Dissolving benomyl in sporulation medium

Andreas Hochwagen, Ph.D.

The maximum concentration of benomyl that can be dissolved completely in SPO (0.3% potassium acetate) is 120 ug/ml. We use Methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate (95%, Aldrich) for our experiments.

Prepare the benomyl medium straight in the flask that will be used for the culture. Use a volume of at least 50 ml SPO medium (even if the experiment would require a smaller volume), it helps with the dissolving. The benomyl stock solution (30mg/ml in DMSO) is prepared freshly on the day of the experiment. Microwave the SPO medium in the flask and add the benomyl immediately after you see the SPO starting to boil. It is important not to swirl the medium while the benomyl is added (swirling while adding causes some of the benomyl to precipitate). First, add the benomyl (a white cloud will form), then swirl gently. This sounds funny, but this probably the most crucial step in the entire preparation.

Make sure that there are ABSOLUTELY NO FLAKES swimming on top of the medium after the benomyl is added. Even if most of the benomyl is dissolved, the flakes usually mean that the benomyl is not going to work (possibly because the flakes act as seeds that allow more of the benomyl to precipitate out over time).

Allow the medium to cool down slowly to 30C or room temperature, depending on the final sporulation temperature. Use the benomyl medium on the same day. Transfer the meiotic cells from normal SPO to benomyl medium by filtration (place the filter into the benomyl medium, and, once the cells are resuspended, remove the filter).

For a typical experiment, prepare at least ten 500ml flasks with 50 ml SPO, and then prepare one after the other. Usually, it takes a while to not get flakes on the surface. Start preparing the benomyl medium at least 2 hours before the filtration. It takes about an hour to cool down and a little extra time is useful in case the preparation needs to be repeated.

For controls, prepare SPO with DMSO treated the same way.