetic information out of the environment and into the laboratory setting and then screening for the activity we are interested in," he says.

Withers' team considered sampling DNA from mosquitoes and leeches, the types of organisms that degrade blood, but ultimately found successful candidate enzymes in the human gut microbiome. Glycosylated proteins called mucins line the gut wall, providing sugars that serve as attachment points for gut bacteria while also feeding them as they assist in digestion. Some of the mucin sugars are similar in structure to the antigens on A- and B-type blood. The researchers homed

in on the enzymes the bacteria use to pluck the sugars off mucin and found a new family of enzymes that are 30 times more effective at removing red blood cell antigens than previously reported candidates.

Withers is now working with colleagues at the Centre for Blood Research at UBC to validate these enzymes and test them on a larger scale for potential clinical testing. In addition, he plans to carry out directed evolution, a protein engineering technique that simulates natural evolution, with the goal of creating the most efficient sugar-removing enzyme.

"I am optimistic that we have a very interesting candidate to adjust donated blood to a common type," Withers says. "Of course, it will have to go through lots of clinical trails to make sure that it doesn't have any adverse consequences, but it is looking very promising."

More information: Discovery of CAZYmes for cell surface glycan removal through metagenomics: Towards universal blood, the 256th National Meeting & Exposition of the American Chemical Society (ACS).

Abstract
Gaining access to enzymes that are able to optimally degrade glycosides of interest under specific conditions has been achieved using two high-throughput methodologies. One involves the synthesis of libraries of genes from within glycoside hydrolase families that contain the general activity of interest. Representation of specificity diversity within that family is achieved bioinformatically by creating phylogenetically distinct sub-families of genes. Representative genes from each sub-family are then synthesised and expressed in E. coli, and the library is screened. In the other approach we screen the diverse gene repertoire present within the "silent majority" of microorganisms that have not been cultured through metagenomic approaches. Total DNA from an

environment of interest is extracted, fragmented into chunks containing either ~ 1 gene (3-5 kB) or ~30-40 genes (40-50 kB) and transformed into E. coli. After picking colonies into 384 well plates we screen them for the activities of interest using custom-synthesized substrates. After hits are validated we sequence interesting fosmids and clone and express any obvious CAZymes: if not obvious we identify the responsible gene through small insert libraries.

Using this approach we have identified useful glycosidases of novel specificity from environments ranging from forest soils to human and beaver feces. In particular we have screened the human gut metagenomic libraries for enzymes that remove the Gal or GalNAc residues that function as the antigenic determinants from A and B type red blood cells, thereby generating "universal" O type blood. High-throughput screening of libraries for glycoside phosphorylases will also be discussed if time permits.

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