

A note on a new defined medium for '*Pseudomonas tomato*'

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A synthetic medium based on D-galactose as a single carbon source and either L-asparagine, L-glutamine or L-threonine as a single nitrogen source has been developed for *Pseudomonas tomato*. The growth of *Ps. tomato* on this medium was equal to its growth on commonly used complex media.

'*Pseudomonas tomato*' (Okabe) Alstatt, the causal agent of the bacterial speck disease of tomato is notorious for its poor growth on defined media based on single carbon and single nitrogen sources [Bergey's *Manual of Determinative Bacteriology* (1974); Okon *et al.* 1978; Bashan *et al.* 1980]. The commonly used media are undefined complex growth media consisting of various peptones and nutrient broth (Schneider & Grogan 1977; Bashan *et al.* 1978; Goode & Sasser 1980). The purpose of this study was to develop a synthetic medium to be used in biochemical, physiological, nutritional, genetic and taxonomic studies of *Ps. tomato*.

Pseudomonas tomato (WT-1) was isolated from infected tomato plants, and was used throughout the procedure. The basal medium used in this study consisted of (g/l): NaCl 5; MgSO₄·7H₂O 1·5; MnSO₄·4H₂O 0·02; FeSO₄ 0·002; ZnSO₄·7H₂O 0·002; CuSO₄ 0·0001; in potassium phosphate buffer 0·06 mol/l, pH 6·8.

The following carbon sources were tested (10 g/l each): arabinose, carboxymethylcellulose, cellobiose, fructose, galactose, glucose, glycerol, lactose, maltose, mannitol, mannose, raffinose, ribose, sorbitol, sucrose, trahalose and xylose.

The following nitrogen sources were used (1 g/l): NH₄Cl, (NH₄)₂SO₄, NH₄NO₃, NH₄H₂PO₄, KNO₃, L-alanine, L-arginine,

L-aspartic acid, L-asparagine, L-glutamine, L-glutamic acid, L-cysteine, L-cystine, L-glycine, L-histidine, L-leucine, L-isoleucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-tryptophan, L-tyrosine, L-threonine and L-valine.

Growth factors were supplied to the medium either as yeast extract (1 mg/l final concentration) or by adding, each at a final concentration of 100 µg/l: ascorbic acid, vitamin B₁₂, biotin, Ca-pantothenate, folic acid, inositol, nicotinamide, pyridoxine, riboflavine, thiamine and vitamin A.

The basal medium and the growth factors were stored in brown bottles at 4°C as concentrated stock solutions (× 100 and × 1000, respectively).

To avoid sedimentation, interactions or denaturation of some of the constituents caused by steam sterilization, media were filter sterilized using Millipore filters (0·45 µm).

Experiments were carried out in 100-ml Erlenmeyer flasks containing 20 ml of medium in each of five replicates. Liquid media were inoculated with 10 µl of a 24-h-old bacterial suspension which had been washed three times with the phosphate buffer, and its absorbance adjusted to 0·1 at 540 nm. Cultures were incubated at 30°C in a rotary shaker (New Brunswick) and results were recorded 24 h after inoculation.

Best growth of *Ps. tomato* occurred in a basal medium supplemented with D-galactose as a single carbon source and with either L-asparagine, L-glutamine or L-threonine as the nitrogen

source. Attempts to use these amino acids as both carbon and nitrogen sources were unsuccessful, and combinations of two or three of these amino acids did not improve *Ps. tomato* growth.

In comparing the newly defined medium described earlier with yeast-peptone broth, nutrient broth, or with a basal medium supplemented with glucose and KNO_3 it was found that the new synthetic medium could replace the complex media for growth of *Ps. tomato* (Fig. 1).

Other *Ps. tomato* isolates such as ATCC 10852, D-1 (Devash *et al.* 1980) and 15 local *Ps. tomato* strains isolated during the last 5 years from all

over Israel, were grown on this medium as well as *Ps. tomato* WT-1.

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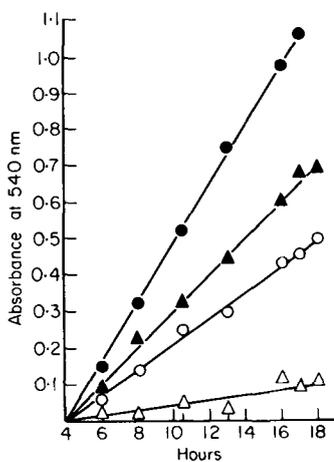


Fig. 1. Growth of *Pseudomonas tomato* on various growth media: ●, yeast-peptone broth; ○, nutrient broth; ▲, synthetic medium; and △, glucose medium.