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Abstract

Use of the bacterial basta resistance gene (*bar*) as a selectable marker in Neurospora was reported by Avalos et al (1989 Curr. Genet. 16:369-372).

Making the selectable marker *bar* tighter and more economical

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Use of the bacterial basta resistance gene (*bar*) as a selectable marker in Neurospora was reported by Avalos *et al* (1989 Curr. Genet. 16:369-372). Unfortunately, phosphinothricin (PPT, also called glufosinate), the active ingredient in basta, is currently expensive in its pure form. PPT is a principal ingredient in the relatively inexpensive herbicide Finale (Hoechst-Roussel Agri-Vet Inc.) commonly found in lawn and garden stores, but we found that Finale prevents growth of both Bar$^+$ and Bar$^-$ Neurospora strains. Marty Pall pointed out to us that since PPT is highly soluble in water, a simple extraction may separate the PPT from the non-specific inhibitory ingredients. We found this to be the case. One simple method is to extract Finale twice with an equal volume of 1-butanol, lyophilize the solution, and then dissolve the resulting gel in water (e.g. to half of the original volume). Bioassays indicated that the extracted PPT worked as well as pure PPT and that little if any PPT was lost during the extractions.

We have also found that the Neurospora *am* (NADP-specific glutamate dehydrogenase) marker can be used to tighten the *bar* selection. PPT is known to act by inhibiting glutamine synthetase, the product of *gln-1* in Neurospora (Dávila, *et al*, 1978 J. Bacteriol. 134:693-698) and it had been reported that *am*; *gln-1* double mutants do not grow on media with ammonium as the sole nitrogen source (Hummelt and Mora, 1980 Biochem. Biophys. Res. Comm. **92**:127-33). We therefore tested whether PPT would cause an *am* mutant to phenocopy an *am*; *gln-1* double mutant. It did and only 10 μg/ml of PPT (5% of the normal level; Pall, 1993 Fungal Genet. Newsl. **40**:58) completely inhibited Bar$^-$ strains on Vogel's minimal medium N but permitted growth of Bar$^+$ strains. Greater than 10 μg/ml PPT inhibited even Bar$^+$ strains. In our hands, this scheme resulted in less background growth than in the original system. Addition of alanine to the medium (which promotes growth of *am* strains; Fincham, 1950 J. Biol. Chem. **182**:61-73) completely countered the effect of *am*.