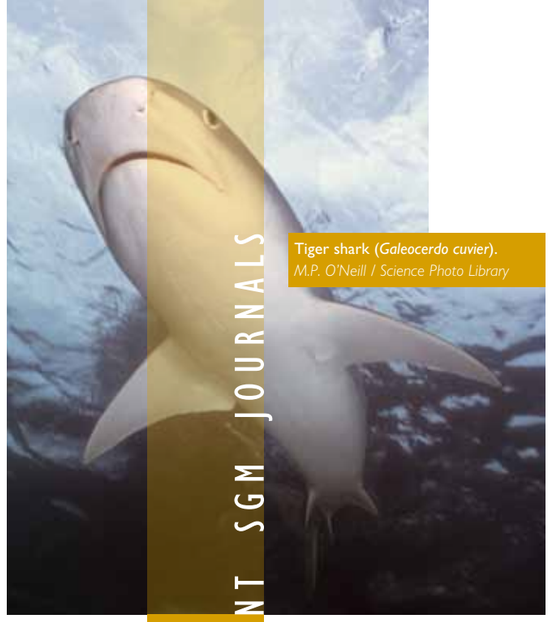
Shark attack!



Tiger shark (*Galeocerdo cuvier*).
M.P. O'Neill / Science Photo Library

Movies set in tropical seas frequently include a dramatic shark attack, with *Jaws* the most celebrated example, but in real life, attacks are not as frequent as the movies suggest. Only 400 fatalities have been recorded since systematic records began in the early 1900s. However, many people who survive an attack suffer from serious infections in their wounds. Treatment includes large amounts of antibiotics as a precaution against the many types of bacteria that might be present in these deep bites. There is surprisingly little information on which bacteria are actually present or their antibiotic sensitivity.

Scientists in Recife, a coastal city in Brazil, have set out to remedy this lack of knowledge so that more targeted treatments can be developed. Recife has one of the highest annual number of shark attacks in the world. A total of 53 incidents were recorded between 1992 and 2009, resulting in 20 people bleeding to death. Two shark species, the bull shark (*Carcharhinus leucas*) and the tiger shark (*Galeocerdo cuvier*), cause most of the damage.

The researchers sourced their bacterial isolates from nine sharks taken from the sea near Recife. After swabbing the surface of the teeth and gums, the researchers used a wide range of culture methods to encourage any bacteria that were present to grow. They isolated individual bacterial colonies and identified 14 species frequently associated with tissue and blood infections. Some might have been long-term inhabitants of the shark's mouth while others could have been picked up from sewage discharged into the sea. However, it was clear from these results that shark bites were full of pathogenic bacteria. Tests of antibiotic sensitivity showed that two species of Gram-negative bacteria were likely to be resistant to several antibiotics. These were *Citrobacter freundii* and *Proteus mirabilis* and the latter is widely reported as a cause of opportunistic infections in wounds. Most strains of the other pathogenic bacterial species were sensitive to many antibiotics so that infections should be readily treatable.

In parallel, the researchers also examined treatment records from the local hospital of people who had survived shark attacks. These suggested that the extent of the wounds was the main factor determining whether bacterial infection occurred. Comparing the antibiotic treatment of the patients with the antibiotic sensitivity of the bacteria from the sharks showed that most were sensitive to the antibiotics used. However, the comparison also indicated that commencing treatment with a different broad-spectrum antibiotic might enhance effectiveness.

Interaminense, J.A., Nascimento, D.C.O., Ventura, R.F., Batista, J.E.C., Souza, M.M.C., Hazin, F.H.V., Pontes-Filho, N.T. & Lima-Filho, J.V. (2010). Recovery and screening for antibiotic susceptibility of potential bacterial pathogens from the oral cavity of shark species involved in attacks on humans in Recife, Brazil. *J Med Microbiol* 59, 941–947.

MERIEL JONES HIGHLIGHTS HOT PAPERS IN RECENT SGM JOURNALS

A juicy tale

Food microbiologists have a particular interest in bacteria that form spores because some of these species cause serious food poisoning. It is a particular problem because the spores are much more difficult to kill than normal bacterial cells, and thus food-processing methods need to be designed specifically to destroy them. However, amongst spore-forming bacteria is one unusual group, called *Sporolactobacillus*. This genus was discovered in 1963 with characteristics of both spore-forming *Bacillus* and lactic acid-producing *Lactobacillus* that are the benign bacteria of yoghurt, cheese, sausages and probiotic drinks. *Sporolactobacillus* bacteria are typically found in soil but sometimes appear in fermented or spoiled food. Their ability to grow in the absence of oxygen, produce lactic acid, grow well at around pH 5.0 and generate spores that can tolerate a temperature of 80°C for 5 minutes, is a unique combination of characteristics of the two groups.

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Researchers in the Laboratory of Quality Control R&D of the Japanese food company Mitsui Norin Co. Ltd. were recently surveying the microbes that spoiled orange juice. They came across unusually heat-tolerant lactic acid bacteria that were the cause of the spoilage, and after running a series of tests, realized that they had found a novel species of *Sporolactobacillus*. As well as testing the conditions in which these new bacteria grew, the researchers also analysed the cell walls, cell lipids and DNA sequence of two genes frequently used to characterize bacteria. Although some of the results instantly matched with the current seven species of *Sporolactobacillus*, others differed enough to make it clear that this was in fact a novel, eighth species.

In naming the species, the researchers thought back to the origin of the bacteria, in spoiled orange juice with an organic, acid odour. As a consequence, they will be forever known as *Sporolactobacillus putidus*, from the Latin word for 'stinking'.

Fujita, R., Mochida, K., Kato, Y. & Goto, K. (2010). *Sporolactobacillus putidus* sp. nov., an endospore-forming lactic acid bacterium isolated from spoiled orange juice. *Int J Syst Evol Microbiol* 60, 1499–1503.

Detecting prions in blood

There continues to be a very practical problem with diagnosis of neurodegenerative spongiform encephalopathy diseases such as BSE and CJD. They have an incubation period of months to decades during which there are no symptoms, even though the pathway of converting the normal brain PrP protein into the toxic, disease-related PrP^{Sc} form has started. At present, there is virtually no way to detect PrP^{Sc} reliably except by examining the brain using neuropathological and immunohistochemical methods after death. Accumulation of the abnormally folded PrP^{Sc} form of the PrP protein is a characteristic of the disease, but it is present at very low levels in easily accessible body fluids like blood or urine. Researchers have tried to develop methods to measure PrP^{Sc}, but there are still no fully accepted methods for use in materials such as blood.

A team from New York has now described detection of PrP^{Sc} even when initially present at only one part in a hundred thousand million (10^{-11}) in brain tissue. The method combines amplification with a novel technology called SOFIA (surround optical fibre immunoassay) and some specific antibodies against PrP^{Sc}. After amplifying and then concentrating any PrP^{Sc}, the samples are labelled with a fluorescent dye using an antibody for specificity and then finally loaded into a micro-capillary tube. This tube is placed in a specially constructed apparatus so that it is totally surrounded by optical fibres to capture all light emitted once the dye is excited using a laser. The technique allowed detection of PrP^{Sc} after many fewer cycles of conversion than others have achieved, substantially reducing the possibility of artefacts, as well as speeding up the assay.

The researchers also tested their method on blood samples from apparently healthy sheep that went on to develop scrapie. The animals' brains were analysed once any symptoms became apparent. The researchers could therefore compare results from brain tissue and blood taken once the animals exhibited symptoms of the diseases, with blood obtained earlier in the animals' lives, and from uninfected animals. The results showed very clearly that PrP^{Sc} could be detected in the blood of animals long before the symptoms appeared.

After further development and testing, this method could be of great value in surveillance for the disease. Although the worst predictions about BSE have not been fulfilled, in particular the amount of transmission from cow to human to cause variant (v) CJD, the disease has not gone away. vCJD has been the definite or probable cause of death of 168 people in the UK in the two decades to 2010. The full incubation period is unknown but can clearly be decades, during which time no symptoms are evident. Unfortunately, it is possible for the disease to be transmitted between humans through organ and blood donations. A rapid method for testing these tissues for PrP^{Sc} would bring reassurance that this can be prevented.

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Rubenstein, R., Chang, B., Gray, P., Piltch, M., Bulgin, M.S., Sorensen-Melson, S. & Miller, M.W. (2010). A novel method for preclinical detecion of PrP^{Sc} in blood *J Gen Virol* 91, 1883–1892.

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Don't spoil the wine

Nardi, T., Corich, V., Giacomini, A. & Blondin, B. (2010). A sulphite-inducible form of the sulphite efflux gene *SSU1* in a *Saccharomyces cerevisiae* wine yeast. *Microbiology* 156, 1686–1696.

There is a long tradition of adding sulfite to wine to improve its quality. During fermentation of grape juice into wine, the yeast cells naturally produce a small amount of sulfite. However, most winemakers add more, typically between 80 and 350 mg per litre, because sulfite is a good preservative: it inhibits the growth of bacteria and removes dissolved oxygen, both of which can spoil wine. A few people are very intolerant of sulfite, so most wine is labelled 'contains sulfites' as a reminder, as are other sulfite-containing foods, such as dried apricots where the antioxidant effect of sulfite allows them to retain their natural orange colour.

Grape juice can ferment to alcohol through the activities of wild yeasts on grape skins. However, most wines are produced by adding a carefully selected strain of the yeast *Saccharomyces cerevisiae* to the grape juice for a more reliable and palatable product. Wild and cultivated yeasts vary substantially in their sensitivity to sulfite and have several mechanisms for resisting its toxicity. The most efficient is a membrane protein (Ssu1p, encoded by the gene *SSU1*) that pumps sulfite out of the cell. Another protein (Fzf1p) seems to have the sole job of controlling production of Ssu1p. The level of activity of the *SSU1* gene varies considerably and one reason is because there are different versions of Fzf1p. However, some yeasts also have a second copy of the *SSU1* gene. This provides an opportunity for another sort of difference, namely in the DNA sequence around the gene that determines when, and how strongly, it is switched on or off. As a consequence, the copies in different locations often behave differently.

Researchers in Montpellier, France, in collaboration with colleagues in Padova, Italy, have been investigating some commercial yeast strains that are particularly resistant to sulfite. They already knew that there are a number of variations in the *SSU1* gene among the different strains of wine yeast. Nevertheless, these *SSU1* genes are in constant use by the cells. Their attention focused on a strain of yeast called 71B and they have discovered that, unlike other yeasts, it has three different versions of the *SSU1* gene. In addition, their level of expression increased during the fermentation process, and also if sulfite was present. To understand the basis of this very different genetic control, they examined the DNA around the three genes. Intriguingly, there were few differences.

The researchers therefore think that the explanation must lie in the signalling pathways that lead to the activation of the gene. This means that they may now have a way to identify how yeasts sense sulfites in their environment, something that is of considerable interest to winemakers. This work will allow the optimization of the industrial process of wine yeast production, preparing the wine yeast according to the sulfites found in grape musts, thus reducing the adaptation time (lag) phase which is of critical technological importance in winemaking.