

Making the selectable marker bar tighter and more economical

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Abstract

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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. Commun.* 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 $\mu\text{g/ml}$ 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 $\mu\text{g/ml}$ 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*.