

TEACHER'S GUIDE

DNA *to Darwin Case Study*

Superbugs

Selecting our enemies

Version 1.1

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Charles Darwin

Superbugs

Selecting our enemies

Studies of the genomes of strains of *Staphylococcus aureus* have provided evidence for the rapid evolution of drug resistance. *S. aureus* is now best known to the UK public as its methicillin-resistant form, MRSA. Researchers have found that this species has evolved a 'Pick'n'Mix' genome that enables it to acquire new characteristics. The ability to share and exchange genes is vital to the *S. aureus*'s spread and success, presenting a major and constantly-changing clinical challenge.

Students will compare the genomes of an MRSA strain and its genetic 'cousin' MSSA to locate DNA differences and mobile elements that could be expected to improve the bacterium's resistance and its ability to cause disease.

Outline of the activity

In this activity, students will compare the genomes of two bacterial strains of *Staphylococcus aureus*, one of the UK's best known 'superbugs'. This will be done using a purpose-made *Adobe Flash* animation, which models software, the *Artemis Comparison Tool (ACT)*, developed for this purpose.

General reading

Reading the story in DNA: A beginner's guide to molecular evolution by Lindell Bromham (2008) Oxford University Press (Paperback) ISBN: 978 0199290918. *An engaging textbook on molecular evolution, which assumes no specialist mathematical knowledge and takes the reader from first principles. Although it's aimed at undergraduates, this superb book contains sufficient detail for PhD students, yet parts will appeal equally to 16–19 year-olds.*

The making of the fittest. DNA and the ultimate forensic record of evolution by Sean B. Carroll (2009) Quercus Books (Paperback) ISBN: 978 1847247247. *A popular lay account of some of the molecular evidence for evolution.*

Scientific publications

Baba, T., F. Takeuchi, *et al* (2002) Genome and virulence determinants of high virulence community-acquired MRSA. *The Lancet*, 359 (9320) 1819–1827.

Ender, M. *et al* (2007) Variability in SCCmecN₁ spreading among injection drug users in Zurich, Switzerland. *BMC Microbiology*, 7:62 doi:10.1186/1471-2180-7-62. This article is available from: www.biomedcentral.com/1471-2180/7/62

- Holden, M. *et al* (2004) Complete genomes of two clinical *Staphylococcus aureus* strains: evidence for the rapid evolution of virulence and drug resistance. *Proceedings of the National Academy of Sciences USA*, **101**, 9786–9791.
- Sergeev, N. *et al* (2004) Simultaneous analysis of multiple staphylococcal enterotoxin genes by an oligonucleotide microarray assay. *Journal of Clinical Microbiology*, **42** (5) 2134–2143.
- Wellcome Trust (2005) Antibiotic resistance: an unwinnable war? *Wellcome Focus*: www.wellcome.ac.uk/stellent/groups/corporatesite/@msh_publishing_group/documents/web_document/wtxo26231.pdf

Requirements

Software

The computer tool for this activity is made in *Adobe Flash*. It will run when you double-click the ACT.exe file (on a PC) or the equivalent on a Macintosh. The interface is a simplified version of the *Artemis Comparison Tool* (ACT) which is a whole genome analysis tool available from: www.sanger.ac.uk/Software/ACT/.

ACT was developed by the Wellcome Trust Sanger Institute for visualising the results of computer-based comparisons of two DNA sequences. In this case, the two sequences are compared automatically using software called *BLAST*, which attempts to line up the sequences where they match.

A related online tool to compare sequences is available at: www.webact.org/WebACT/home (needs *Java*). To authenticate the data used in this activity, you can use the ‘pre-computed’ tab in *WebACT* to preload sequences of *Staphylococcus aureus* strain MW2 (*GenBank* code: BA000033) vs. *Staphylococcus aureus* strain MSSA476 (*GenBank* code: BX571857).

The simplified *Adobe Flash* simulation of ACT allows students to view a comparison of the two whole genomes, and then to scroll through the entire genome to click on regions of difference. This will activate an additional zoom, showing individual gene elements in each of the regions, and allow students to find information about the gene elements, DNA sequences, amino acid sequences and protein structures.

Examining the genes

The software has been set up to provide DNA and amino acid sequence data for each of the interesting genes in these regions. For many of the genes, you will see a link to the *Protein Data Bank* (www.pdb.org). If you click on this link, if you have an internet connection you will be taken to the correct page on the PDB site. We recommend clicking on the word ‘Jmol’ under the image on the right of the screen to see a 3-D version of the protein structure without having to download extra viewing software.

The DNA and amino acid sequences can be cut and pasted from the results pages for use in, for example, *BLAST* searches to find similar bacterial genes.

Students' worksheets

Students will require copies of Student's Guide, pages 2–12. For Exercise 2, they will also need print-outs of the ACT cards PDF file.

Using this activity in the classroom

This activity is essentially a guided exploration of a genome. Depending on your students' IT literacy and your flexibility with computer use, you may want to choose an online exploration followed by an offline task that classifies the genes found into their strains and functions.

Answers to the questions on the worksheets

Exercise 1

Computer-based genome comparison of antibiotic-resistant and sensitive strains of *Staphylococcus aureus*

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- a. You can see that there are relatively few sites where DNA has been inserted or deleted between these two strains, and this shows that they are very closely-related.
- b. The answer to this question will vary according to the region the student is examining.
- c. There are three regions in the MW2 strain which differ significantly from the entire MSSA476 genome, and two regions in the MSSA476 genome that differ from MW2.

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- c. The non-matching sections may be independent insertions into the sequence; the whole region may have begun identical, but the larger sections mutated over time so that they are no longer similar; the whole region might be an insert, but from two origins (with only minimal similarity).
- d. This section of MRSA MW2 carries the *mecA* gene encoding a penicillin binding protein, which makes the strain resistant to methicillin and other penicillin-based antibiotics. This section of MSSA476 carries *fusB1*, a fusidic acid resistance gene. The other genes in this region are generally associated with the act of incorporating new sequences into the bacterial genome (*ccrA* and *ccrB*), or recognising and chopping up 'foreign' DNA. In different environments, with different inserted sequences, these could be beneficial to the *S. aureus* strain.
- e. *sec4* produces an enterotoxin precursor that busts holes in cells; *ear* and *sel* also produce toxins that create holes in cell membranes. They can attach to cells, including B- and T-cells, and appear to be involved in altering the immune response.
- f. The *int* gene indicates that this set of genes may have been horizontally transferred from another part of the genome or another related bacterium.
- g. *lukF* — Panton-Valentine leukocidin chain F precursor

lukS — Panton-Valentine leukocidin chain S precursor

Both of these toxins act by killing the white blood cells produce in response to infection. The two subunits of Panton-Valentine leukocidin are secreted by the bacterium and meet up in the cell membranes of target cells. There they form a ring through which the contents of the cell can leak out, causing the cell to die.

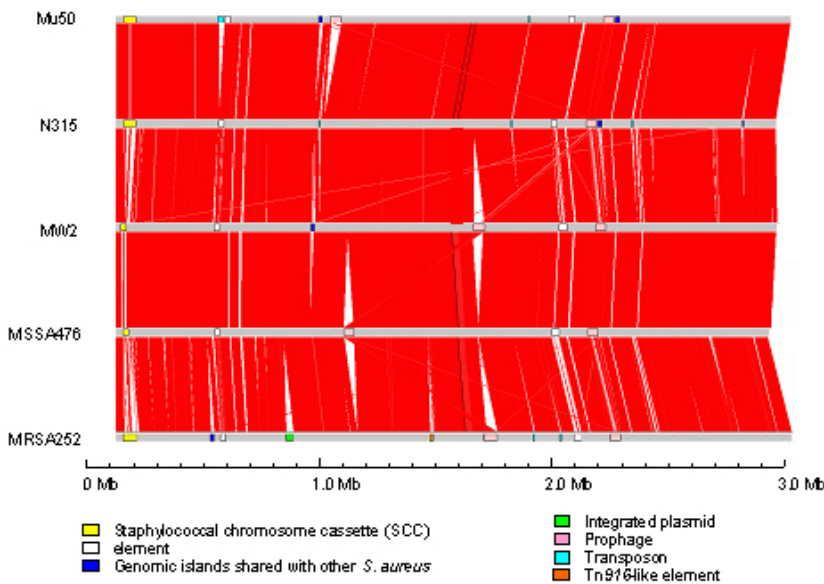
- h. The presence of *int* (an integrase gene) suggests that the piece of DNA carrying the instructions to make the two toxins might have been inserted. This specific integrase comes from a bacteriophage, so although the *ints* present in the *MSSA 476* and *MRSA MW2* strains are slightly different from one another, they appear to have a similar origin.

Exercise 2

Categorising the genes of antibiotic-resistant and sensitive strains (offline activity)

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- i. All of the toxins and the two antibiotic resistance genes (*mecA* for *MRSA MW2* and *fusB1* for *MSSA 476*) would be expected to help the bacterium cause disease under different environmental stresses.
- j. The genes that help to incorporate others (e.g., *int*, *orfX*, *ccrA* and *ccrB*) enable the bacterial ‘Pick n’mix’, while the *hsdM*, *hsdR*, and *hsdS* genes could help protect against unfavourable transfers.
- k. *S. aureus* is able to acquire genes for toxins, antibiotic resistance and combating foreign insertion by transferring genes in ‘cassettes’. Some of the genes a bacterial strain acquires seem to have come from quite distantly-related bacteria. Different strains also appear to have incorporated genes from bacterial viruses. The ability to transfer genes horizontally provides *S. aureus* with a ‘genetic plasticity’ that helps it to adapt to different environmental situations and stresses. This is supported by the view obtained if you compare the genomes of these strains with three other types of *S. aureus* collected since 1961:



A screenshot of the ACT, showing five different bacterial strains.

Possible extension activities

A gene in *MRSAMW2*, called *sdrD* (SD repeat gene D), codes for one of the cell surface proteins used in a recently-developed *MRSA* vaccine. However, versions of this gene in other strains, including *MSSA 476*, have already shown small alterations in the number of repeats of amino acids SD (serine-aspartate).

Why might variation of this type occur?

Some researchers have postulated that by varying repeats in this protein, *S. aureus* may be able to improve its ability to bind to cells; others believe that this generational change could already be in response to the medical targeting of this protein. By exploring this topic with your students, you may be able to uncover some of the misconceptions about genetic variation and selection.

Acknowledgements

This activity was developed by Bronwyn Terrill and Steve Cross with Matt Holden (Pathogen Sequencing Unit, Wellcome Trust Sanger Institute). The *Flash ACT* viewer was created by Preeti Deshpande with programming support from Elizabeth Cooper-Williams.