L. A. Hazelwood, J.-M. Daran, A. J. A.van Maris, J. T. Pronk and J. R. Dickinson

A Review on Fusel Alcohol Formation by Yeast

Based on a lecture given at the 32nd European Brewery Convention Congress in Hamburg, 10–14 May 2009

Fusel alcohols and the esters derived therefrom are important flavour and aroma constituents in beers. Consistent batch-to-batch maintenance of the desired concentrations of these compounds is essential. The formation of fusel alcohols is one of the longest-studied biochemical processes: investigations having been begun by *Ehrlich* in 1907. The aims have been (i) to define the steps of the biochemical pathway (the 'Ehrlich pathway'), (ii) to identify the enzymes involved and the genes which encode them, and (iii) to understand the biochemical and genetic regulation associated with changes in yeast's growth and environmental conditions.

The methods included the use of amino acids specifically labelled with ^{13}C followed by ^{13}C NMR spectroscopy to identify the metabolic sequences, specific mutants suspected or known to encode particular enzymes/isoenzymes, overexpression of structural genes and transcriptome profiling.

Leucine, isoleucine, valine, phenylalanine, tyrosine, tryptophan and methionine that are present in wort serve as the starting materials for the formation of isoamyl alcohol, 'active' amyl alcohol, isobutanol, 2-phenylethanol, tyrosol, tryptophol and methionol (respectively). The steps of the Ehrlich pathway are transamination in which the amino acid is converted into an α-keto acid, then decarboxylation in which the α-keto acid is converted to an aldehyde. The aldehyde is then reduced resulting in formation of the appropriate fusel alcohol. In aerobic conditions (not found in beer production), the aldehyde could be oxidized to the corresponding fusel acid. Four transaminases, 5 TPP-dependent decarboxylases, 16 alcohol dehydrogenases, 6 aldehyde dehydrogenases and 2 broad-spectrum reductases have roles in the pathway depending mainly upon the amino acid, growth phase of the yeast and other cultivation conditions. Transcriptional regulation of the structural genes explains most, but not all of the regulation observed. Posttranslational modification(s) of enzymes remain to be discovered. Timely use of the Ehrlich Pathway likely offers both metabolic and developmental advantages to a yeast.

Recent work has led to a far more complete understanding of fusel alcohol formation and its regulation. Consequently, the development of elite strains dedicated to specific processes and with greatly-improved consistency of performance is now a realistic possibility.

Descriptors: Amino acid, fusel alcohol, genetics, physiology

1 Introduction

Fusel alcohols (compounds such as isoamyl alcohol, active amyl alcohol, isobutanol, 2-phenylethanol, tyrosol, tryptophol and methionol, see Fig. 1) are important flavour and aroma compounds in beers. In addition, a variety of esters derived from them, e.g. isoamyl acetate (described as a having a 'banana' note) and phenylethyl acetate (variously sensed as 'roses' or 'honey') are also important constituents of all yeast-fermented products. Fusel alcohols are all produced via the Ehrlich Pathway (see Fig. 2.) Although it was initially proposed by Ehrlich in 1907 [1] and modified by Neubauer and Fromherz in 1911 [2], the pathway was not formally proven until the late 1990s. Numerous experimental results reported between these dates were consistent with it, and many more workers assumed it to be correct (reviewed in [3] and [4]). The proof came from growing yeast in minimal media with individual amino acids specifically labelled with ^{13}C as the sole nitrogen source; ^{13}C nuclear magnetic resonance ('NMR') was used to identify the metabolic sequences involved [5-9]. This NMR method allows the fate of individual atoms in each intermediate from the amino acid to the end product to be monitored. Further confirmation of the Ehrlich Pathway and the non-participation of other routes, was obtained by testing specific mutants in which potential alternative metabolic pathways were blocked [5–9].

Authors:

Hazelwood, L.A., Department of Biotechnology, Delft University of Technology; Kluyver Centre for Genomics of Industrial Fermentation, Delft, The Netherlands; current address: NIZO Food Research B.V., Ede, The Netherlands; Daran, J-M., van Maris, A. J. A., Pronk, J. T., Department of Biotechnology, Delft University of Technology; Kluyver Centre for Genomics of Industrial Fermentation, BC Delft, The Netherlands; Dickinson, J. R., Cardiff School of Biosciences, Cardiff University, Cardiff, United Kingdom; corresponding author: jrichard dickinson@hotmail.com

Tables and figures see Appendix

2 The Ehrlich Pathway: An Outline

The Ehrlich Pathway (Fig. 2) has three main stages. The first is transamination in which the amino acid is converted into an α-keto acid; α-ketoglutarate accepts the $-NH_2$ group from the

amino acid, resulting in the formation of glutamate. In every yeast culture in which the preferred nitrogen sources such as glutamine, ammonia or asparagine have been depleted or are absent, this is an important activity because the glutamate formed can serve as the starting material for the formation of nearly every other amino acid needed in protein biosynthesis, as well as in the many other metabolic pathways that must operate.

The next step is decarboxylation in which the α-keto acid is converted into what we have called a 'fusel aldehyde' [11] and carbon dioxide is evolved. The final step is reduction of the aldehyde using NADH and/or NADPH to yield the fusel alcohol. Thus, for each amino acid we can explain the corresponding aldehyde and fusel alcohol (Table 1). (The reader will note that Table 1 also refers to 'fusel acids'; these are described later.)

In the case of methionine catabolism only, an enhanced version of the Ehrlich Pathway operates (Fig. 3). Besides the 'conventional' Ehrlich Pathway in which transamination yields α-keto-γ-(methylthio)butyrate ('α-KMBA') and decarboxylation produces β-(methylthio)propionaldehyde (often called 'methional') followed by reduction into methionol, there is also a demethiolase activity acting on both methionine and α-KMBA to produce methanethiol (with its accompanying unpleasant smell) and α-ketobutyrate [9]. α-ketobutyrate is a carbon skeleton that can be re-utilized by yeast, e.g. as a precursor in isoleucine biosynthesis [12]. Hence, methionine catabolism is both more complex and more economical for the cell than the other catabolic pathways which use the Ehrlich Pathway and result solely in the formation of fusel alcohols whose carbon skeletons cannot be recycled.

3 Recent Developments

Much interest in recent years has focussed on identifying the structural genes for the relevant enzymes involved. Starting with the amino acid aminotransferases (Fig. 4), *TWT1* and *TWT2* (also known as *BAT1* and *BAT2*) encode the mitochondrial and cytosolic **b**ranched-chain amino acid **a**mino**t**ransferases respectively [13–14], i.e. those involved in transamination of leucine, isoleucine and valine. The mitochondrial isoenzyme is highly expressed in batch cultures during exponential growth and is repressed during stationary phase. The cytosolic isoenzyme (Twt2) has the opposite expression pattern. Hence, in a batch culture, such as in a beer fermentation, yeast always has an enzyme available for this reaction. *ARO8* and *ARO9* encode broad substrate specificity amino acid aminotransferases [15–16]. Their *in vitro* activities are mirrored by transcriptome profiling of cells in a glucose-limited chemostat when phenylalanine, methionine or leucine were the sole nitrogen source, *ARO9* and *TWT2* were up-regulated compared to growth in ammonia, proline or asparagine where catabolism does not use the Ehrlich Pathway [17].

For decarboxylation of the α-keto acids the yeast genome apparently contains 5 genes encoding thiamine diphosphate-dependent decarboxylases. Four of these are shown in figure 4: *PDC1*, *PDC5* and *PDC6* (known as different isoenzymes of **p**yruvate **dec**arboxylase) and the broad substrate specificity *ARO10* [18–21]. Another gene, *THI3*, is not indicated in figure 4 because despite the protein's predicted structure looking very like another decarboxylase, it is perhaps a regulator that works by controlling *in vivo* levels of thiamine diphosphate which is needed by the other four decarboxylases for activity. Hence, it appears that Pdc1, Pdc5, Pdc6 or Aro10 can all function in the decarboxylation of the α-keto acids derived from leucine, valine, isoleucine, tryptophan, tyrosine or phenylalanine; as long as one of these decarboxylases is present, the reaction will occur [11]. However, it appears that in methionine catabolism, the decarboxylation of α-keto-γ-(methylthio)butyrate is achieved only by Aro10 [9, 21]. Thus, despite their names, Aro10 is not specific to **aro**matic amino acid catabolism and Pdc5 is not just a **p**yruvate **dec**arboxylase – both have broad-substrate specificity α-keto acid decarboxylase activity *in vitro* and *in vivo*.

The metabolism of aldehyde to alcohol (Fig. 4) is a reduction reaction and a wide range of enzymes are available. Whether the fusel aldehyde is converted to a fusel alcohol or a fusel acid depends on the cultivation conditions. In anaerobic culture (as in brewing) there is an almost complete conversion to the fusel alcohols. However, in aerobic conditions (not used in brewing) the aldehydes are converted predominantly to fusel acids. Fusel acids (Table 1) include compounds such as isovaleric acid (derived from leucine catabolism) and isobutyric acid (derived from valine catabolism). The fusel acids can be transported out of the cell by the ATP-dependent transporter Pdr12 [17, 22]. The transcriptional induction of *PDR12* is well-understood and is mediated by War1 [23]; however, further consideration of fusel acids is beyond the scope of this brief review.

There are 16 alcohol dehydrogenases, 6 aldehyde dehydrogenases and at least 2 other broad-spectrum reductases that catalyze the pyridine nucleotide-dependent interconversion of aldehydes and alcohols (Fig. 4). Adh1 is the main cytosolic alcohol dehydrogenase that is chiefly responsible for the production of ethanol from acetaldehyde in cells grown anaerobically or in the presence of glucose excess [24]. Adh2 is also cytosolic, it is glucose-repressed and is used for growth on ethanol in aerobic conditions (i.e. the conversion of ethanol to acetaldehyde) [25]. Adh3 is mitochondrial and its role seems to be that of a redox shuttle [26–27] – i.e. shuttling mitochondrial NADH to the cytosol, where it is handled by other NADH dehydrogenases. Adh4 is present at very low levels in laboratory strains but expressed at high levels in brewing strains where ethanol production rates are high [28]. The role of Adh5 is still uncertain [29]. Adh6 is an NADPH-dependent enzyme [30–31]. Adh7, as it has recently been named (previously known only by its ORF number – YCR105w), is also NADPH-dependent; it is 64 % identical to Adh6 [32]. The reductase activity of Adh7 is about 5-fold greater than its dehydrogenase activity. Hence, the specificity of the substrate and the cofactor support the involvement of Adh6 and Adh7 in aldehyde reduction. From the point of view of their importance to yeast, we should perhaps regard NADP(H) homeostasis as their true role [33]. The 7 *AAD* genes encode putative **a**ryl **a**lcohol **d**ehydrogenases [34–35]. No-one knows their true purpose but there is speculation that they could be useful activities in an environment where lignin, cellulose, and/or their breakdown products are abundant (i.e. the natural environment). YPL088w is another putative aryl alcohol dehydrogenase – an assignment made solely on the basis of sequencing information. *SFA1* encodes a formaldehyde dehydrogenase [36]. Using strains

containing all possible combinations of mutations affecting the seven *AAD* genes, five *ADH* genes (*ADH1–ADH5*) and *SFA1*, we showed that the final step of the Ehrlich Pathway can be catalysed by any one of the ethanol dehydrogenases Adh1–Adh5 or Sfa1 [8]. In addition, Ypr1 and Gre2 have been shown to have activity towards 2-methylbutanal (in isoleucine degradation) and isovaleraldehyde (in leucine catabolism) respectively [37–38]. Despite what has just been stated, one must always be aware that ascribing precise roles to alcohol dehydrogenases and aldo-ketose reductases is very problematic because of overlapping specificities and the presence of other related enzymes.

Of course, there are a plethora of transporters (often called 'permeases') responsible for the uptake of amino acids into the cell. These are described in the excellent review [39], so we mention only one here (Fig. 4): *BAP2* which encodes the **b**ranched-chain **a**mino acid **p**ermease in *Saccharomyces cerevisiae* [40]. Lager yeast (*Saccharomyces pastorianus*) has two very different Bap2 enzymes. One is identical to that of *Saccharomyces bayanus*, the other is very similar to that of *Saccharomyces cerevisiae* [41]. This agrees with the idea that lager yeast is a hybrid of *S. bayanus* and *S. cerevisiae*. The regulation of these two genes is very different. *Kodama et al.* [41] studied the transcription of the two variants in a beer fermentation. They found that addition of leucine to the growth medium induced the *cer-BAP2* but did not induce *Lg-BAP2*; ethanol or weak acids left expression of *cer-BAP2* unaffected but *Lg-BAP2* was repressed; and early in the fermentation *Lg-BAP2* was repressed whereas *cer-BAP2* was highly expressed throughout the fermentation [41]. These results suggest that most of the branched-chain amino acid uptake (and hence most of the resulting fusel alcohol formation) in lager beers is due to the *S. cerevisiae* parentage.

It should be added that our ^{13}C labelling studies have shown that anabolic and catabolic pathways share a common pool of α-keto acid [6]. Hence, a molecule of fusel alcohol may have arisen from *de-novo* synthesized rather than catabolically-derived α-keto acid.

4 The Cellular Purposes Of The Ehrlich Pathway

A yeast cell apparently achieves three distinct advantages from operating the Ehrlich Pathway. First, it can salvage NH_2 groups from less-preferred nitrogen sources to enable the biosynthesis of all of the other amino acids needed for protein synthesis and growth. Second, is the maintenance of redox balance. It is widely-known that in yeast excess NADH formed during anaerobic growth can be oxidized by glycerol-3-P-dehydrogenase to form glycerol [42–43]. The formation of glycerol from glucose requires the expenditure of ATP. However, the Ehrlich Pathway may be a more energy-efficient way to do this because it has been reported that respiro-fermentative cultures produce less glycerol when valine is the nitrogen source instead of ammonia [44]. Third, fusel alcohols induce morphological change in yeasts [45–47]. For example, isoamyl alcohol induces yeast-form cells to become pseudohyphal/filamentous [45, 48]. Some workers have sought to explain filamentation as either a foraging respsonse or an escape mechanism [46, 49]. Unlike a motile organism, yeast, upon sensing either a deficiency in its nutrient supply or the presence of a high concentration of toxin or waste product, cannot move-away large distances in search of fresh nutrients or to escape its polluted environment. Thus, when growing as a colony, filament formation is a means by which those cells most distal to the original colony may encounter better conditions. As stated earlier, fusel alcohols are produced via the Ehrlich Pathway when yeast does not have access to its preferred nitrogen sources. Thus, the metabolic end products are acting as signalling molecules for yeast to undergo morphological change [48, 50]. Many brewers have seen these forms in 'stuck' fermentations or when isoamyl alcohol concentrations were high.

5 Conclusions

Viewing the Ehrlich Pathway as a whole, there is decreasing specificity as the pathway is traversed. The amino acid transporters (which bring the amino acids into the cell) and the amino acid aminotransferases are highly specific (not so for Aro8 and Aro9). The decarboxylases display broad substrate specificity; this is especially true of Aro10. However, this does not mean that the cell deploys the enzymes non-specifically. Fusel aldehydes can be reduced by a very large number of enzymes.

6 Future Perspectives

If we wish to alter fusel alcohol production in a yeast strain or process, we should target the earlier parts of the pathway for either genetic or physiological manipulation (respectively) because these offer the best way of achieving the desired result.

7 Acknowledgements

Many thanks to all the students and researchers in Delft, Cardiff and other laboratories around the world who did the work upon which this short review is based.

8 References

1. Ehrlich, F.: Über die Bedingungen der Fuselölbildung und über ihren Zusammenhang mit dem Eiweissaufbau der Hefe, Berichte der Deutschen Chemischen Gesellschaft, **40** (1907), pp. 1027-1047.

2. Neubauer, O. and Fromherz, K.: Über den Abbau der Aminosaüren bei der Hefegärung, Hoppe-Seyler's Zeitschrift für Physiologische Chemi, **70** (1911), pp. 326-350.

3. Webb, A. D. and Ingraham, J. L.: Fusel Oil, Advances in Applied Microbiology, **5** (1963), pp. 317-353.

4. Large, P. J.: Degradation of Organic Nitrogen Compounds by Yeasts, Yeast, 2 (1986), pp. 1-34.

5. Dickinson, J. R.; Lanterman, M. M.; Danner, D. J.; Pearson, B. M.; Sanz, P.; Harrison, S. J. and Hewlins, M. J. E.: A ^{13}C Nuclear

Magnetic Resonance Investigation of the Metabolism of Leucine to Isoamyl Alcohol in *Saccharomyces cerevisiae,* Journal of Biological Chemistry, **272** (1997), pp. 26871-26878.

6. Dickinson, J. R.; Harrison, S. J. and Hewlins, M. J. E.: An Investigation of the Metabolism of Valine to Isobutyl Alcohol in *Saccharomyces cerevisiae*, Journal of Biological Chemistry, **273** (1998), pp. 25751-25756.

7. Dickinson, J. R.; Harrison, S. J.; Dickinson, J. A. and Hewlins, M. J. E.: An Investigation of the Metabolism of Isoleucine to Active Amyl Alcohol in *Saccharomyces cerevisiae*, Journal of Biological Chemistry, **275** (2000), pp. 10937-10942.

8. Dickinson, J. R.; Salgado, L. E. J. and Hewlins, M. J. E: The Catabolism of Amino Acids to Long Chain and Complex Alcohols in *Saccharomyces cerevisiae,* Journal of Biological Chemistry, **278** (2003), pp. 8028-8043.

9. Perpète, P.; Duthoit, O.; De Maeyer, S.; Imray, L.; Lawton, A. I.; Stavropoulos, K. E.; Gitonga, V. W.; Hewlins, M. J. E. and Dickinson, J. R.: Methionine catabolism in *Saccharomyces cerevisiae,* FEMS Yeast Research, **6** (2006), pp. 48-56.

10. Dickinson, J. R.: The Catabolism of Branched-Chain Amino Acids to Fusel Alcohols, European Brewery Convention Monograph **28**, Fachverlag Hans Carl, Nürnberg, Germany, (1999), pp. 74-82.

11. Hazelwood, L. A,; Daran, J-M.; van Maris, A. J. A.; Pronk, J. T. and Dickinson, J. R.: The Ehrlich Pathway for Fusel Alcohol Production: a Century of Research on *Saccharomyces cerevisiae* Metabolism, Applied and Environmental Microbiology, **74** (2008), pp. 2259-2266.

12. Jones, E. W. and Fink, G. R.: Regulation of Amino Acid and Nucleotide Biosynthesis, in: The Molecular Biology of the Yeast *Saccharomyces cerevisiae,* Metabolism and Gene Expression, Cold Spring Harbor Laboratory, Cold Spring Harbor, U.S.A., (1982), pp. 181-299.

13. Eden, A.; Simchen, G. and Benvenisty, N.: Two Yeast Homologs of *ECA39*, a Target for c-Myc Regulation, Code for Cytosolic and Mitochondrial Branched-chain Amino Acid Aminotransferases, Journal of Biological Chemistry, **271** (1966), pp. 20242-20245.

14. Kispal, G.; Steiner, H.; Court, D. A.; Rolinski, B. and Lill, R.: Mitochondrial and Cytosolic Branched-chain Amino Acid Transaminases from Yeast, Homologs of the *myc* Oncogene-regulated Eca39 Protein, Journal of Biological Chemistry, **271** (1996), pp. 24458-24464.

15. Urrestarazu, A.; Vissers, S.; Iraqui, I. and Grenson, M.: Phenylalanine- and tyrosine-auxotrophic mutants of *Saccharomyces cerevisiae* impaired in transamination, Molecular and General Genetics, **257** (1998), pp. 230-237.

16. Iraqui, I.; Vissers, S.; Cartiaux, M. and Urrestarazu, A.: Characterization of *Saccharomyces cerevisiae ARO8* and *ARO9* genes encoding aromatic aminotransferases I and II reveals a new aminotransferase subfamily, Molecular and General Genetics, **257** (1998), pp. 238-248.

17. Boer, V. M.; Tai, S. L.; Vuralhan, Z.; Arifin, Y,; Walsh, M. C.; Piper, M. D.; de Winde, J.H.; Pronk, J. T. and Daran, J-M: Transcriptional responses of *Saccharomyces cerevsiae* to preferred and nonpreferred nitrogen sources in glucose-limited chemostat cultures, FEMS Yeast Research, **7** (2007), pp. 604-620.

18. Schaaf, I.; Green, J. B.; Gozalbo, D. and Hohmann, S.: A deletion of the *PDC1* gene for pyruvate decarboxylase of yeast causes a different phenotype than previously isolated point mutants, Current Genetics, **15** (1989), pp. 75-81.

19. Hohmann, S. and Cederberg, H.: Autoregulation may control the expression of yeast pyruvate decarboxylase structural genes *PDC1* and *PDC5*, European Journal of Biochemistry, **188** (1990), pp. 615-621.

20. Hohmann, S.: Characterization of *PDC6*, a third structural gene for pyruvate decarboxylase in *Saccharomyces cerevisiae*, Journal of Bacteriology, **173** (1990), pp. 7963-7969.

21. Vuralhan, Z.; Luttik, M. A.; Tai, S. L.; Boer, V. M.; Morais, M. A.; Schipper, D.; Almering, M. J.; Kotter, P.; Dickinson, J. R.; Daran, J-M. and Pronk, J. T.: Physiological characterization of the *ARO10*-dependent, broad-substrate-specificity 2-oxo acid decarboxylase activity of *Saccharomyces cerevisiae*, Applied and Environmental Microbiology, **71** (2005), pp. 3276-3284.

22. Hazelwood, L.A.; Tai, S. L.; Boer, V. M.; de Winde, J. H.; Pronk, J. T. and Daran, J-M.: A new physiological role for Pdr12 in *Saccharomyces cerevisiae*: export of aromatic and branched-chain organic acids produced in amino acid catabolism, FEMS Yeast Research, **6** (2006), pp. 937-945.

23. Gregori, C.; Bauer, B.; Schwartz, C., Kren, A.; Schuller, C. and Kuchler, K.: A genetic screen identifies mutations in the yeast *WAR1* gene, linking transcription factor phosphorylation to weak-acid stress adaptation, FEBS Journal, **274** (2007), pp. 3094-3107.

24. Ciriacy, M.: Genetics of Alcohol Dehydrogenase in *Saccharomyces cerevsiae*: I. Isolation and Genetic Analysis of *adh* Mutants, Mutation Research, **29** (1975), pp. 315-325.

25. Lustorf, U. and Megnet, R.: Multiple Forms of Alcohol Dehydrogenase in *Saccharomyces cerevisiae*. I. Physiological Control of ADH-2 and Properties of ADH-2 and ADH-4, Archives of Biochemistry and Biophysics, **126** (1968), pp. 933-944.

26. Young, E. T. and Pilgrim, D.: Isolation and DNA Sequenc of *ADH3*, a Nuclear Gene Encoding the Mitochondrial Isozyme of Alcohol Dehydrogenase in *Saccharomyces cerevisiae,* Molecular and Cellular Biology, **5** (1985), pp. 3024-3034.

27. Bakker, B. M., Bro, C., Kotter, P., Luttik, M A., van Dijken, J. P. and Pronk, J. T.: The Mitochondrial Alcohol Dehydrogenase Adh3 is Involved in a Redox Shuttle in *Saccharomyces cerevisiae,* Journal of Bacteriology, **182** (2000), pp. 4730-4737.

28. Drewke, C. and Ciriacy, M.: Overexpression, Purification and Properties of Alcohol Dehydrogenase IV from *Saccharomyces cerevisiae,* Biochimica et Biophysica Acta, **950** (1988), pp. 54-60.

29. Kruckeberg, A.L and Dickinson, J. R.: Carbon metabolism in "The Metabolism and Molecular Physiology of *Saccharomyces cerevisiae*" (Dickinson, J.R. and Schweizer, M. eds), CRC Press, Boca Raton, U.S.A., (2004), pp. 42-103.

30. Larroy, C., Fernández, M. R., González, E., Parés, X. and Biosca, J. A.: Characterization of the *Saccharomyces cerevisiae* YMR318C (*ADH6*) Gene Product as a Broad Specificity NADPH-Dependent Alcohol Dehydrogenase: Relevance in Aldehyde Reduction, Biochemical Journal, **361** (2002), pp. 163-172.

31. Larroy, C., Fernández, M. R., González, E., Parés, X. and Biosca, J. A.: Properties and Functional Significance of *Saccharomyces cerevisiae* ADHVI, Chemico-Biological Interactions, **143-144** (2003), pp. 229-238.

32. Larroy, C., Parés, X. and Biosca, J. A.: Characterization of a *Saccharomyces cerevisiae* NADP(H)-Dependent Alcohol Dehydrogenase (ADHVII), a Member of the Cinnamyl Alcohol Dehydrogenase Family, European Journal of Biochemistry, **269** (2002), pp. 5738-5745.

33. De Smidt, O.; du Preez, J. C. and Albertyn, J.: The Alcohol Dehydrogenases of *Saccharomyces cerevisiae*: a Comprehensive Review, FEMS Yeast Research, **8** (2008), pp. 967-978.

34. Delneri, D., Gardner, D. C. J. and Oliver, S. G.: Analysis of the Seven-Member *AAD* Gene Set Demonstrates that Genetic Redundancy in Yeast may be More Apparent than Real, Genetics, **153** (1999), pp. 1591-1600.

35. Delneri, D., Gardner, D. C. J., Bruschi, C. V. and Oliver, S. G.: Disruption of Seven Hypothetical Aryl Alcohol Dehydrogenase Genes from *Saccharomyces cerevisiae* and Construction of a Multiple Knock-Out Strain, Yeast, **15** (1999), pp. 1681-1698.

36. Wehner, E. P., Rao, E. and Brendel, M.: Molecular Structure and Genetic Regulation of *SFA*, a Gene Responsible for Resistance to Formaldehyde in *Saccharomyces cerevisiae*, and Characterization of its Protein Product, Molecular and General Genetics, **237** (1993), pp. 351-358.

37. Ford, G. and Ellis, E.M.: Characterization of Ypr1p from *Saccharomyces cerevisiae* as a 2-Methylbutyraldehyde Reductase, Yeast, **19** (2002), pp. 1087-1096.

38. Hauser, M.; Horn, P.; Tournu, H.; Hauser, N. C.; Hoheisel, J. D.; Brown, A. J. P. and Dickinson, J. R.: A Transcriptome Analysis of Isoamyl Alcohol-Induced Filamentation in Yeast Reveals a Novel Role for Gre2p as Isovaleraldehyde Reductase, FEMS Yeast Research, **7** (2007), pp. 84-92.

39. Paulsen, I. T., Sliwinski, M. K., Nelissen, B., Goffeau, A. and Saier, M. H. Jnr.: Unified Inventory of Established and Putative Transporters encoded within the Complete Genome of *Saccharomyces cerevisiae*, FEBS Letters, **430** (1998), pp. 116-125.

40. Grauslund, M.; Didion, T.; Kielland-Brandt, M. C. and Andersen, H. A.: *BAP2*, a Gene Encoding a Permease for Branched-Chain Amino Acids in *Saccharomyces cerevisiae,* Biochimica et Biophysica Acta, **1269** (1995), pp. 275-280.

41. Kodama, Y.; Omura, F. and Ashikari, T.: Isolation and Characterization of a Gene Specific to Lager Brewing Yeast That Encodes a Branched-Chain Amino Acid Permease, Applied and Environmental Microbiology, **67** (2001), pp. 3455-3462.

42. Overkamp, K. M., Bakker, B. M., Kotter, P., van Tuijl, A., de Vries, S., van Dijken, J. P. and Pronk, J. T.: *In Vivo* Analysis of the Mechanisms for Oxidation of Cytosolic NADH by *Saccharomyces cerevisiae* Mitochondria, Journal of Bacteriology, **182** (2000), pp. 2823-2830.

43. Van Dijken, J. P., van den Bosch, E., Hermans, J. J., de Miranda, L. R. and Scheffers, W. A.: Alcoholic Fermentation by 'Non-Fermentative' Yeasts, Yeast, **2** (1986), pp. 123-127.

44. Derrick, S. and Large, P. J.: Activities of the Enzymes of the Ehrlich Pathway and Formation of Branched-Chain Alcohols in *Saccharomyces cerevisiae* and *Candida utilis* grown in Continuous Culture on Valine or Ammonium as Sole Nitrogen Source, Journal of General Microbiology, **139** (1993), pp. 2783-2792.

45. Dickinson, J. R.: 'Fusel' Alcohols Induce Hyphal-Like Extensions and Pseudohyphal Formation in Yeasts, Microbiology, **142** (1996), pp. 1391-1397.

46. Lorenz,M. C., Cutler, N. S. and Heitman, J.: Characterization of Alcohol-Induced Filamentous Growth in *Saccharomyces cerevisiae,* Molecular Biology of the Cell, **11** (2000), pp. 183-199.

47. La Valle, R. and Wittenberg, C.: A Role for the Swe1 Checkpoint Kinase During Filamentous Growth of *Saccharomyces cerevisiae,* Genetics, **158** (2001), pp. 549-562.

48. Dickinson, J. R.: Filament Formation in *Saccharomyces cerevisiae* – A Review, Folia Microbiologica, **55** (2008), pp. 3-14.

49. Gimeno, C. J., Ljungdahl, P. O., Styles, C. A. and Fink, G. R.: Unipolar Cell Divisions in the Yeast *Saccharomyces cerevisiae* Lead to Filamentous Growth: Regulation by Starvation and *RAS*, Cell, **68** (1992), pp. 1077-1090.

50. Chen, H. and Fink, G. R.: Feedback Control of Morphogenesis in Fungi by Aromatic Alcohols, Genes and Development, **20** (2006), pp. 1150-1161

Received 29 July, 2009, accepted 01 October, 2009

Appendix

Table 1 Ehrlich pathway intermediates

Amino acid	α-keto acid		'Fusel aldehyde'		Fusel alcohol		'Fusel acid'	
	systematic	traditional	systematic	traditional	systematic	traditional	systematic	traditional
Leu	4-Methyl-2-oxo-pentanoate	α-Keto-isocaproate	3-Methyl-butanal	Isovaleral-dehyde	3-Methyl-butanol	Isoamyl alcohol	3-Methyl-butanoate	Isovalerate
Val	3-Methyl-2-oxo-butanoate	α-Keto-isovalerate	2-Methyl-propanal	Isobutanal	2-Methyl-propanol	Isobutanol	2-Methyl-propanoate	Isobutyrate
Ile	3-Methyl-2-oxo-pentanoate	α-Ketomethyl-valerate	2-Methyl-butanal	Methylvaleral-dehyde	2-Methyl-butanol	Active amyl alcohol	2-Methyl-butanoate	Methyl valerate
Phe	3-Phenyl-2-oxo-propanoate	3-Phenyl-pyruvate	2-Phenyl-ethanal	2-Phenylacetal-dehyde	2-Phenyl-ethanol		2-Phenyl-ethanoate	2-Phenyl-acetate
Tyr	3-(4-Hydroxy-phenyl) 2-oxo-propanoate	*p*-Hydroxy-phenyl-pyruvate	2-(4-Hydroxy phenyl) ethanal	*p*-Hydroxy-phenylacetal-dehyde	2-(4-Hydroxy-phenyl) ethanol	Tyrosol	2-(4-Hydroxy phenyl) ethanaoate	*p*-Hydroxy - phenyl-acetate
Trp	3-(Indol-3-yl) 2-oxo propanoate	3-Indole pyruvate	2-(Indol-3-yl)-ethanal	3-Indole acetal-dehyde	2-(Indol-3-yl)-ethanol	Tryptophol	2-(Indol-3-yl)-ethanoate	
Met	4-Methylthio-2-oxo-butanoate	α-Keto-γ-(methylthio) butyrate	3-Methylthio propanal	Methional	3-(Methylthio) propanol	Methionol	3-(Methylthio) propanoate	

'Fusel aldhyde' denotes the aldehyde formed after decarboxylation of the indicated amino acid-derived α-keto acid. The table is reproduced from [11] with corrections.

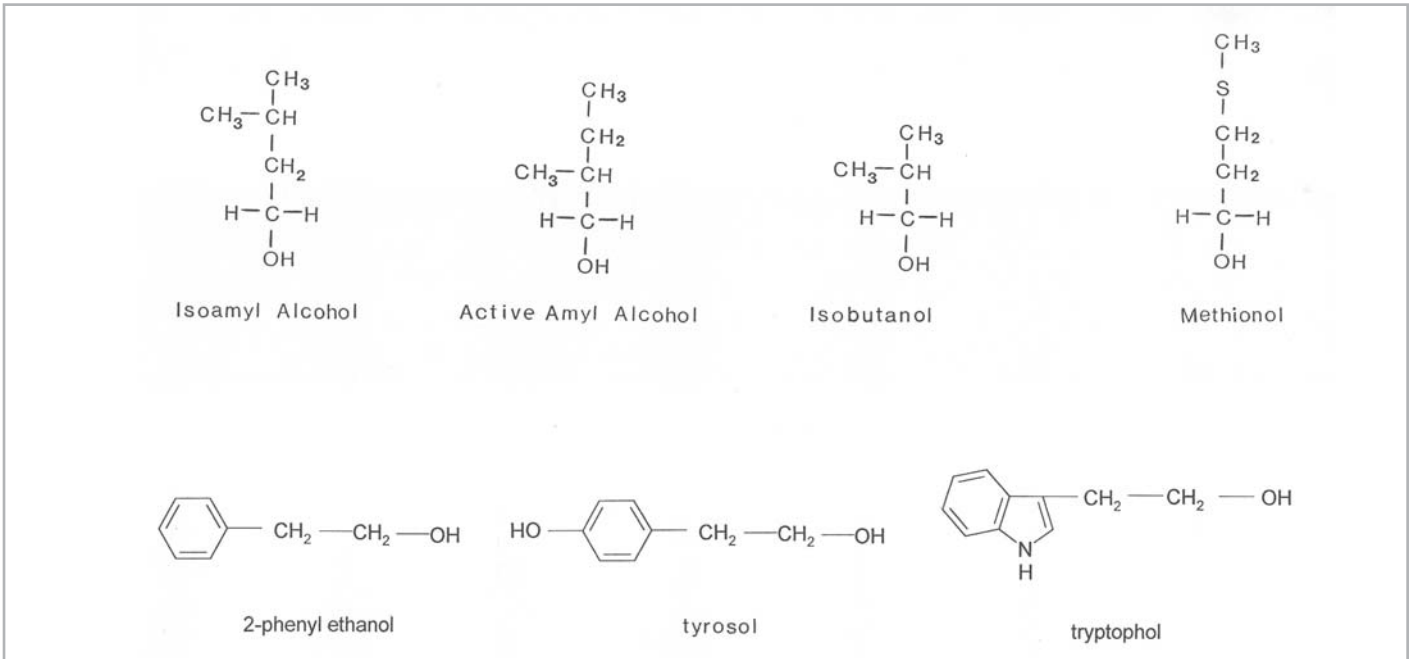

Fig. 1 The chemical structures of the fusel alcohols

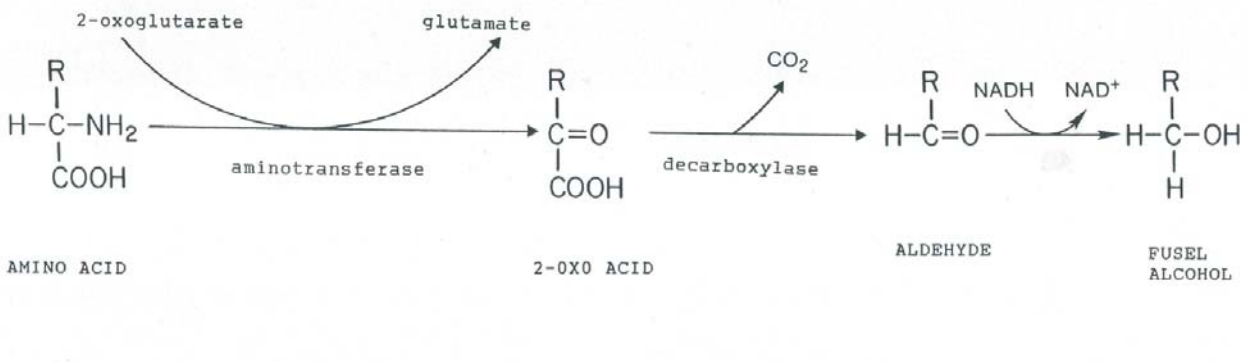

Fig. 2 The Ehrlich Pathway. (Re-drawn from [10])

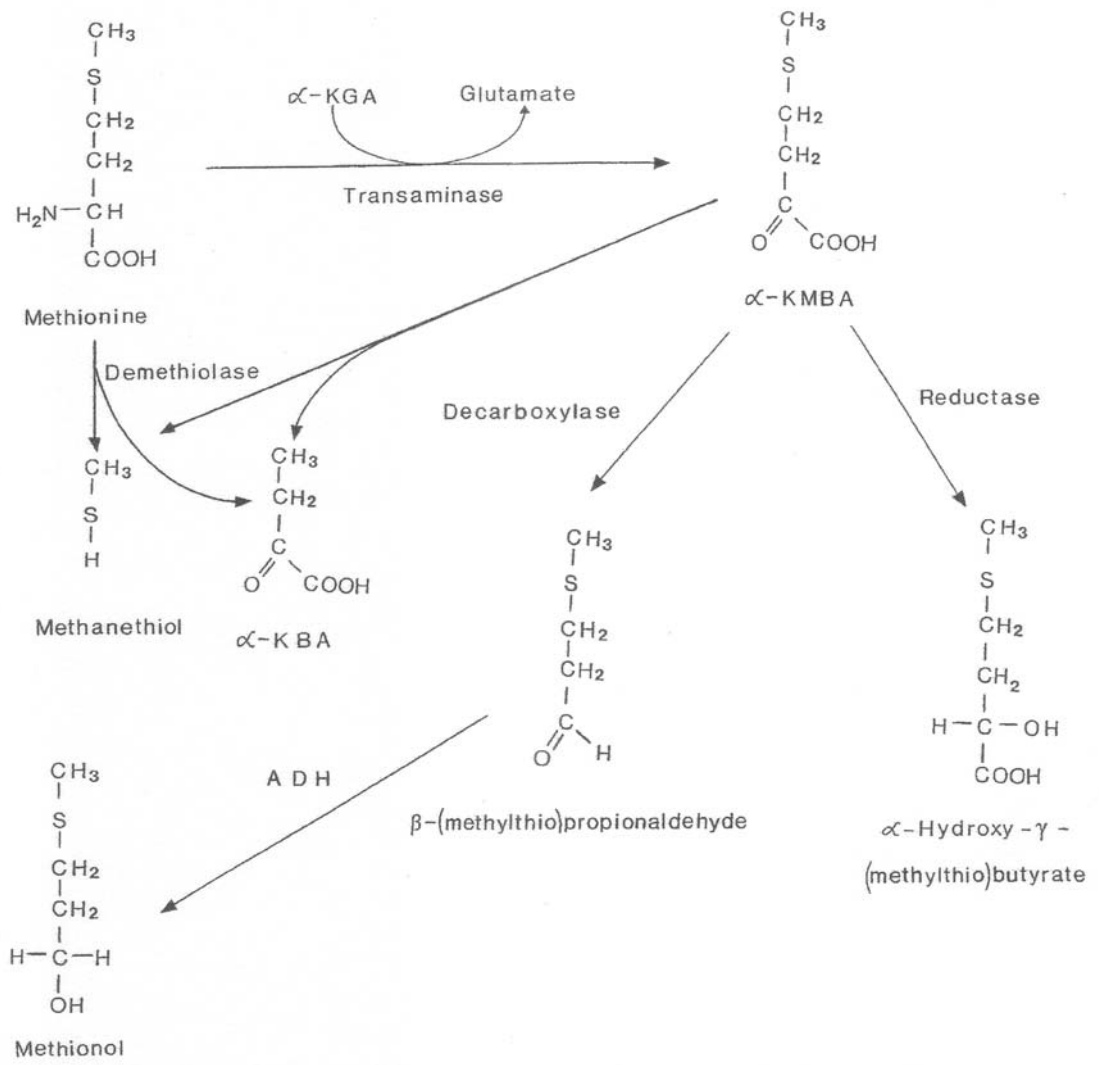

Fig. 3 The pathways of methionine catabolism. The abbreviations used are: α-KMBA: α-keto-γ-(methylthio)butyrate; α-KBA: α-ketobutyrate; ADH: alcohol dehydrogenase. (Reproduced from [9])

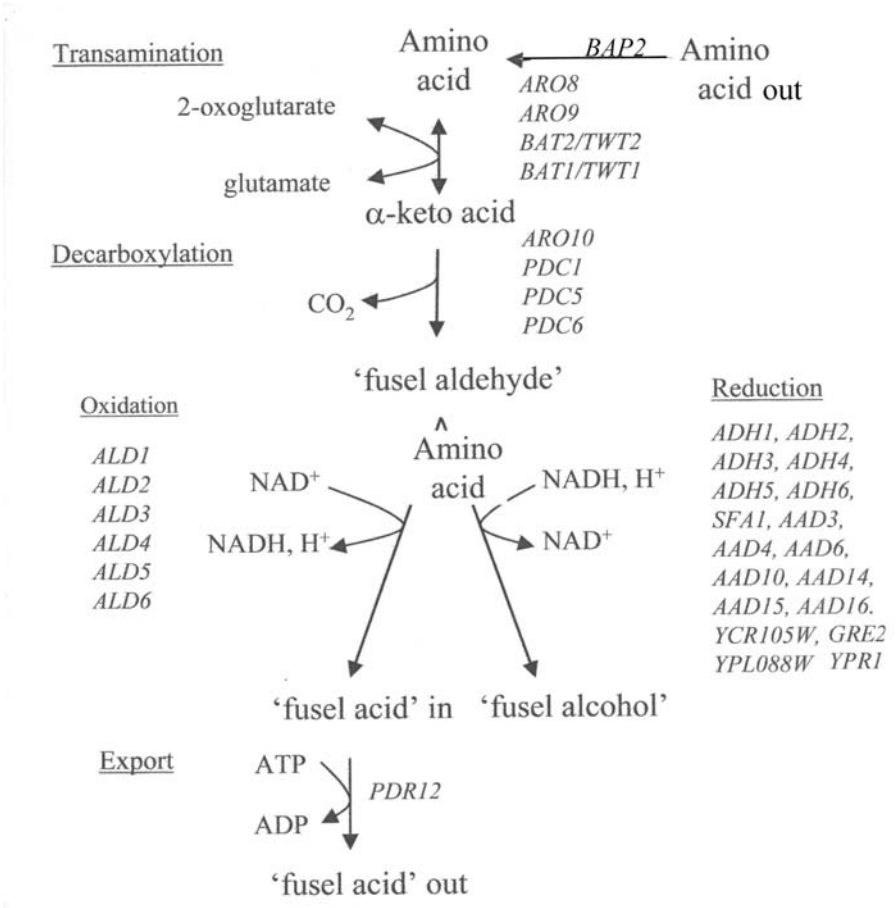

Fig. 4 The Ehrlich Pathway showing the genes encoding all of the enzymes that are potentially involved at each step. The figure also shows *BAP2* which encodes the branched-chain amino acid permease responsible for the uptake of leucine, isoleucine and valine. (Many other permeases exist but these are not considered here.) (Re-drawn from [11])