

Wilson, J. F. and W. K. Bates. Ethylene glycol treatment of *Neurospora conidio*.

disruption of these cells yields large numbers of intact nuclei and mitochondria, while gradual removal of the ethylene glycol results in approximately 75% germination within one hour. We now present some details of the methodology involved.

The conidio routinely used are from seven-to-fourteen-day-old cultures, grown at 30°C on Vogel's minimal agar medium, with supplements as required for mutants. The strain used for most of our studies is a m-isolate of the Oak Ridge wild type. Additional studies with me-3 (36104) FGSC#502, inos (37401) FGSC#406, and [mi-1] (poky, mi-1-1.8) FGSC#1578, with appropriate supplements, have indicated that the effect is not limited to one strain, although variations do occur in the degree of the response.

Recently we described some effects of treatment of *Neurospora conidio* with ethylene glycol (Bates and Wilson 1972 Genetics 68:54). This treatment results in conidio which enlarge, with concomitant weight gain, and which become osmotically sensitive after two or more days. Osmotic

Conidia ore harvested in sterile **water**, filtered through four **layers** of sterile **gauze** to remove hyphal fragments, and the **concentration** is determined with a **hemacytometer**. The **conidial** suspension is allowed to stand for **at least** one hour at **25°C** before the **conidia** ore transferred to ethylene glycol medium. This **pretreatment** with **water** results in foster and more uniform enlargement of the conidia in response to ethylene glycol. **Pre-treatment** periods longer than one hour produce no **additional** effect,

The formulation for **100 ml** of the ethylene glycol medium is: 2 ml of **50X** Vogel's **minimal** medium; 80 ml of distilled water; **18 ml** ethylene glycol, **reagent grade** (**20 grams**); 1.5 g sucrose. We routinely double these **amounts** to obtain **200 ml** ethylene glycol medium, and use this volume in 500 ml Erlenmeyer **type** flasks with stainless steel closures (**DeLong** culture **flasks**). All components are **autoclaved** together in the flask.

We **inoculate** at **1-3x** 10^8 conidio per ml medium ($2-6 \times 10^9$ per flask) by centrifuging the volume of **aqueous** suspension of **conidia** necessary for each flask in a sterile screw-capped tube and decanting the water from the conidial pellet. The conidio ore then re-suspended in a **part** of the contents of a **flask** of ethylene glycol medium and **transferred** back to the **flask**. Thus, inoculation is **achieved** without dilution of the medium. **Flasks** ore then placed on a **rotary shaker** at **25°C** with **carriers** mounted at a **15°** angle and ore shaken continuously at 150 rpm. Osmotic sensitivity is **demonstrable** at 48 hrs, and both size and osmotic sensitivity continue to **increase** for at least **10 days**. We have observed more than 80% viability after 8 days of this **treatment**.

Osmotic disruption is **accomplished** by centrifuging a **suitable** portion of the suspension and re-suspending the pellet in a **hypo-tonic** solution to **approximately** 10% of the **original** volume. Disruption occurs within a few seconds. For mitochondrio, the pellet is resuspended in 2% **sucrose-1mM** EDTA at 5% of the **original** volume, followed by an equal volume of 28% **sucrose-1mM** EDTA at 30 seconds. It should be noted, however, that such mitochondrio ore not identical to those prepared by **sand grinding**.

For studies involving **germination** (including **sorbose** plating) it is **necessary** to dilute the ethylene glycol **gradually**, allowing the conidio to **equilibrate** at the lower **concentrations**. We have accomplished this with **minimal** disruption by non-linear rates of **addition** of **water** or minimal Tedium, according to the following schedule:

10 ml conidial suspension in 20% ethylene glycol in 125 ml Erlenmeyer **flask** on magnetic stirrer at room temperature.

Add **diluent** at **1 ml/min** for 10 min to yield 10% solution,

Add **diluent** at **2 ml/min** for 10 min to yield 5% solution,

Add **diluent** at **4 ml/min** for 15 min to yield 2% solution.

Diluent is added by a peristaltic pump, **aseptically** if necessary. If faster dilution is required, **rates** of addition can be doubled with only a slight **increase** in disruption.

Ethylene glycol **treated** conidio ore much more susceptible to disruption by sand grinding than ore untreated conidia, as judged by **comparative** extraction yields. This **allows** preparation of **extracts** when osmotic shock is not desirable, or in the **preparation** of mitochondrio. The **procedure** is: dilute as described above; centrifuge to concentrate the conidio; re-suspend in the extraction medium; and grind with sand with a mortar and pestle.

Although **various** **modifications** will be **necessary** to suit specific experiment conditions, the methods outlined above should prove **adequate** for **preliminary** studies. A more complete **characterization** of these conidio and the extracts obtained from them will be presented elsewhere. - - - (Supported by grants from the Research Corporation and the UNC-G Research Council) - - -
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