The Mystery of the Warehouse Stains

A black fungus, with a liking for distilleries and bakeries, has a love-hate relationship with ethanol in the atmosphere

Bernard Dixon

Anyone being shown around a distillery in France may notice, in the warehouse area where cognac and armagnac are held for ageing, characteristic sooty stains on the walls. Nearby fences and tree trunks are sometimes affected too. The same thing can occur at whisky distilleries in Scotland, and occasionally around commercial bakeries elsewhere in the world.

But what causes this so-called warehouse staining? Thanks to diligent work by James Scott and colleagues at the University of Toronto and Sporometrics Inc. in Toronto, we now have a proper answer to this longstanding question. Their investigations have centred on another factor that is common to distilleries, storage facilities for alcoholic beverages, and bakeries—the vapor of alcohol in the ambient air.

In Scotland, it is common knowledge that even within the best regulated facilities a proportion of alcohol escapes into the atmosphere during the years when whisky is maturing. It is popularly known as the "angels' share," and composer Robert Matheson recently wrote a bagpipe tune to mark the regrettable loss of this proportion of Scotland's national drink. What the Canadian team has discovered is that the organism responsible for warehouse staining does indeed have a close relationship with ethanolic vapours—though not one that is entirely straightforward.

It has been over 100 years since French mycologists carried out the first truly scientific work on the sooty stains in brandy stores. They found that the black material was a fungus, which they characterized as *Torula compniacensis*. However, mycologists thereafter virtually ignored it over many decades because the organism grew frustratingly slowly and was overgrown by competitors and thus proved very difficult to isolate in pure culture. Another reason for neglect was that the fungus was, in James Scott's words, "microscopically nondescript."

Natural curiosity aside, his group was motivated to reinvestigate the organism because in areas such as North America (though not in France) the black staining is considered to be both ugly and unwholesome. An understanding of its physiology, especially of the link with alcohol, might lead to effective methods of control.

Using modern methods, principally nuclear ribosomal gene sequencing, Scott and his coworkers first managed to cultivate the fungus in pure culture and then discovered that it had been improperly classified. Reporting in *Mycologia* (**99:**592, 2007), they described it as the type species of a new genus, *Baudoinia,* within the order *Capnodiales*.

As well as investigating the association with ethanolic vapors, the researchers hoped to uncover the physiological basis of the hardiness of an organism they believed to be ecologically unique. It lives in conditions where it may be exposed to very low temperatures but also very high temperatures—up to 65°C on asphalt roofs, for example. In many of its biota-poor habitats, it has to make do with simple sources of nitrogen made available through rainfall.

When these investigations got under way, there were surprises from the very start. Scott and his colleagues (*Mycol. Res.* **111:**1422, 2007) found that *Baudoinia* did indeed use ethanol, though not other simple alcohols, as a source of carbon for growth. On the other hand, it was not addicted to ethanol. It proved just as capable of obtaining its carbon from glucose in alcohol-free medium. However, ethanol did appear to be important in the life of *Baudoinia*, activating stress response proteins overlapping with those activated by heat shock.

The authors suggested that this process might facilitate the establishment and growth of the organism in the extreme, bare locations, exposed to the sun, where it often occurs around facilities such as spirit maturation warehouses. "The fungus does not ordinarily survive temperatures of 52°C or higher when moisture is present, but can be pre-adapted to survive this temperature by prior heat or ethanol exposure," they wrote. "SDS-PAGE analysis of cellular proteins reveals that heat and ethanol pre-adaptation appear to induce the formation of putative heat shock proteins."

In their most recent paper (*Mycol. Res.* **112:** 1373, 2008), the Toronto workers show that, despite countless observations of *B. compniacensis* in places characterized by low concentrations of ethanol in the surrounding air, greater amounts had an adverse effect. Whereas up to about a day's exposure to 10 ppm of ethanol in vapor form enhanced germination of the fungus, exposure to higher concentrations for shorter durations strongly inhibited growth. Indeed, a level of 5%—well below that tolerated by most of the species of *Saccharomyces* which themselves produce alcohol—suppressed growth totally. These findings and other evidence indicate that, notwithstanding its favored habitat, *B. compniacensis* is relatively intolerant of ethanol.

Enzymological sleuthing then led James Scott and his group to identify a role in their warehouse stainer for the glyoxylate pathway first identified just over 50 years ago when Hans Krebs and Hans Kornberg recognized the capacity of certain bacteria to proliferate efficiently on two-carbon compounds. The Toronto team found that the specific activities of two glyoxylate cycle enzymes, isocitrate lyase and malate synthetase, in *Baudoinia* grown on acetate were comparable with those found in various other microorganisms. These results appear to indicate that the glyoxylate cycle plays a crucial role in replenishing TCA intermediates, such as gluconeogenesis precursors, when this fungus grows on acetate or ethanol.

"It is likely that the glyoxylate cycle of *Baudoinia*, conferring the ability to utilize ethanol, is a critical factor involved in the abundant growth of this organism in the nutrient-deprived conditions found on exposed surfaces around distillery warehouses and bakeries," the authors write. "Whether ethanol serves as a major carbon source under these conditions or only as a signal stimulating growth, it is likely that the glyoxylate cycle is involved: the level of simple sugars in the environment is unlikely to be high enough to switch it off."

As Scott and his coworkers observe, further work is required to determine what overall contribution the glyoxylate cycle makes to the metabolism of the sooty fungus. There have been several previous reports showing that the cycle's enzymes can be significant in functions other than basic metabolism on two-carbon substrates. For example, they contribute to the synthesis of riboflavin in *Ashbya gossypii*, of protein in the nitrogen-fixing bacterium *Bradyrhizobium japonicum* and of oxalate in the plant pathogen *Scerotium rolfsii*.

The Toronto studies might suggest that distillery warehouses could, by cutting their output of alcoholic vapor, prevent *B. compniacensis* from colonizing their premises. On the other hand, it is possible that even vanishingly small levels of alcohol in the air can induce it to its proliferate. We simply do not know. "If ethanol functioned mainly as a carbon source, any reduction in emission should lead to a corresponding reduction in growth of *Baudoinia*," Scott and his colleagues write. "However, if low levels of ethanol triggered colony establishment even when ethanol levels were insufficient to provide all the carbon needed for growth, then only the reduction of ethanol to very low levels would be likely to reduce the environmental proliferation of *Baudoinia*."

One other question remains, but it is probably one to be answered not by a microbiologist but by a psychologist or sociologist. What accounts for the contrast in our human response to the warehouse-staining fungus? Some years ago, when I was being shown around a distillery in Scotland producing fine malt whisky, the guide pointed with pride at the blackened, mouldy marks around the area where the Scotch was maturing. I've heard of similar experiences in cognac distilleries in France, where the stains are seen as a sign of quality. Yet in North America they are viewed as unclean, even hazardous. In truth, they are neither. So why the difference?