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# Guidelines for the Preparation of a Starter Culture for Cucumber Fermentation

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## Guidelines for producing a starter culture for cucumber fermentation

1. Obtain a cucumber jar inoculated with *Lactobacillus plantarum* (a mother culture) from the USDA-ARS Food Science Research Unit, Raleigh, NC. This jar will be ready for use as a source of inoculum for 1 gallon jars as soon as turbidity develops in it. This will normally take 3-4 days from the day it was inoculated at standard room temperature (25 °C / 77 °F). Once the culture has grown, jars may be refrigerated for up to 4 months and used as needed. The bacteria slowly die off beyond this point, therefore, the cultures cannot be relied upon.

2. Pasteurized 1-gallon fresh pack dill pickles (whole or spears) are used to grow the culture. No preservative would be preferred for this, but it works if benzoate is present. The presence of yellow no. 5 in such jars can help in determining the end point of fermentation in the 1-gallon jars at the end of this process.

*Follow the next step only the first time this protocol is followed with a minimum of two independent (two jars) brine samples. This step may be omitted on subsequent preparations, given that the amount of baking soda added is recorded in a secure place for future reference and that the same source for 1-gallon jars is used.*

3. Aseptically (without touching the neck of the jars, adding saliva or inserting any non-sterile object into the jars), pour off 100 ml of equilibrated brine from two independent 1-gallon pasteurized fresh pack cucumber jars into two clean containers (need not to be sterile). The pH of both samples should be adjusted to 5.2 by adding KNOWN quantities of sodium hydroxide using a calibrated pH meter. The total amount of sodium hydroxide added must be recorded. The amount of base added is enough for 100 ml of brine. Since a 1-gallon jar is ~3785.4 ml, the amount of base added to the 100 ml sample should be multiplied by 37.8 to obtain the amount to be added to each 1-gallon jar (about 120 grams or 4.25 oz). *Optional:* Addition of 6% grape juice to the 100 ml brine samples generates an orange color that changes to dark green when pH levels reach 5.2 due to the addition of the base (see picture below).

From left to right: Original Brine Color, Brine with 6% Grape Juice pH 3.83, Brine with Grape Juice at pH 5.65, and Brine with Grape Juice at pH 7.01.

Thus, the addition of grape juice may serve as an indicator of the pH in the 100 ml brine samples. If grape juice is added, a change in color from orange to dark green should be observed. Addition of grape juice will not affect the amount of base required.

4. Add the pre-determined amount of base or sodium hydroxide to 1-gallon jars that are to be used for tank inoculations. Leave jars at room temperature (25 °C / 77 °F) for 48 hours with occasional shaking to allow for complete equilibration of the added base with the cucumbers. After this pH adjustment to ~5.2, aseptically transfer 10-20 ml of brine from the mother culture (from USDA-ARS FSRU) using a sterile syringe with a sterile needle attached. Clean the rubber septum with rubbing alcohol and then insert the above sterile needle and pull up the brine with the culture. When manipulating the syringe, the parts that will be submerged in the brine should not be in contact with any surface or hands prior to completing the process. The 1-gallon jar should be opened without touching the edges of the neck using aseptic techniques as briefly described above.

5. Store the 1-gallon jars at room temperature (25 °C / 77 °F) for 3-4 days until high turbidity develops in each jar. If the original brine contains Yellow No. 5, a red tint appears at the tips of the cucumbers, or onto damaged cucumber skin spots (see picture below), and the brine will turn colorless when high enough numbers of *Lactobacillus plantarum* are present in the jars. At this time the jars are ready to serve as inocula for the tanks.

6. Add 1 gallon of inoculated brine per 2000 gallons to a fermentation tank as you begin purging. We don't know the best way to add culture to large tanks, so we suggest adding a gallon of culture to the cushion brines when you begin putting cucumbers into a tank and then adding the rest of the culture to the top of the tank as you begin purging. The idea is to get the bacteria distributed in the tank, but not to let them sit in the high salt cover brine with no sugar available for food.