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bubbles, foam and gush

Vivienne Baillie Gerritsen

A ting of metal against glass. The lights are dimmed. Voices turn to a murmur. All eyes are set on the bottle of champagne and its cork as it is gradually eased out. Then a pop, and your host is nervously juggling with glasses and a challenging flow of bubbles. These are bubbles we expect. However, sometimes bubbles emerge in an uncontrollable gush. Take a can of beer; one that has been waiting patiently in the fridge. It hasn't been shaken. It hasn't fallen. The can itself is pristine. You slip a finger under the tab and pull. You expect some foam but not the untamed rush of bubbles that spills over your hands and onto the ground. When it finally ends and you peer into the can, there's little left to drink. Gushing such as this is a defect which occurs in carbonate beverages. At the beginning of the 20th century, scientists speculated that something biological was probably the culprit, but they were unable to put their finger on it. Today, researchers postulate that gushing is most likely caused by hydrophobins. Hydrophobins are proteins synthesized by fungi that have infected the grain used in the production of beer. In particular, class II hydrophobin 5 from *Fusarium culmorum*.

'Running Through the Barley'
by Rob Barnes, www.robbarnesart.co.uk

Courtesy of the artist

For carbonate beverages including sparkling wines, gushing is defined as an unwanted flow of foam that does not stem from the mishandling of bottles or cans. In the realm of beer, two types of gushing are distinguished. The first is caused by technological setbacks during the brewing process. The second has to do with the malt. Malt is grain – usually barley or wheat – which has been soaked in water to germinate and then heated to halt germination. It is one of the early stages in the production of beer (and whisky). In damp weather during the flowering season, barley and wheat are prone to infestation because fungi thrive in humidity. Researchers discovered that one species of fungus in particular seems to be at the heart of beer gushing: the plant pathogen *Fusarium*.

Humans have been making and drinking beer for thousands of years. The art of beer brewing is thought to have originated with the Sumerians (who apparently enjoyed over twenty types of beer) and gradually made its way to the West where monasteries were among the major brewers. In those days, beer probably did not have the foam we are now accustomed to. Today, beer foam is a must and defines the beverage's aesthetics – its density, its colour and its longevity. Proteins are at the heart of beer foam (*read all about it and the history of beer in Protein Spotlight issue 'One Beer Please'*). So could they also be at the heart of too much foam? The first scientist to describe 'the wildness of malt beer' was the German chemist H. Kastner in an article published in 1909. Though he couldn't explain how it happened, he did mention that the cause was most probably biological. He was right. In the 1930s, 'the overflowing of beer' was shown to depend on barley, its variety and the years it was harvested. In the 1960s, the phenomenon was described as 'beer gushing' and owed to microorganisms that grow in barley during storage. It took another forty years for scientists to understand that the infection of barley by fungus is what causes beer to gush. Today we know that the culprit are indeed proteins known as hydrophobins, that are secreted by *Fusarium.*

Beer gushing may be caused by hydrophobins but it is not what *Fusarium* synthesizes them for. Hydrophobins are small proteins, produced and secreted only by filamentous fungi. Their roles are multiple but largely protective as they surround fungal cells with a hydrophobic monolayer to preserve them from external aggression – thus promoting development and dispersion. Hydrophobins spontaneously aggregate at water/gas interfaces. As fungal hyphae emerge from moist soil to continue their growth in the air, hydrophobins wrap them in a protective shield. Hydrophobins aggregate differently at the actual water/air interface and around hyphae. Two classes have thus been identified. Class I hydrophobins self-assemble into filamentous structures called rodlets, which are present on aerial hyphae and fungal spores, the conidia. Class II hydrophobins, however, simply gather into an ordered monolayer at the water/air interface without forming rodlets.

Hydrophobins show little sequence conservation save for the presence of eight cysteine residues that form four cysteine bridges in the protein's tertiary structure. The purpose of hydrophobins is to change the 'wettability' of the fungal surface. How? Hydrophobins are both hydrophilic and hydrophobic (amphipathic) and spontaneously assemble at hydrophilic/hydrophobic interfaces like water/gas interfaces. When the fungus pushes its hyphae from the humid soil out into the air, the secreted Class II hydrophobins weaken the water's surface tension thus enabling the hyphae to cross the interface. As the fungal cells emerge into the air, Class I hydrophobins rapidly form protective rodlets around the aerial hyphae.

Hydrophobins are also thought to be involved in plant infection by interfering with the host's immune system. This would explain their presence in barley malt whose grain has been infected, in particular by *Fusarium culmorum*. Class II hydrophobin 5 seems to be the main offender in beer gushing. How is it explained? There are several hypotheses – some of which seem to be contradictory, so we will only describe one. After having survived both the malting and the brewing of barley, it is assumed that hydrophobins spontaneously aggregate on the surface of tiny beer bubbles – where CO_2 and water meet – just as they aggregate where water and air meet in a developing fungus. In doing so, the protein stabilises thousands of beer bubbles. When pressure is released from a can or a bottle as you open it, the pressure inside suddenly drops. This would cause thousands of tiny bubbles to rise, expand and burst releasing gas in a sort of explosive chain reaction, i.e. beer gushing.

Though the physics of beer gushing still needs to be understood, researchers already know that hops may provide a way of regulating it. Hops are the flowers of *Humulus lupulus* and give beer its aroma and flavour, besides acting as a natural preservative. It turns out that hops have a suppressant effect on beer gushing. The more hops it seems, the less the gush – which may be due to hop oil, one of the flower's constituents. Hydrophobins themselves prove to be useful in various applications thanks to their capacity to form amphipathic membranes. They can be used to coat nanoparticles and provide a way of delivering drugs that avoid the host's immune system for instance. They can also be used in the food industry to help create foams that remain stable far longer than food emulsifiers. So the next time you open a beer can or dip a spoon into your strawberry mousse, spare a thought for hydrophobins.

Cross-references to UniProt

Class II hydrophobin 5, *Fusarium culmorum* : A9NIV5

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