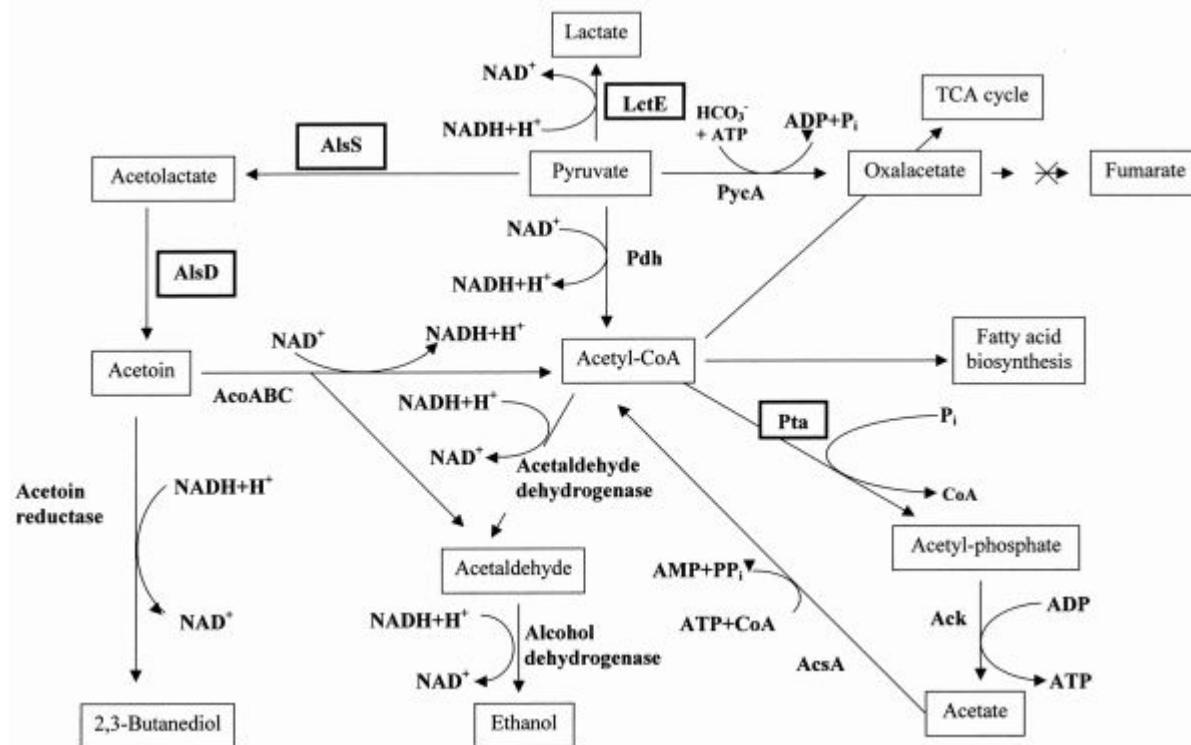


## Quiz: metabolic pathways

This is a practice quiz on metabolism. The general theme is for you to explore a pathway you have not seen before, and extract useful information from it. The quiz does not depend much on knowledge of specific biochemical pathways, but it does use general principles you have learned. It requires you to apply ideas you have learned to try to sort out some unfamiliar information. The first point is simply to not be intimidated by the Figure, but rather to dissect it, and see individual pieces of information in it one at a time. (This question was used as a final exam question recently; it has been modified some for this quiz.)

I encourage you to read over the entire question (p 2) before starting. But then, do one part, and check yourself (p 3) before continuing. That will help guide you. This is a fairly difficult question, especially in the later parts. You will get the most benefit from it by working through it slowly yourself. Simply reading the answer key will be of little value in helping you to analyze pathways. You need to do the practice yourself.

The Figure summarizes some parts of the metabolism in the bacterium *Bacillus subtilis*. The emphasis in the Figure is on what happens during “fermentation” -- anaerobic metabolism, when oxidative phosphorylation is not available. In glycolysis, glucose (a C<sub>6</sub> compound) is converted to pyruvic acid (2-oxopropanoic acid); two NADH are generated per glucose in glycolysis. A key issue in fermentative pathways, which start with the pyruvate from glycolysis, is getting rid of those two NADH, to regenerate NAD<sup>+</sup>. (No other knowledge about glycolysis is required.)



Each part below addresses some aspect of this Figure. The parts are substantially independent, except for parts d-e, as noted below.

In the Figure, all relevant cofactors (NAD etc) are properly shown for parts a-c. (However, they are not complete for the entire Figure.)

a. Which is the “high energy” compound, acetate or acetyl CoA? How can you tell, based on information shown in the Figure? (You may know which is the higher energy compound, but that is not the point here. Use evidence from the Figure; one particular reaction is the key. Of course, the evidence should agree with what you otherwise know.)

b. What is the “simplest” fermentative pathway shown in the Figure?

- Does this pathway “get rid of” two NADH per glucose, as required? Explain.

c. A popular fermentative pathway is the one that makes ethanol. Write out this pathway (free of all the other stuff from the Figure, which is not relevant at the moment). Include all relevant NAD/NADH.

- Does this pathway properly get rid of two NADH per glucose? Explain.

- What other C compound does this pathway make (as an end product) besides ethanol?

Parts d-e are closely related; you may want to work on them as a unit, since the answers to them must fit together into one coherent story.

The Figure shows that *B. subtilis* can make 2,3-butanediol by fermentation. This pathway, from pyruvate to butanediol, contains a couple of compounds with common names that probably are unfamiliar to you. However, you should be able to figure them out, by analyzing the pathway as a whole.

d. Draw the structure of ...

- acetolactate (Hint... the name suggests that it contains ?? and ??; however, the exact form of this compound requires that you also see how it is related to those just before and after it. Don’t try to propose how it is made.)

- acetoin (Hint... think about what it becomes, and what type of reaction is involved.)

e. Two other C compounds are involved in the conversion of pyruvate to butanediol, but are not shown in the Figure. One is a reactant, and one is a product. What are they, and where do they fit in? (This has nothing to do with co-factors, etc.)

The Figure on this quiz is Figure 1 from: H C Ramos et al, Fermentative metabolism of *Bacillus subtilis*: physiology and regulation of gene expression. *J Bacteriol*, 182(11):3072-80, 6/00.

Key and discussion

a. Acetyl CoA is the high energy compound. Look at the Figure, from acetate to acetyl CoA. The Figure shows that going from acetate to acetyl CoA uses ATP, which is an energy source. (You can also see it from the reverse pathway, which makes ATP, but that is more complex.)

b. The simplest pathway is pyruvate → lactate. This involves simply reducing the keto group of pyruvate to the hydroxy group of lactate. This uses one NADH per pyruvate. Since there are two pyruvates (C<sub>3</sub>)/glucose (C<sub>6</sub>), this pathway uses up the two NADH per glucose, as required.

Note that you also learn here what lactate is, which you will need in a later part. Lactate is the product of treating pyruvate with NADH. Using your basic organic chem knowledge (Ouellette Ch 10), this presumably reduces the keto group to an alcohol. Yes. Lactic acid is 2-hydroxypropanoic acid.

c. Ignoring the carrier... Pyruvate → acetate → acetaldehyde → ethanol. The first step generates another NADH. The second and third steps use NADH. So overall, this pathway net consumes one NADH per pyruvate or two per glucose -- as required. The pathway also generates CO<sub>2</sub>. Pyruvate is a C<sub>3</sub> compound; the others are C<sub>2</sub>. Thus C<sub>1</sub> is lost; in context, CO<sub>2</sub> is most likely. If you want to go through the details of the H count, you can show that rigorously, but doing it by “seems most likely” is fine.

d & e. You do need to think about these together, to get the pieces to fit together. Overview... Pyruvate → acetolactate → acetoin → 2,3-butanediol. You know the structure of pyruvate, and the final product has a nice IUPAC name. The final step is a simple reduction, using one NADH, which suggests that acetoin is like 2,3-butanediol, but with one carbonyl group. Thus it would seem that acetoin is 3-hydroxybutanone. Pyruvate is C<sub>3</sub>; the final two compounds are C<sub>4</sub>. So what is acetolactate. Well the name might suggest that it is somehow aceto (C<sub>2</sub>) + lactate (C<sub>3</sub>), both of which are familiar. Thus it is C<sub>5</sub>. This implies that the first reaction is pyruvate + acetate → acetolactate, and that the acetolactate (C<sub>5</sub>) is decarboxylated to make acetoin (C<sub>4</sub>). So now you know that acetolactate has 5 C, arranged in such a way that loss of a CO<sub>2</sub> will give acetoin.

Therefore...

d. 2-hydroxy-2-methyl-3-oxobutanoic acid; 3-hydroxybutanone

If you have an alternative for acetolactate that seems “plausible”, please show it to me. I think it is possible to draw more than one structure that is consistent with the information given here. The one I give is the one used in Nature, but you may have insufficient information to narrow it down that far. So let me look at proposed alternative answers. If what you drew reasonably seems to contain “aceto” and “lactate”, and is organized in a way that simple decarboxylation (and it isn’t oxidative in this case) gives acetoin, then that is ok here. The compound I listed is the actual one used; it is a normal metabolite, as a precursor to making valine.

e. Need a C<sub>2</sub> unit (“aceto” group) in the first reaction; second reaction makes CO<sub>2</sub>. Both of these are familiar.