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Review

Synthetic biology stretching the realms of possibility in wine yeast research

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ABSTRACT

It took several millennia to fully understand the scientific intricacies of the process through which grape juice is turned into wine. This yeast-driven fermentation process is still being perfected and advanced today. Motivated by ever-changing consumer preferences and the belief that the 'best' wine is yet to be made, numerous approaches are being pursued to improve the process of yeast fermentation and the quality of wine. Central to recent enhancements in winemaking processes and wine quality is the development of *Saccharomyces cerevisiae* yeast strains with improved robustness, fermentation efficiencies and sensory properties. The emerging science of Synthetic Biology – including genome engineering and DNA editing technologies – is taking yeast strain development into a totally new realm of possibility. The first example of how future wine strain development might be impacted by these new 'history-making' Synthetic Biology technologies, is the de novo production of the raspberry ketone aroma compound, 4-[4-hydroxyphenyl]butan-2-one, in a wine yeast containing a synthetic DNA cassette. This article explores how this breakthrough and the imminent outcome of the international *Yeast 2.0* (or *Sc2.0*) project, aimed at the synthesis of the entire genome of a laboratory strain of *S. cerevisiae*, might accelerate the design of improved wine yeasts.

1. History in the making

1.1. Making wine-history through continuous cycles of innovation

Continuous innovation is the corkscrew which allows grapegrowers, winemakers and scientists to open the bottled mystique of wine quality and consumer satisfaction. With every twist and turn the underpinning sciences uncork novel ideas of how to improve the quality of wine while minimizing resource inputs, production costs and environmental impact. For > 7000 years there has been a continuing evolution in grape and wine production. Working symbiotically, grapegrowers, winemakers and scientists have raised fresh questions and revealed new possibilities regarding old problems, ingeniously overcoming emerging challenges regarding the way grapes are grown, how wine is made, and how consumers respond to their products.

The Human race was imbibing alcohol long before writing was even invented. Fermented beverages were not a mere byproduct of civilization – they were central to it. Since at least 9000 years ago, the hedonic properties of alcoholic beverages sparked the creativity of our ancient ancestors that led to the development of language, the arts and religion. In fact, there is a link to alcohol to nearly all great transitions in human history from the origin of farming to the origin of writing.

The oldest evidence of an alcoholic beverage comes from Jiahu in China where farmers were fermenting a cocktail of rice, hawthorn berries, wild grapes and honey in clay jars by 7000 BCE (McGovern et al., 2004). Evidence has also been found that, in the Caucasus Mountains of modern-day Georgia and the Zagros Mountains of Iran, grapes were one of the earliest fruits to be domesticated. We can trace the evolution of grape-derived wine from its earliest archaeological origins in Ancient Persia, the Caspian Sea shores (ca. 6000 BCE), and ancient Egypt (ca. 5000 BCE) (Fig. 1). For an overview of the history of winemaking and timelines of developments in the wine industry, see the following articles and references therein: Borneman et al., 2013b; Curry, 2017; Chambers and Pretorius, 2010; McGovern et al., 2004; Pretorius, 2000, 2010, 2016; Pretorius et al., 2015). The following paragraphs represent a synthesis of information drawn from the latter publications and references therein.

Based on how wine was written into the literature of the day, portrayed through the art, and from fossilized grape seeds and skins found in the remains of long-gone buildings and cities, we know that by approximately 4000 BCE, wine was fully embedded in the diet and culture of the ancient Greeks. By 1500 BCE, winemaking was popular throughout southern Europe and in northern Africa, following colonization by the Greeks, the Phoenicians and the Romans. Just over

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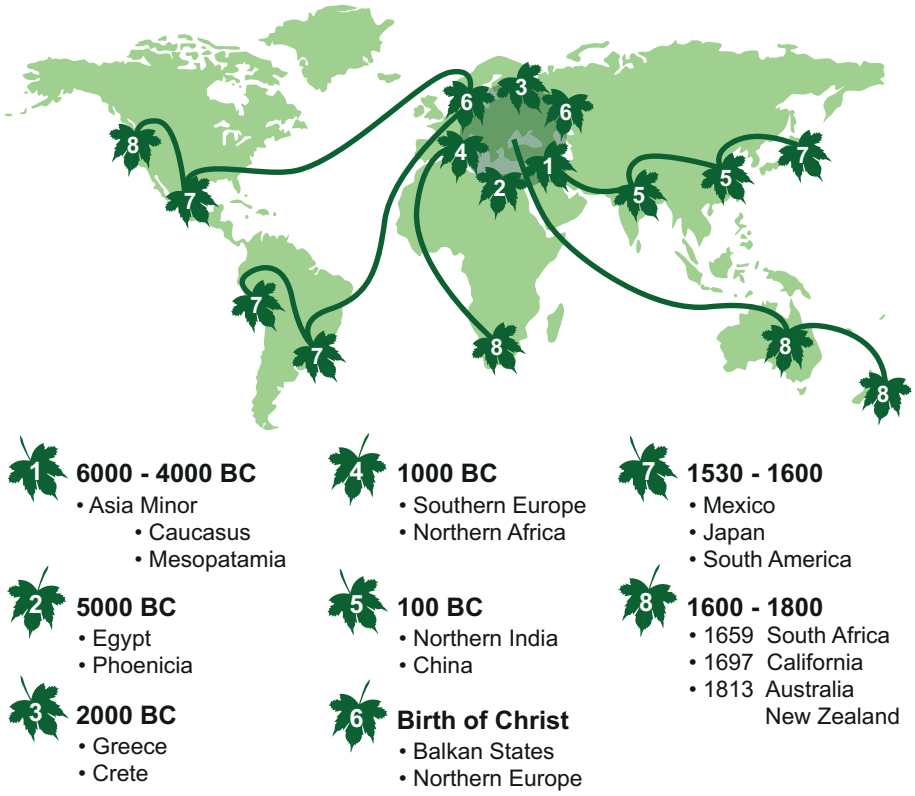

Fig. 1. The early spreading and world distribution of the know-how of grape cultivation and winemaking (adapted from Pretorius, 2000). It is widely believed that *Vitis vinifera* wine grapes originated at the foot of the Caucasus Mountains in Georgia and the Zagros Mountains of Iran. Georgia has > 500 indigenous grape varieties and, for millennia, Georgians have fermented wine in beeswax-lined clay jars called *qvevri* (Curry, 2017).

1000 years later, by 500–100 BCE, wine was also produced in China and India, coinciding with the development of large earthenware pots for storage and transport by Roman potters. The Romans went on to develop barrels (around 3 CE) following their contact with the Gauls, who had occupied much of western Europe and were experienced at fashioning barrels from wood.

Admittedly, ancient Persian and Pharaonic wine might well be considered too historically remote to be usefully compared with wine-making today; however, it does offer valuable insights of how the ancients founded a tradition of imaginative ingenuity, inventiveness and innovation.

The decline of the Roman Empire was followed by a 'dark' period of economic and cultural decay that aligned with little innovation in wine production practices. Advances in production methods (such as vine cultivation, pottery production and winemaking practices) peaked around 200–400 CE and was followed by a period of 1200 to 1400 years during which progress in wine technology slowed and was generally restricted to monastic religious orders in western Europe. But then things started to change. Spanish explorers and conquistadors took vines to the New World in the 1500s, planting *Vitis vinifera* varieties in Mexico and South America. Halfway through the 1600s, Dutch settlers planted French vine cuttings in South Africa, followed by plantings in California and, by the mid-1800s, vineyards were also plentiful in Australia and New Zealand.

Geographic exploration was accompanied by a search for scientific knowledge. The Age of the Enlightenment – reflected in European art, culture and science through the 17th and 18th centuries – was also evident in wine research as early scientists sought to understand the chemical composition of wine. In 1768, for example, the French chemist Antoine Baumé published his hydrometer scale, allowing the concentration of sugar in grapes to be accurately measured for the first time. Today, winemakers still refer to *Baumé*, preferring this measurement term to 'sweetness' or 'sugar'.

Yeast cells were observed for the first time under a microscope in 1680 by the Dutchman Antonie van Leeuwenhoek, and by 1789 another French chemist, Antoine-Laurent de Lavoisier, had analyzed the process of fermentation, and showed that through fermentation sugar produces carbon dioxide and ethanol. Knowledge of grape composition took another step forward, as researchers isolated sugar as well as tartaric acid from grapes. Wine was no longer a mysterious ferment, produced from haphazard vines. It was now ripe for development backed by scientific research.

The pursuit of technological innovation that transformed industry, agriculture and daily life throughout Europe and North America in the 19th and early 20th centuries also heralded dramatic change in the world's fledgling wine industry. The discovery and adoption of pasteurization facilitated storage and transport. This was not the only significant contribution famed French biochemist, Louis Pasteur, made for the wine industry; he also proved that yeast are living cells, responsible for wine fermentation. Pasteur also went on to recognize the importance of wine phenolics and how oxygen played a pivotal role in the development of color and in the production of red wine. The term 'enzyme' was used for the first time by the German pathologist Wilhelm Kühne in the late 1800s. These discoveries spurred researchers to further investigate the chemical composition of wine, with a view to understanding its creation and production in more detail. Chemists, biochemists and microbiologists analyzed and quantified sugars, acids, alcohol, sulfur dioxide and color in wine and reached a deeper understanding of fermentation enzymes, wine yeast and malolactic bacteria. The concept of inoculating grape juice with pure *Saccharomyces cerevisiae* yeast cultures was introduced in 1890; a practice that would only catch on more than half a century later.

In the vineyard, plant pathologists confronted fungal diseases such as powdery and downy mildew. By the end of the 1800s, it had become widely accepted to graft *V. vinifera* noble variety scions onto *Phylloxera*-resistant rootstocks. By the 1920s and 1930s, plant geneticists had