

OBJECTION IN RESPECT OF THE APPLICATION TO  
THE NATIONAL DEPARTMENT OF AGRICULTURE, SOUTH AFRICA BY  
THE WINE RESEARCH CENTRE (UBC) AND WARRENCHAM SPECIALTIES  
FOR THE COMMERCIAL APPLICATION OF GE WINE YEAST ML01

PREPARED BY



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# ASSESSMENT OF ML01

## BACKGROUND

This document is a brief assessment of the salient features of genetically engineered (GE) yeast ML01 and documents our concerns regarding the application for general release.

### Application by WRC

An application has been made by the Wine Research Centre (WRC) in the Faculty of Land and Food Systems at the University of British Columbia in Vancouver through their South African representative from Warrenchem Specialties, for the application of genetically enhanced malolactic wine yeast ML01 for the commercial production of wine in South Africa (The Mail and Guardian, September 29 2006).

The application is for the use of GM malolactic yeast ML01 in wineries in 20 wine producing regions in the Southern and Western Cape, including Vredendal, Citrusdal, Piketberg, Malmesbury, Tulbagh, Wellington, Worcester, Paarl, Montagu, Robertson, Bonnievale, Swellendam, Franschoek, Stellenbosch, Villiersdorp, Durbanville, Cape Town, Hermanus, Botrivier and Elgin.

### Winemaking

Winemaking has been carried out for thousands of years with evidence of wine production as far back as 6000 BC. Winemaking has evolved over time and the final outcome is dependent on fairly precise monitoring with a dash of mystery. The first steps in winemaking are measurements of the amount of sugar to enable an estimate of the alcohol content of the finished product and acidity levels in the grape.<sup>1</sup> Crushed grapes or must is pressed to separate the skins, seeds, and any other non-juice must item from the juice. After racking, to separate the juice from the settled lees, fermentation is initiated by the addition of 1-2% by volume of yeast to the juice. The fermentation process is closely monitored and once fermentation ceases, aging begins, usually in wooden barrels. Wine from different must batches, varieties, vineyards, fruit maturities, and wine making treatments may occasionally be blended by the winemaker in order to produce a more uniform final product. Certain fining agents may then be added to the wine to reduce haziness and the final product will be filtered to clarify and stabilize the wine. The final product is then bottled.

In the production of red wine, wine passes through two fermentation processes.<sup>2</sup> The first is the alcohol fermentation of sugars to produce alcohol and carbon dioxide by the introduction of a yeast strain.<sup>3</sup> This is followed by malolactic fermentation where lactic acid bacteria which have a permease for malic acid are applied to the conversion of malic acid



into lactic acid.<sup>4</sup> The acidity of lactic acid is about half that of malic acid and malic acid conversion renders a product that is softer on the palate.<sup>5</sup>

Post alcohol fermentation conditions, including reduced nutrients, high alcohol levels and low pH occasionally inhibit or delay successful establishment of malolactic bacteria, enabling other opportunistic bacteria to establish themselves. These unwanted bacteria might convert naturally occurring amino acids into bioamines which can cause severe migraines, asthma attacks and skin rashes in consumers of the wine.<sup>5,6</sup> A well known example of a bioamine is histamine, which is a protein that causes many allergic reactions.<sup>5</sup>

## MOLECULAR CHARACTERIZATION OF ML01

### ML01 in Brief

The ML01 yeast is a commercial wine yeast which has two gene insertions, a gene from a bacterial species and a gene from a wild yeast. Its proposed role is the elimination of a need for a bacterial inoculum during winemaking and hence elimination of the headache-causing chemicals. The two inserted genes come from organisms typically associated with foods and beverages.<sup>7</sup> The first is the malate permease gene from the yeast *Schizosaccharomyces pombe*, which is found in many alcoholic beverages, and the second, the malolactic gene from *Oenococcus oeni*, which is used routinely in the wine industry for malolactic fermentation. The ML01 yeast is being marketed and sold by Springer Oenologie, a division of Lesaffre Yeast Corporation. The U.S. Food and Drug Administration (FDA) has designated *Saccharomyces cerevisiae* strain ML01 as a GRAS (generally recognised as safe) organism in their response to Lesaffre's submission to that office." Further, the developer of ML01, has co-authored a paper that states that that the ML01 yeast is substantially equivalent to the parental industrial wine yeast.<sup>8</sup>

### ML01 Construction

A shuttle vector containing an integration cassette with genes encoding malate permease from *S. pombe*, malolactic enzyme from *O. oeni*, regulatory genes and integration sequences, directing homologous recombination at an unspecified chromosomal locus, was used to modify *S. cerevisiae* strain S92. To enable screening of the transformed yeast, this integration cassette was co-transformed with a plasmid (pUT322) carrying a selectable marker conferring resistance to the antibiotic phleomycin. In terms of the strategy, it was believed that cells transformed with plasmid pUT322 were more likely to also have been transformed with the integration cassette. The first round of screening was therefore for resistance to phleomycin with these phleomycin-resistant yeast then screened for the ability to produce lactic acid. The original phleomycin plasmid did not contain integration gene sequences, rendering it unstable and frequently lost from the yeast. Lesaffre confirmed that the



phleomycin-sensitive isolate they had identified was free of plasmid pUT332 sequences and designated this strain as ML01.”

### **Elimination of Bacteria from Wine Making**

The biggest change in the application of ML01 yeast to winemaking would be the elimination of the need for a bacterial inoculum. Whilst malolactic transformation is the most obvious outcome from the addition of bacteria to the winemaking process, these bacteria add new flavours and aromas to the wine. Some amino acids, for example, may be used following pathways restricted to strains carrying the adequate enzymes, whilst some strains can produce exopolysaccharides. All of these transformation influence the sensory and hygienic quality of the final product.<sup>9</sup> Sensory analyses have shown that many other reactions may change the aromas and make malolactic fermentation beneficial, but what these are are not yet known. Skilled winemakers can avoid creating the headache-causing amines without sacrificing flavour.

### **Persistence of Yeast Cells**

Wine yeasts are unstable<sup>10</sup> resulting in losses in heterozygosity, the occurrence of translocations and hyperactivity in mitotic recombination. In yeast which have transgenes, such chromosomal rearrangements may result in unexpected toxicity.

After a yeast cell is exhausted and dies, autolysis occurs when proteolytic enzymes in the yeast cells rupture the cell and degrade the cell into its basic constituents.<sup>11</sup> These products of autolysis include ribonucleotides<sup>12</sup> that may persist in the wine for many years. Cummins reports on a study to investigate whether wine still contains DNA during must fermentation. In this study, PCR primers from the yeast chitinase gene and the chlorophyll a/b binding protein from plants as well as microsatellite markers<sup>13</sup> showed that large DNA markers were present in must, while 250 base pair micro satellites were present in both must and young wine up to six months. The CFSAN Agency Response Letter to the Lesaffre notification of GRAS status, states that “Lesaffre considers that exposure to the yeast itself or to the newly introduced proteins would be negligible because the processing procedures used in winemaking remove intact yeast cells, debris associated with autolyzed yeast cells, and proteins released during autolysis of yeast cells.” No evidence has been provided to support this statement and the evidence in the literature suggests otherwise.

Outside of the wine barrels, commercial wine yeasts have been found at fairly close proximity (10-200m) to a winery. These results are from a three year study in 6 wineries. 296 of 3780 isolates had a genetic profile identical to that of commercial yeast strains. Dissemination of these strains was largely post-harvest and was favoured by water run-off. These strains did show periods of natural fluctuation on an annual basis.<sup>14</sup> Yeast comparisons between a winery abandoned in 1914 and a modern winery showed that the genetic characteristics of the yeast in the abandoned winery persisted for over ninety years.



Examinations of wine jars from the tombs of ancient Egypt indicated that yeast had been used in winemaking at least as far back as 3150 BC. The persistence of yeast does not appear to have been considered in the Response Letter to the GRAS notification.

## RESPONSES TO THE USE OF ML01

### What is GRAS?

GRAS, as mentioned above, is the acronym for “generally recognised as safe”. It is an American designation for substances that are considered for use as direct or indirect additives to food.<sup>15</sup> Prior to 1958, this referred to substances in general use as food additives, based on a history of use on as food additives, with no further testing necessary. Post 1958, substances put into use were to be recognised as safe based on scientific procedures. Recognition of safety may be based on the views of industry experts and need not necessarily require FDA affirmation, though this may be acquired through what is considered to be a very lengthy process, not often sought by applicants.<sup>16,15</sup>

On 17 April 1997, the Center for Food Safety and Applied Nutrition (CFSAN) and Center for Veterinary Medicine (CVM) published a proposed rule in the Federal Register (62 FR 18938) to amend the regulations to replace the current GRAS affirmation process with a notification procedure. Under the new notification process, applicants could notify the agency that they deemed use of a particular substance to be GRAS. Applicants would have to include in their notification a description of the substance, the conditions of use, and the basis of the GRAS determination. The FDA would **not** conduct its own detailed evaluation of the data, as was done previously, but would evaluate the applicant’s notice to ascertain if there was sufficient basis for a GRAS determination.<sup>17</sup> It is in accordance with this revised requirement for GRAS notification, that Lesaffre Yeast Corporation submitted an application to the US FDA CFSAN.<sup>18</sup> The US FDA Agency Response Letter makes clear that “as always, it is the continuing responsibility of Lesaffre to ensure that food ingredients that the firm markets are safe, and are otherwise in compliance with all applicable legal and regulatory requirements”.<sup>18</sup>

### Applicability of GRAS

It is important to stress that GRAS is not a recognised global standard and the designation of a substance as GRAS in the US does not negate the requirements for thorough testing and risk assessment in South Africa. The applicant has repeatedly stressed the GRAS status of ML10 in the application to the South African Department of Agriculture. This should not be viewed as endorsement of the GE yeast and the applicant should not be exempt from full and complete scrutiny for biosafety.

Further, because the GRAS status is designated for substances considered safe in foods, alternate routes of entry into the body other than in food are not examined. Mode of

administration of a substance impacts greatly on toxicity. For example, substances that might be safe for ingestion may not be safe for inhalation. In the fragrance industry, GRAS status indicates that a substance may be safely used to fragrance a product but does not take into consideration the effect of that substance on the skin, the respiratory system, or the nervous system.

### **The USA**

ML01 malolactic yeast has been approved for use in the USA and Canada. In the United States, yeasts are classified as processing agents and wines made with any genetically modified yeast would therefore need no declaration that they contained GM ingredients.<sup>19</sup> Therefore, in the absence of any labeling requirements, the use of GE yeast might go undetected. This raises very important questions for those countries with more stringent regulations which consider yeast to be an additive or one of the key ingredients in wine. The key exporter of wine in the USA is California with wine exports in 2005 totaling \$625 million.<sup>20</sup> The Wine Institute in San Francisco which has as its members, most of the state's wineries has adopted a policy statement saying that no genetically modified materials can be used in the production of California wines. Whilst just a guide, this policy statement carries a great deal of weight. The Institute has made clear its support for the effort to make the ruling about genetic modification a state-wide or even federal decision rather than the current county-by-county arrangement.<sup>21</sup> It is claimed by the California distributor of the yeast, American Tartaric Products Inc. that a few wines made with ML01 yeast already are reaching consumers. Which brands those are have not been identified and the wines in question do not have to carry any special label.<sup>22</sup> The lack of labeling in those countries where risk assessments are required and where markets are sensitive to the GMO content of food might very well scupper the export of US wines to those countries.

### **Australia**

The Australian Wine Industry has taken an official position against the use of the new GM yeast and stated that “no genetically modified organisms be used in the production of Australian wine”.<sup>7</sup> Prior to the adoption of any new technology in wine production, safety assessments have to be carried out. Further, there has to be a level of public acceptance. The industry takes the view that: “there are potentially great benefits in employing gene technology” yet is cognisant of the “need for safety, openness and quality assurance in any use of gene technology”.<sup>7</sup> Any change in this position will be dependent on the outcome of detailed risk assessments and the receptiveness of the Australian local and export markets to wines containing GE yeasts.

### **South Africa**

The South African wine industry through Winetech (Wine Industry Network of Expertise and Technology) supports a GMO policy that “promotes innovative research and dynamic



science in a responsible, intelligent and perceptive manner to the benefit of all interested parties”.<sup>23</sup> Research into GMOs has also been supported in order that this industry remains on the 'cutting edge' of international research and technical innovation. Despite this, the SA Wine Industry Council (SAWIC) has decided to oppose this request to the Registrar: Genetically Modified Organisms for permission for the commercial use of malolactic yeast ML01 for production of wine in South Africa.<sup>24</sup> The drive behind this decision is the lack of proven international acceptability of the use of such GM yeast. Professor Kader Asmal, the chairperson of SAWIC has stated that South African wine is GMO free and that the time is not ripe for such commercial applications and must therefore be rejected.<sup>24</sup>

### **FDA Approval Not a Rubberstamp.**

The FDA has come under considerable criticism from the British Medical Journal *The Lancet*. In an editorial by the Lancet's Richard Horton, the FDA was criticized as being a servant of industry. The editorial in particular speaks about the FDA's handling of GlaxoSmithKline Plc's controversial bowel drug Lotronex.<sup>25</sup> The FDA approved Lotronex in February 2000, but GlaxoSmithKline voluntarily withdrew the drug nine months later after the deaths of five patients who had been taking it. In his editorial, Horton wrote that “This story reveals not only dangerous failings in a single drug's approval and review process but also the extent to which the FDA, its Center for Drug Evaluation and Research (CDER) in particular, has become a servant of the industry,”. Despite evidence of serious side effects from Lotronex during the pre-approval process and shortly afterward, the FDA kept the product on the market.

The Lotronex scandal is not the only example of potentially dangerous failing by the FDA in the product review and approval process. As many as eleven recent examples of drugs withdrawn because of safety reasons have been cited. For at least four of these drugs, namely bromfenac (Duract), mibefradil (Posicor), troglitazone (Rezulin), and alosetron (Lotronex), clear evidence of danger existed before approval. This pre-existing evidence of danger of these drugs was not adequately heeded. For 4 more of these drugs, namely terfenadine (Seldane), astemizole (Hismanal), cisapride (Propulsid), and phenylpropanolamine (PPA) initial indications of serious adverse effects were not acted upon immediately.<sup>26</sup>

This puts in question the claims by the American Biotechnology industry that genetic engineering of products and processes is well regulated, and that the US government oversees thorough testing to prove safety.<sup>27</sup> According to the Lancet, international faith in the FDA is fast eroding because approvals are frequently influenced by political pressure.



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## MAIN FINDINGS

In summary, the main concerns related to the potential persistence of the GE yeast, potential differences in taste from using ML01 instead of a bacterium, uncertainty regarding the receptiveness of consumers to the use of GE modified yeast in wine production, the lack of proven acceptance of the yeast in the US and Australia, disquiet regarding the implications of a GRAS designation and a reluctance to rely on the FDA as being an arbiter of the Biosafety of GE products.

1. Winemaking is a centuries old activity, and is in many respects unique. Wine production is both science and art and as such cannot be precisely controlled. The final product is subject to the grape cultivar, regional variations, a lack of sterile conditions and the touch of the vintner. Any modified organism has the potential to become an enduring resident of the winery flora.
2. The lack of a bacterial inoculum might impact on the flavour and development of the wine and the use of ML01 might alter the taste of the final product
3. Part of the commercial success of the wine industry hinges on its artisan reputation and the whole mystique of wine consumption. The receptiveness of consumers is paramount in the commercial success of wine production. The refusal of the Wine Institute in San Francisco to endorse ML01 or any other genetically modified material in winemaking is related in part to the Institute's reluctance to jeopardise the lucrative export market.
4. The Australian Wine Industry has officially taken a position against the use of the new GE yeast. Any change in this position would require detailed risk assessments and a level of comfort as to the receptiveness of the Australian local and export markets to wines containing GE yeasts
5. The lack of proven international acceptance has resulted in the SA Wine Industry Council (SAWIC) opposing the request to the Registrar: Genetically Modified Organisms for permission for the commercial use of malolactic yeast ML01 for production of wine in South Africa
6. The requirements of GRAS notification places the onus for ensuring safety on the applicant, It is important to stress that GRAS is not a recognised global standard and the designation of a substance as GRAS in the US does not negate the requirements for thorough testing and risk assessment in South Africa.



7. FDA endorsement is neither without bias nor is it a wholly objective process and is subject to both industry and political pressure. Several instances of approval granted despite pre-knowledge of proven adverse effects have raised serious questions about the efficiency of the regulatory authority to effectively assess applications.

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