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Use of partially purified Polyoxin B
for forming protoplasts.

The formation of stable protoplasts from os-1 (NM233t) (FGSC #4493 or 4494) mycelial cultures involves the germination of macroconidia in Vogel's medium N containing 10% sorbose and 200-400 µg/ml purified Polyoxin B at 37° C (Selitrennikoff et al., 1981 Exper. Mycol. 5: 155-161). Polyoxin B is purified from Polyoxin AL Wettable Powder (Kaken Chemical Co.) by Norite and Dowex chromatography. Although not overly difficult, complete purification is time consuming. As an alternative, partially purified (40%) polyoxin was used and found to be as effective in promoting protoplasts as was purified (b 70%) polyoxin. Simply, AL Wettable Powder is made to 10% in water (100 mls), the insoluble material filtered using Whatman #1 paper and the dark brown filtrate adsorbed to a granular Norite column, washed with H₂O, and eluted with 60% acetone. The acetone was removed and the brown powder dissolved in H₂O, filtered, sterilized, and stored frozen at -20° C until used (up to six months without detectable loss of activity). A granular Norite column is extremely fast flowing so that the entire partial purification procedure requires less than a day. If the Norite column step is omitted, i.e., if crude polyoxin is used, then protoplast formation is inhibited. (Supported by National Science Foundation award PCM 8112212.)

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