Saccharomyces kluveri is a petite-negative yeast, which is less prone to form ethanol under aerobic conditions than is S. cerevisiae. The first reaction on the route from pyruvate to ethanol is catalysed by pyruvate decarboxylase, and the differences observed between S. kluveri and S. cerevisiae with respect to ethanol formation under aerobic conditions could be caused by differences in the regulation of this enzyme activity. We have identified and cloned three genes encoding functional pyruvate decarboxylase enzymes (PDC genes) from the type strain of S. kluveri (Sk-PDC11, Sk-PDC12 and Sk-PDC13). The regulation of pyruvate decarboxylase in S. kluveri was studied by measuring the total level of Sk-PDC mRNA and the overall enzyme activity under various growth conditions. It was found that the level of Sk-PDC mRNA was enhanced by glucose and oxygen limitation, and that the level of enzyme activity was controlled by variations in the amount of mRNA. The mRNA level and the pyruvate decarboxylase activity responded to anaerobiosis and growth on different carbon sources in essentially the same fashion as in S. cerevisiae. This indicates that the difference in ethanol formation between these two yeasts is not due to differences in the regulation of pyruvate decarboxylase(s), but rather to differences in the regulation of the TCA cycle and the respiratory machinery. However, the PDC genes of Saccharomyces/Kluyveromyces yeasts differ in their genetic organization and phylogenetic origin. While S. cerevisiae and S. kluveri each have three PDC genes, these have apparently arisen by independent duplications and specializations in each of the two yeast lineages.