Specialist enzymes make E. coli antibiotic resistant at low pH

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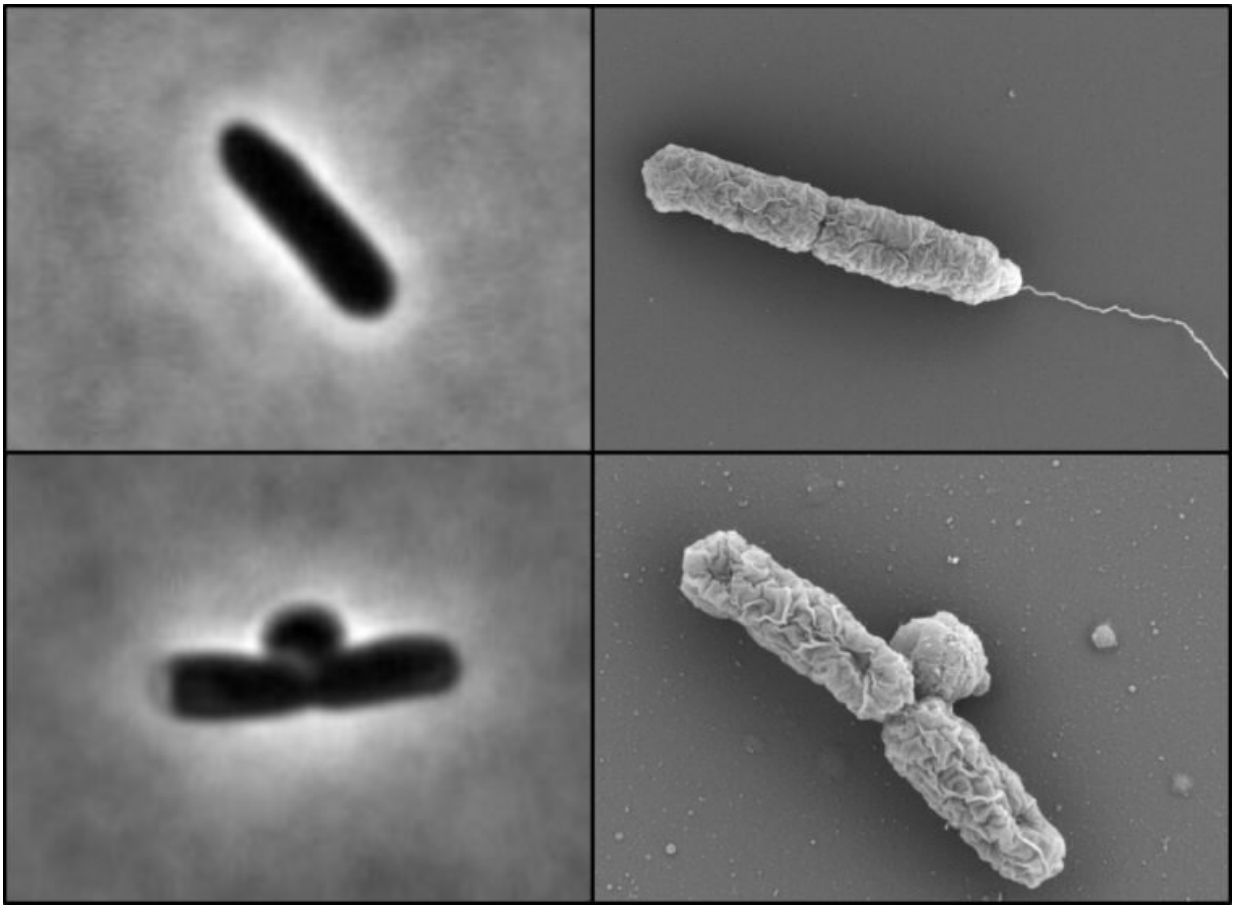

Mutant E. coli strain imaged by phase contrast microscopy (left) and scanning electron microscopy (right) at pH 7.0 (top) and pH 4.5 (bottom). Scanning electron microscopy images taken in collaboration with the Washington University Center for Cellular Imaging. Credit: Elizabeth Mueller

Scientists long puzzled over why bacteria contain so many "redundant" enzymes. Why make several molecules that do the same job, interchangeably, when it would be much more efficient to make just one?

New research from Washington University in St. Louis suggests that many of these so-called redundant enzymes are actually specialists that ensure maximal growth across different environments.

Further, these specialist enzymes were found to increase E. coli's resistance to antibiotics at low pH conditions, such as those found in the GI tract or urinary tract—raising concerns that current antibiotic susceptibility tests are inadequate. The research from the laboratory of Petra Levin, professor of biology in Arts & Sciences, is published April 9 in the journal *eLife*.

"Some enzymes that appear to be redundant for bacterial growth and fitness under standard laboratory conditions are specialized for particular environmental conditions," said Elizabeth Mueller, a Ph.D. candidate and first author of the new study. "We probably miss a lot of interesting and clinically relevant biology by studying bacterial cells predominately during growth in nutrient-rich, neutral-pH, aerated-growth media."

Mueller found that a subset of enzymes involved in making E. coli 's cell wall are pH specialists that ensure robust growth and cell wall integrity in a wide pH range. The work was completed with collaborators at Newcastle University in Britain and Utrecht University in the Netherlands.

Why so many?

It's like opening someone's closet, finding a pile of shoes, and asking, "Why so many?" If a person can get along fine with just one pair—and it

doesn't matter which one—why keep all the others? Upon closer inspection, however, you see that pile of shoes is made up of running sneakers and hiking boots, wool slippers and rain boots, flip-flops and stilettos. They're all shoes, but different styles suit different occasions. You could wear a pair of wellies on a 3-mile run, but you'd get fewer blisters in sneakers. The same appears to be true for E. coli cell-wall enzymes in different pH conditions.

For this study , the authors generated strains of E. coli that were missing nonessential cell-wall enzymes. These strains were then grown in medium with pH representative of what E. coli would find in the lower GI tract and urinary tract. The authors found that in these conditions, instead of being interchangeable, several of these enzymes helped the E. coli grow better.

The study focused on two pH specialist enzymes: PBP1a and PBP1b. PBP1a was required for maximal growth in alkaline conditions, while PBP1b was necessary in acidic conditions. If a cell is missing one of these enzymes and grown in that enzyme's "specialist" pH condition, then that cell will have decreased viability.

Interestingly, redundancy in cell wall synthesis appears to have consequences for E. coli's sensitivity to certain cell wall-active antibiotics. The activity of PBP1b in acidic conditions increases the cell's resistance to specific beta-lactam antibiotics by as much as 64-fold, compared to growth in standard culture conditions.

"Most clinical labs test antimicrobial susceptibility by growing bacterial cultures in nutrient-rich media at around neutral pH," Mueller said. "These conditions poorly reflect those experienced by pathogens at most sites in the human body."

"Our study supports the idea that environmental conditions at the

infection site may affect the efficacy of antibiotic treatment," she added.

Future work into the mechanism behind how PBP1b protects the cell may reveal new antimicrobial targets that can be inhibited across pH conditions. Additionally, researchers in the Levin laboratory predict that future research will identify similar enzyme specialists in other bacteria. These specialists could explain high levels of redundancy in other classes of enzymes, particularly those located outside of the cell that are exposed to the environment.

More information: Elizabeth A Mueller et al. Plasticity of Escherichia coli cell wall metabolism promotes fitness and antibiotic resistance across environmental conditions, *eLife* (2019). DOI: 10.7554/eLife.40754

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