Nutrient modifications for improved growth of *Brassica nigra* cell suspension cultures

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Abstract. Cell pellet yield of two *Brassica nigra* suspension cultures was stimulated by amino acid supplements in the growth medium. This could confound the interpretation of amino acid feeding studies involved in characterizing amino acid metabolism mutants. The nutritional requirements of one of the *Brassica nigra* suspension cultures growing in modified Murashige & Skoog medium were therefore reviewed. Sucrose at 2% w/v was growth limiting and amino or organic acid supplements stimulated growth rate and yield. Increasing sucrose to 6% and supplementing with 15 mM sodium succinate increased maximum cell pellet volume by 2.7 times and maximum dry weight by 2.8 times, stimulated cell enlargement and produced similar maximum numbers of cells per culture. The further addition of an amino acid supplement of 4 mM alanine, 4 mM glutamine and 1 mM glutamate produced no further improvement. The revised medium was more strongly buffered, supported cell growth for a longer period and permitted a 30-fold reduction in the minimum cell inoculum. Cells grown in the revised medium are 10-fold more resistant to growth inhibition by the tryptophan analogue 5MT. These advantages recommend the revised medium for amino acid feeding, mutant isolation and similar studies.

Introduction

Characterization by amino acid feeding studies of a 5-methyl-DL-tryptophan (5MT)-resistant cell line isolated from a *B. nigra* (L.) Koch suspension culture was complicated by a growth response of the parental cells to amino acids added to the growth medium even in the absence of 5MT [12]. Four other observations suggested that this response to amino acids might be symptomatic of a general nutritional deficiency affecting amino acid metabolism. Cell pellet yield in this and a second independently derived *B. nigra* cell line was relatively low and increased 2 to 3-fold on addition of 1–4 g l$^{-1}$ of casein hydrolysate to the medium (Molnar, unpubl.). The minimum

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inoculum required for reproducible growth was relatively large with both cell lines. *B. nigra* cells grown in the standard medium were relatively sensitive (0.1–1.0 μg ml\(^{-1}\)) to growth inhibition by 5MT compared to a range of inhibition concentrations (1–50 μg ml\(^{-1}\)) reported for 11 other species [12]. A 5MT-resistant variant isolated from this cell line had a novel phenotype [12]. The objective of the present study was to derive a growth medium in which cell pellet yield was insensitive to added amino acids and to assess the growth-supporting potential of the revised medium.

**Materials and methods**

*B. nigra* line 15/2 was obtained from K. Klimaszewska and W.A. Keller [9]. The culture was routinely maintained on MSmod2D, a modified MS medium [13] supplemented with 2 mg l\(^{-1}\) 2,4-dichlorophenoxyacetic acid (2,4-D) [9]. This medium contains the macro- and micronutrients of MS medium, vitamins of B5 medium [5], iron as 40 mg l\(^{-1}\) of Sequestrene Fe-330 (Ciba Geigy) and 2% w/v sucrose, pH 5.8. Unless otherwise noted, supplements were autoclaved with the medium at 121 °C for 17 min. Stock cultures were subcultured weekly by one-quarter to one-sixth dilution and maintained at 50-ml cultures in 125-ml Erlenmeyer flasks. Flasks were incubated at 25 °C with a 16-h photoperiod on gyratory shakers (135 rpm). Test media included Kao [8], SH [17], B5 [5], B5H [1], MK [11], N6 [3] and KAM. KAM was Kao’s medium supplemented with tryptophan, phenylalanine, and tyrosine (each at 0.1 mM) plus lysine, threonine, methionine, arginine, isoleucine, leucine, valine, cysteine, histidine, serine, proline and glycine (each at 0.2 mM) and glutamine, glutamate, aspartate and asparagine (each at 0.3 mM) after Negrutiu [14]. All were prepared with 2% w/v sucrose, pH 5.8.

Growth of cells in suspension was monitored by transferring cultures to sterile calibrated 50-ml centrifuge tubes and measuring settled cell pellet volume after 60 min (\(V_{60}\)). All growth experiments were initiated by inoculating one ml of \(V_{60}\) of mid-growth phase cells (3–4 days after subculture). Such an inoculum was determined to be equivalent to 1.4 × 10\(^7\) cells using chromic acid dissociation of cell clumps and counting in a haemocytometer. Dry weights were determined by collecting 4 ml \(V_{60}\) of cell pellet on a pre-weighed filter in a Buchner funnel with multiple distilled water washings. Loaded filters were dried in a microwave oven until there was no further weight change, allowed to equilibrate to room humidity and re-weighed.