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THE ABILITY OF PSEUDOMONAS FLUORESCENS TO UTILISE VARIOUS CARBON SOURCES FOR GROWTH.

BY:

AJIBADE A. ROKOSU

Department of Biochemistry, University of Benin,
P.M.B. 1154, Benin City, Bendel State ,
Nigeria

ABSTRACT

The ability of *Pseudomonas fluorescens* to use carbon and nitrogen sources for growth was investigated by growing the organism in various carbon sources (Histidine, glucose, succinate and glutamate) and nitrogen sources (Histidine, and ammonium sulphate) at pH 7.2 and 30°C temperature. The fastest growth rate was observed when glutamate served as the carbon source followed by glucose, while growth in succinate or histidine medium was relatively slow. The presence of a nitrogen source in addition to the carbon source did not affect growth pattern appreciably than when only carbon source was present. *Pseudomonas fluorescens* seemed quite adaptable in terms of carbon or nitrogen sources for growth.

INTRODUCTION

Pseudomonas belongs to the family *Pseudomonasceae,* which is characterised by being gram-negative and non spore formers (Bergey, 1957). Broadly speaking the genus *Pseudomonas* can be divided into fluorescent and non-fluorescent species (Stanier, *et al,* 1966). Pigments such as pyocyanin and fluorescein are common in the fluorescent species. However the ability of these fluorescent organisms to produce pigment depends on the nutritional state of the organisms (Stanier, *et al,* 1966). Previous work had shown the presence of CO_2 – fixing enzymes, pyruvate carboxylase and phospho-enol pyruvate carboxylase in *P. fluorescens* (Higa, *et al,* 1976) when the organism was grown on glucose. *P. fluorescens* degrades histidine via forminino glutamate and formyl glutamate when the organism is presented with this amino acid (Tabor, *et al,* 1952). It has subsequently been shown that this pathway is common to other species of *Pseudomonas* so far studied, namely *P. aeruginosa* (Lessie, et al, *1967);* P. *putida* (Hug, *et al,* 1968) and *P. testosteroni* (Coote, *et al,* 1973). Some carbohydrates, glucose, ribose and fructose in low or high concentrations were found to shorten the lag period required for the synthesis of protocatechuate oxygenase in *P. fluorescens* (Kirkland, *et al,* 1965). Friede, *et al* (1976) showed that when *P. fluorescens* is presented with hydroxylsine, the organism metabolised this amino acid by two possible pathways. *P. fluorescens* utilized maltose by hydrolizing it to glucose alone by means of an inducible alpha – glucosidase (Guffanti, *et al,* 1975). Reick, *et al* (1971) studied the growth rate of colonies of *P. fluorescens* in the presence of glucose and observed a linear increase in bacterial population with time. From the various observations so far, it seems that the genus *Pseudomonas* is nutritionally diverse baving the ability to adapt when presented with varying substrates for growth. The present study examines the ability of *P. fluorescens* to adapt when presented with a variety of carbon or nitrogen sources.

MATERIALS AND METHODS

Chemicals

All chemicals were purchased commercially from British Drug House Limited, Poole, Dorset, England.

Media

Sole carbon sources:
Glucose (2 g/l); Succinate (2 g/l); Glutamate (2 g/l) and Histidine (2 g/l). Sole nitrogen sources: Ammonium sulphate (2 g/l): and Histidine (2 g/l).

Methods

Organism: The organism used for the study was *Pseudomonas fluorescens* NCIB 9046 (obtained commercially from the National Collection of Industrial Bacteria, England).

Maintenance of Organism.
The organism was normally maintained on nutrient agar slopes at 0°C. It was frequently subcultured by streaking on sterile nutrient agar plates, and incubated for 24 h at 30°C to check for homogeneous colonies.

Growth of starter cultures
Each medium (10 ml) containing 2 g/l of a carbon source (either histidine, glucose, succinate or glutamate, depending on the one to be used as the starter culture, 5 g/l potassium dihydrogen phosphate with pH adjusted to 7.2 with 5-M-sodium hydroxide was placed in four separate boiling tubes and plugged with cotton wool before sterilizing. A few drops of sterile 10% w/v magnesium sulphate solution was added to each medium in the boiling tube.

Each medium was inoculated with the organism from a nutrient agar plate and the organism was allowed to grow in each tube under shaking conditions (30 cycles/min) for 24 h at 30°C.

If histidine was used as the source of carbon, the starter culture was termed 'histidine starter culture' in the text. Similarly this description was applied to other compound sources.

Growth of large cultures.
Four conical flasks (250 ml vol.) contained 100 ml of each of the following defined media
Histidine medium contained 0.2 g/100 ml histidine and 0.5 g Potassium dihydrogen phosphate.
Glucose medium contained 0.2 g/100 ml glucose and 0.5 g potassium dihydrogen phosphate.
Succinate medium contained 0.2 g/100 ml succinate and 0.5g potassium dihydrogen phosphate.
Glutamate medium contained 0.2 g/100 ml glutamate and 0.5 g potassium dihydrogen phosphate.

The pH of each medium was adjusted to 7.2 with 5 M-sodium hydroxide and the tops plugged with cotton wool before sterilizing. Sterile 10% (w/v) magnesium sulphate solution was added to each medium to give a final concentration of 0.2% (w/v) magnesium sulphate. Each 100 ml medium was brought to the appropriate temp. (30°C) and each was inoculated with the appropriate starter culture (10 ml) from the boiling tube. Growth of the organism in the various media proceeded at 30°C under shaking conditions. Growth of the organism in each medium was monitored at stated intervals by measuring the absorbance at 580 nm of small samples using a Pye Unicam SP 500 Spectrophotometer. Growth curve for each given condition was obtained by plotting log A580 nm against duration of growth for 10 h.

RESULTS

Glutamate starter culture was grown for 24 h at 30°C. This was used to inoculate histidine, glucose, succinate and glutamate media. Growth proceeded at 30°C under shaking conditions as described in Methods and the pattern of growth in each medium was followed by reading the

absorbance at 580 nm. The growth patterns are shown in fig. 1. There was no lag phase observed when the organism was transferred from the glutamate starter medium. The exponential phase of growth in such medium lasted for about 6 h and the organism reached the stationary phase in each medium after 7 h growth (fig. 1). The organism showed a slightly better growth in glutamate medium than other media. It is observed that the growth response was poorer when the succinate starter culture was used to inoculate the four growth media (fig. 2). There was no lag phase observed when the organism was transferred to these media. There was variation in the time elapsed to reach the stationary phase as the organism reached it after 8 h growth in glutamate (fig. 2). When histidine starter culture was used to inoculate the four media, the growth curves gave similar shape except that the exponential phase was less steep. Generally the stationary phase was reached after 7 h growth and there was no lag phase (fig. 3). When the glucose starter culture was used to inoculate the four media (fig. 4) it is observed that the rate of growth was faster in the media and the stationary phase was attained quicker. When we included ammonium sulphate as a nitrogen source in the various starter cultures to inoculate the various media to observed generally a short lag phase of up to 1 h growth. Earlier we had observed that growth of the organism was generally poor in all media at pH 6.8 and 26°C temperature.

DISCUSSION

The growth pattern of *Pseudomonas fluorescens* was observed by growing in various carbon sources and nitrogen sources. Growth is the sum of all the processes of catabolism and anabolism leading to net synthesis of cellular substances. Normally when actively growing cells are transferred from one medium to the other they undergo an adaptive phase during which the organism produces the enzymes necessary for the metabolism of the new medium. From the present study it seems that *P. fluorescens* NCIB 9046 could metabolise the carbon sources glucose, glutamate, succinate and histidine without any appreciable lag phase. Generally glutamate on its own supported growth faster than the other carbon sources. In instances where the medium for the starter culture was different from the growth medium – histidine starter culture transferred to glucose medium – the organism did not show appreciable lag phase or the lag phase was rather short. The ability of *P. fluorescens* to metabolise glutamate rapidly could be explained in the sense that this amino acid can be rapidly transaminated to ketoglutarate, a key metabolite in Kreb's cycle. Possibly the presence of this keto acid could induce the enzymes of the citric acid pathway. Glucose supported growth of the organism faster after glutamate. It is doubtful whether glucose itself is the inducer of the Etner – Duodoroff pathway enzymes in this organism. There is evidence that gluconate served as the inducer of the enzymes of this path-way in a species of *P. flourescens* (Quay, *et al,* 1973). It was shown that gluconate, but not glucose induced the synthesis of glucokinase in some mutants. It is surprising that succinate as a carbon source did not support a faster growth rate in *P. fluorescens* NCIB 9046, although this substrate is an intermediate in the citric acid pathway. Succinate as such may not be a powerful inducer of the citric acid pathway enzymes. The pathway of histidine metabolism is well established (Meister, 1967). *P. fluorescens* metabolises histidine to glutamate (Tabor, *et al,* 1952). It is quite likely that the slow response of the organism to growth on histidine is due to this. The organism has to convert histidine to glutamate, and then this amino acid can be transaminated. Normally glutamate on its own induced a faster growth rate. From these results it seems that *P. fluorescens* is quite adaptable in its ability to utilise the carbon sources glutamate, glucose, succinate and histidine. The presence of a nitrogen source in addition to the carbon source did not significantly affect this ability. We did not observe "transition points" as observed by Rieck, *et al,* (1973) when *P. fluorescens* was grown on glucose. It is quite reasonable to suggest that some of the enzymes of the metabolic pathways of these carbon sources are present at constitutive levels, and that they are fully induced when a particular substrate is presented.

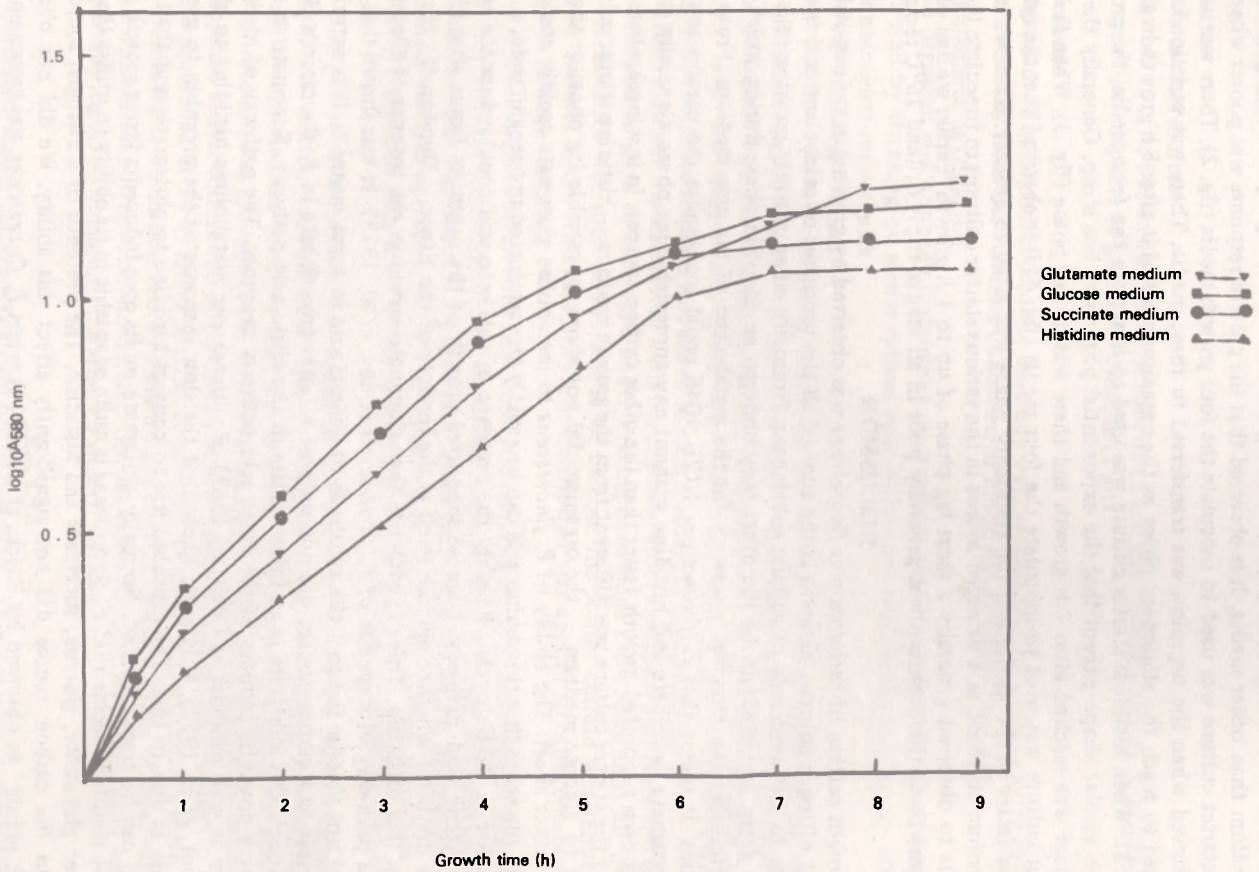


Fig. 1. Growth of *P. fluorescens* on glutamate, glucose, succinate and histidine using glutamate starter culture.

The organism was grown on glutamate starter culture for 24 h at 30°C and then transferred to the various media as described in the text. At 1 h intervals samples were taken from each medium for the determination of A_{580} nm.

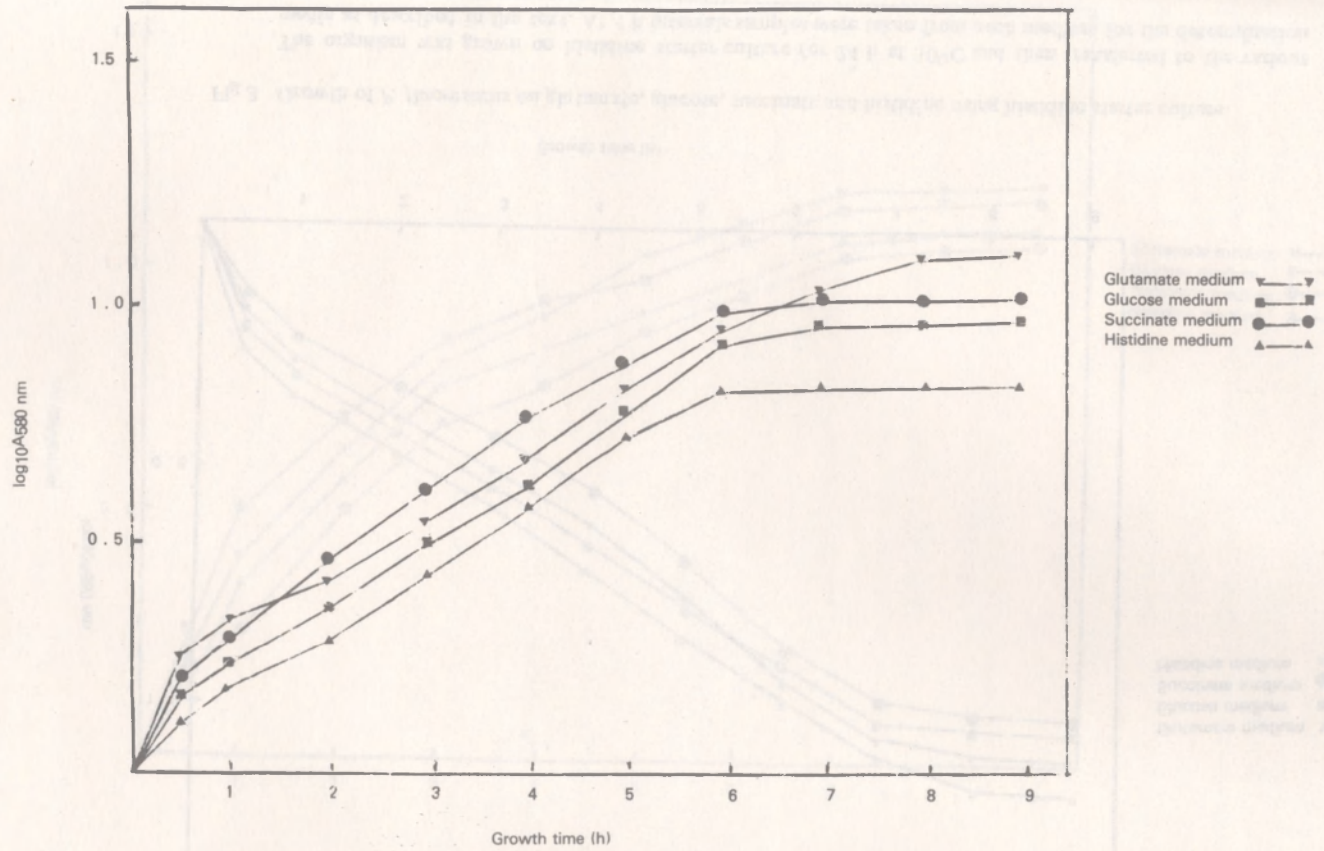


Fig. 2. Growth of *P. fluorescens* on glutamate, glucose, succinate and histidine using succinate starter culture.

The organism was grown on glutamate starter culture for 24 h at 30°C and then transferred to the various media as described in the text. At 1 h intervals samples were taken from each medium for the determination of A_{580} nm.

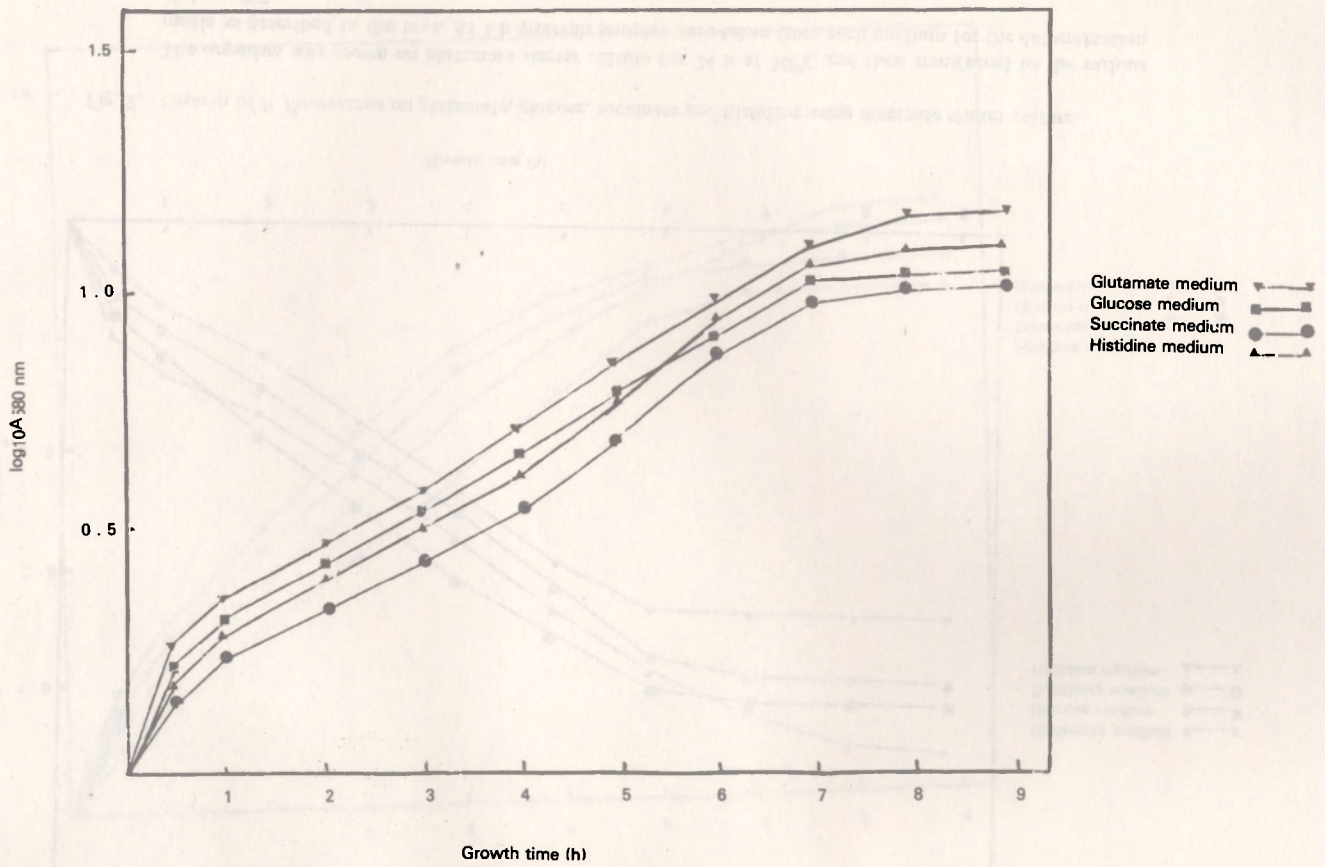
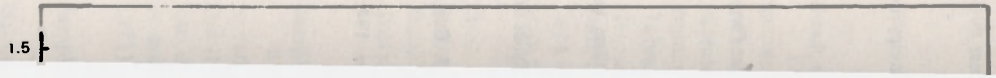


Fig 3 Growth of *P. fluorescens* on glutamate, glucose, succinate and histidine using histidine starter culture.

The organism was grown on histidine starter culture for 24 h at 30°C and then transferred to the various media as described in the text. At 1 h intervals samples were taken from each medium for the determination at A_{580} nm.



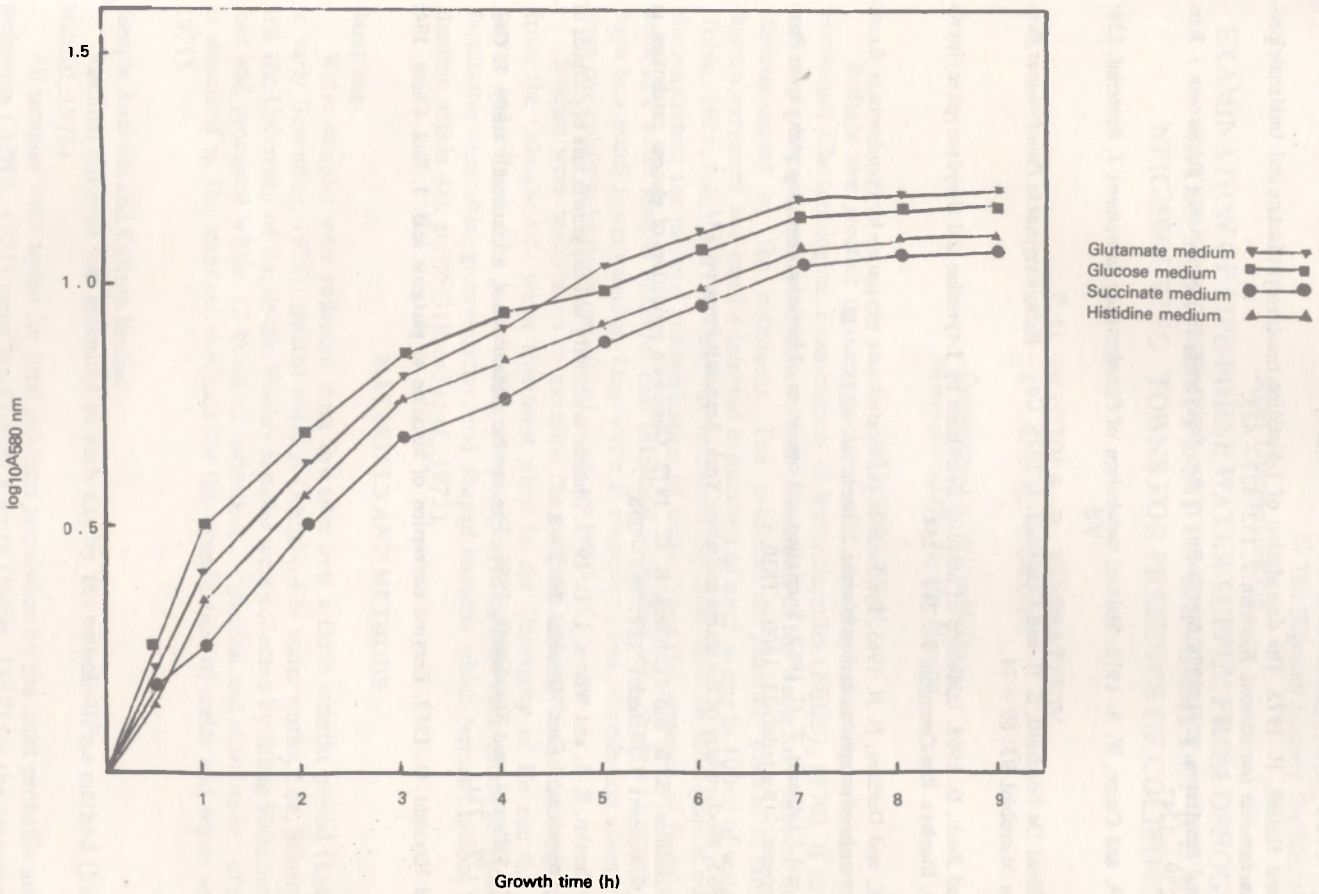


Fig. 4. Growth of *P. fluorescens* on glutamate, glucose, succinate and histidine using glucose starter culture.

The organism was grown on glucose starter culture for 24 h at 30°C and then transferred to the various, media as described in the text. At 1 h intervals samples were taken from each medium for the determination of A_{580} nm.

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