VANILLIN DEGRADATION AND NITROGEN FIXATION BY AZOTOBACTER VINELANDII

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(Manuscript received 15 January 1991)

Abstract

Azotobacter vinelandii grown on Burk's medium containing glucose and vanillin , was able to degrade vanillin at two different conentrations in the Warburg appparatus as a sole source of carbon. It was also capable to fix nitrogen under similar conditions.

INTRODUCTION

Azotobacter is one of the most imoprtant free living diazotrophs in soils . its high energy consumption during nitrogen fixation made it regarded as less important than the symbiotic diazotrophs. Hussein *et al.*, (1975) stated that the co-metaboilism of ferulic acid by *Azotobacter vinelandii* cells grown on Burk's medium containing 1/ u mole / ml ferulic acid, degraded ferulic, vanillic and protocatechuic acids in the Warburg apparatus.

The aim of this work was to test the ability of *Azotobacter vinelandii* to degrade vanilin in two different concentrations. The cells were also tested to fix nitrogen in the presence of the two concentrations of vanillin under similar conditions in comparison with glucose. Hegazi *et al* (1979) tested the effect of some carbon sources on the nitrogenase activity in soil without using vanillin.

MATERIALS AND METHODS

A locally isolated strain of *Azotobacter vinelandii* was used in this work. Burk's medium (Allen 1959) solified with 2 % agar was used for the maintenance of the culture. The same liquid medium was enriched with 1μ mole / m 1 of vanillin and used to grow the cells in liquid culture. Inoculated liquid culrures were incubated on a rotarory shaker at 180 rpm for 18 days at 30^{o}C. cells were havested at 5^{o}C for ten min. at 4000 g . The bacterial growht was washed and resuspended in distilled water (10 ml / 1 of culture) kept at 3-4^{o}C for use.

The oxygen uptake of 1.5 ml of the cell suspension was determined by the Warburg techinque at 38^{o}C (Umbreit *et al.*, 1964).

One ml of a three fold concentrated Burk's medium without glucose was placed in the main well . One or two μ mole / ml of vanillin was given in the side arm. A 0.2 ml of 10 % NaOH was placed in the centre well. Distilled water was added to give a final reaction mixture of 3 ml . The oxygen uptake was measured after 15 min. equilibration at intervals of 10 min. for 5 hours. Calculations were based upon the 3.2 ml flask-contents volume. All experiments were run in triplicates.

The samples were taken from the Warburg after 5 hours; acidified to pH 1 wih HCL and extracted twice with 30 ml ether. The ether extract was combined , concentrated and used for TLC (0.4. mm). The plates were developed with dibutyl ether: acetic acid (90 : 10) and sprayed with diazotized sulphanilic acid as a location reagent . The spots were identified using authentic compounds as markers.

Nine ml of the same constituents tested in the Warburg were prepared in 100 ml flasks . Vanillin was also used in two concentrations, 1 and 2μ mole / ml. Glucose in the same concentrations was also used for comparison. All the experiments, were run in triplicates. Nitrogenase activity was estimated after : 0 , 24 and 48 hours of incubation at 30^{o}C on the shaker according to the procedure of Hegazi *et al.*, (1979) using GCV pye Unicum gas chromatography.

RESULTS AND DISCUSSION

The oxygen uptake of *Azotobacter vinelandii* in the presence of vanillin is given in Fig 1 . It is clear that the vanillin was directly consumed without a lag phase. The first concentration tested, 1 M mole / ml was thoroughly utilized during 5 hours. The oxygen uptake became less and equal to the control. The second concentration , 2 μ mol / ml was not yet utilized.

After 5 hours, the experiments were stopped and the samples were extracted and chromatographed. The first concentration gave negative results. The doubled concentration showed some spots similar to vanillin and protocatechuic acids.

The results of the nitrogenase activity are shown in Table 1. It is clear that the two concentrations of glucose were more effective than similar concentations of vanillin. The nitrogentase activity was still detectable from zero time until 48 hours. The doubled concentration of both vanillin and glucose gave higher activity than the first concentration.

Table 1. C H production n moles / culture / hour by *Azotobacter vinelandii* in the presence of vanillin and glucose at the rate of 1 and 2μ mole / m.

Substrate	Conccentation μ mole / ml	C H μ moles / culture / hour after :		
		0h	24h	48 h
Vanillin	1	0.03	0.05	0.30
	2	0.06	0.06	0.51
Glucose	1	1.54	1.65	5.40
	2	1.84	2.20	16.50

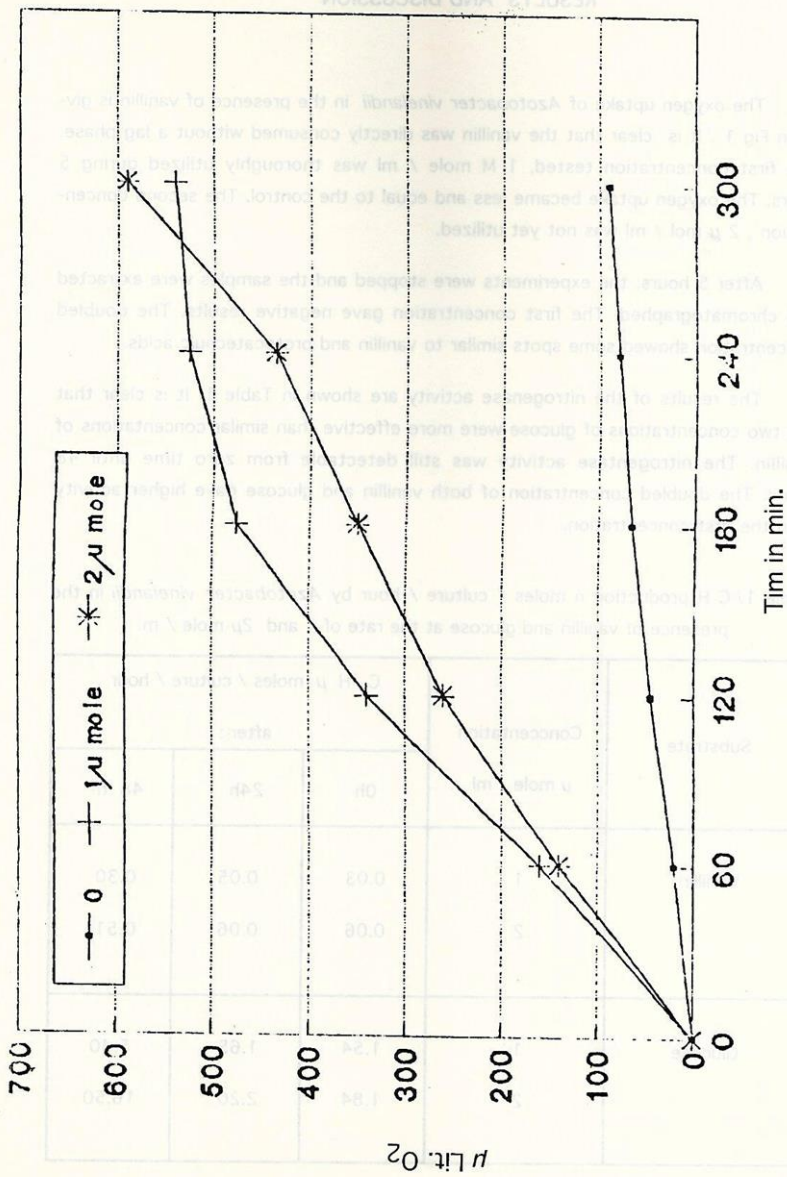

Fig 1. The oxygen uptake by Azotopacter in the presence of vanillin 1 and 2μ mole / ml during 5 hours.

The strain of *Azotobacter vinelandii* used in this work was capable to degrade vanillin at the rate of 1M mol / ml comptlety during 5 hours. A doubled concentration (2μ mol /ml) was also assimilated but somewhat slower. It was not completely degraded in 5 hours. It also gave traces of intermediates by TLC. The intermediates of the vanillin were similar to vanillic and protocate chuic acids.

The nitrogen fixation was easily detectable by gas chromatorgaphy , even by the first concentration and without any incubation period.

Similar results were obtained with glucose for comparison . The glucose was more effective than vanillin because glucose is easy to be assimilated (Allen 1959) . The strain tested in this work can utilize vanillin as the sole source of carbon as detected by the Warburg experiments. It can also assimilate vanillin and fix nitrogen as shown by gas chromatography. This gives free living diazotrophs some more imprtance in soils. They can fix nitrogen but not on the cost of glucose alone. They seem to be able to degrade aromatic substances and cause ring fission. They may be also capable to avoid pollution by degrading aromatic pesticides, and use them as carbon sources for nitrogen fixation.This is in agreement with the work of Walker and Harris (1970). who found that *Azotobacter* sp. grown on benzoate could oxidize 3 chlorobenzoate forming 3 - chlorocatechol. Umarov (1982) reported that, nitrogen fixing bacteria are numerous in the soil and their activity depends mainly on the availability of energy sources, root exudates and root decomposition, which was the main purpose of this work.

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تحليل الفانيلين وتثبيت نيتروجين الهواء الجوى بواسطة ميكروب الأزوتوباكتر

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استهدفت هذه الدراسة اختبار قدرة ميكروب الأزوتوباكتر على استعمال مادة الفانيليين كمصدر وحيد للكربون . ولقد وجد ان للميكروب المختبر القدرة على استعمال مادة الفانيلين بتركيزين مختلفين . وفى نفس ظروف التركيزين كان لهذا الميكروب القدرة على تثبيت نيتروجين الهواء الجوى.