APO 2- A metabolism case study: soy sauce

Team Members _____
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Scenario:
Brewing soy sauce is one of the original biotech industries. Soy sauce was shipped in barrels within Asia over 500 years ago, and in bottles to Europe by the 1600s. Now soy sauce is used all over the world. About 5000 years ago in China, people grew soybean crops for food and animal feed. Because soybeans spoil easily, salt was added as a preservative. Over time the beans fermented much like pickles or sauerkraut. Unlike pickles, however the soy beans turn into a paste called miso as they ferment. The paste is easier to digest than the unfermented soy beans, and people have been eating it for centuries. About 500 years ago, someone discovered that instead of discarding the sauce at the bottom of the barrels, theycould use it for cooking. Thus, soy sauce was invented.

Unlike making wine from grapes, soy sauce brewing is performed in two stages. First, the soy beans are steamed and mixed with toasted crushed wheat. Fungi *Aspergillus oryzae* and *Aspergillus sojae* are added to the mixture to make *koji* (the first step in the soy sauce-making process) that is then left uncovered for a couple of days. Next, salt and water are added to koji to form a mash called *moromi*. Moromi is then put in airtight containers where it is allowed to ferment for at least 6 months. The mash is then squeezed to get the liquid soy sauce. Finally, the sauce is filtered, pasteurized, and tightly bottled for distribution.

Questions

I. Koji phase

1. In the koji phase, the mixture is inoculated with two species of *Aspergillus* fungi, and the mixture is left uncovered.

i) If you analyze the microbial populations found in koji, what do you expect to find?

A. *Aspergillus* fungi only
B. *Aspergillus* and other fungi
C. bacteria only
D. various fungi (including Aspergillus) and bacteria
E. no microbes

ii) If you expect to find microbes other than the ones with which the mixture was inoculated, explain where they come from and why they thrive in koji.
If you do not expect to find additional populations, explain why not.

II. Moromi phase

Once *Aspergillus* has broken down the macromolecules in the soybeans and wheat into monomers, the koji phase ends. Moromi is then made by mixing koji with water and enough salt to make a 16-20% concentrated salt solution, or brine.

1. When brine is added, the populations of microbes found in koji change. Do you expect greater or lesser microbial diversity?

A. Greater
B. Lesser

2. How does adding brine lead to these changes?

3. Another challenge to the microbes in moromi is that it is placed in airtight containers for a number of months. What types of microbes will survive under these conditions?

Explain how they obtain energy for life processes.

4. Both lactic acid and ethanol are found in soy sauce after the moromi phase is complete. At a minimum, how many species of microbes thrive in moromi?

A. 0
B. 1
C. 2
D. 3
E. can't tell

Justify your answer.

5. Lactic acid and ethanol are produced from the same starting material.

i) What is the starting molecule?

A. Glucose
B. ATP
C. NADH
D. CO_2
E. Other

ii) What other waste products are produced along with lactic acid and/or ethanol?

iii) Do lactic acid and ethanol result from oxidation or reduction of their respective precursors?

A. Lactic acid and ethanol both result from oxidation of the precursors
B. Lactic acid and ethanol both result from reduction of the precursors
C. Lactic acid results from oxidation, while ethanol results from reduction
D. Lactic acid results from reduction, while ethanol results from oxidation