香川大学農学部学術報告第47巻 第2号 127～132, 1995

EFFECT OF A RARE SUGAR, D-PSICOSE ON GROWTH OF AN OLIGOTROPHIC BACTERIUM V-16

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Summary

An oligotrophic bacterium, V-16, which was a gram positive rod, grew twice well by supplement with a rare keto-hexose, D-psicose, as well as L-tagatose and D-sorbose in 100-fold diluted nutrient broth as a basal medium. The organism utilized about 44% of the supplemented D-psicose for growth carbon source in the diluted basal medium. But the growth of V-16 was remarkably inhibited by the supplement of D-psicose in higher concentrations of basal media during early cultivation term. After the about 10 days cultivation the growth recovered from the inhibition by D-psicose.

The organism is not useful for bacterial source for D-psicose metabolic enzymes, because it is difficult to obtain abundantly cells capable of inducing these enzymes with as small amount of D-psicose as possible.

Key words : rare sugar, D-psicose, bacterial growth, oligotroph

Introduction

Although rare sugars are rarely distributed in nature, there are some bacteria capable of utilizing various rare sugars in soil.[1] L-Sorbose and D-tagatose of rare keto-hexoses have been reported to be utilized by some bacteria.[2, 3, 4, 5] But little is known of the microbial utilization of D-psicose, L-tagatose and D-sorbose of rare keto-hexoses. Therefore, metabolism of these rare keto-hexoses in bacteria is a subject of considerable interest.

An oligotrophic isolate, V-16, is one of a few bacteria capable of utilizing D-psicose.[1] We, therefore, examined the effect of D-psicose on growth of V-16. D-Psicose stimulated the growth of V-16 in lower concentrations of basal media, but inhibited the growth in the higher concentrations of the basals.

Materials and Methods

Organisms. The bacteria, V-16, was isolated from the vineyard soil in Kagawa university farm. This isolate can grow in the 10,000-fold diluted nutrient broth, but can not in the standard nutrient broth. This organism, therefore, should be called an obligate oligotrophic bacterium.[6]

Media and cultivation. The 100-fold diluted solution of nutrient broth (NB)[6] was used as a basal medium for growth of V-16. The synthetic medium[6] was also used for the growth inhibition examination. The seed culture, 0.1 ml, of V-16 was inoculated into 10 ml of the basal medium supplemented with 0.1% of D-psicose or other sugars and incubated statically at 27℃ for 10 days or longer.

Identification methods. Gram staining was carried out by the Hucker's modification.[7] The oxidase

test was done using a cytochrome-oxidase reagent (Nissui Co.). Catalase was tested by dripping a 3 % H_2O_2 solution on the collected cells. Sporulation was checked by Synder's staining and heat treatment at 85℃ for 10 min.[8] The 100-fold diluted NB supplemented with 0.008 % bromothymol blue, 0.2 % agar, and 1.0 % glucose was used for oxidation-fermentation test. Culture for nitrate reduction test was done using NB／100 supplemented with 0.1 % $NaNO_3$. API 20 NEkit (MERIEUX S. A.) was also used for the other biochemical properties of the isolate. Cells were observed with a transmission electron microscope (Hitachi, H-7100).

Assay methods. The degree of bacterial growth was estimated by measuring the optical density of the cultures at 600 nm with light length of 5 cm or 1 cm. The amount of reducing sugars in the cultures was estimated by the method of Nelson-Somogyi.[9]

Chemicals. D-Psicose and D-tagatose were synthesized from galactitol by microbial transformation reaction.[10,11] Other sugars were purchased from Sigma Chemical Company. Agar noble and yeast extract were purchased from Difco Laboratories. All other chemicals were obtained from Wako Pure Chemical Industry, Japan and were reagent grade.

Results

Characteristics of the isolate, V-16.

Differencial characteristics of the isolate, V-16, are shown in Table 1. The organism was a gram positive oligotrophic bacterium. The three oligotrophic bacteria characterized previously in our laboratory were gram negative and were identified to genera *Aeromonas* and *Chromobacterium*.[12] The V-16 cells grown in NB／100 were rods possessing a single polar flagella (Fig. 1). Chemical taxonomical properties of V-16 were not examined. Therefore, the isolate was not identified.

Utilization of natural and rare sugars.

The isolate, V-16, was grown in NB／100 supplemented with 0.1 % of each sugar for 10 days. As shown in Table 2, this organism grew well in the media supplemented with every one of fifteen mono-saccharides without regard to rare or natural sugars, but not so well in the media with four di-saccharides. It was worth noting that this organism consumed more than 40 % of the supplemented D-psicose and L-tagatose. The pH value of the culture supplemented with D-psicose after the 10 days cultivation was 6.5, while pH values of those supplemented with the others were 7.1 ～ 7.4.

Table 1. Differencial characteristics of an oligotrophic bacterium V-16.

Cell shape	Rod	Nitrate reduction	+
(0.7－1.2 μm×1.5－2.2 μm)		Methyl red test	－
Motility	+	Glucose oxidation	－
	(polar flagella)	Oxidase	+
Sporulation	－	Catalase	+
Gram stain	+	Urease	+
O-F test	O	β-Galactosidase	+
V-P test	－	β-Glucosidase	+
H_2S production	－	Arginine dehydrogenase	－
Indole production	－	Proteinase	－

+, positive ; －, negative.

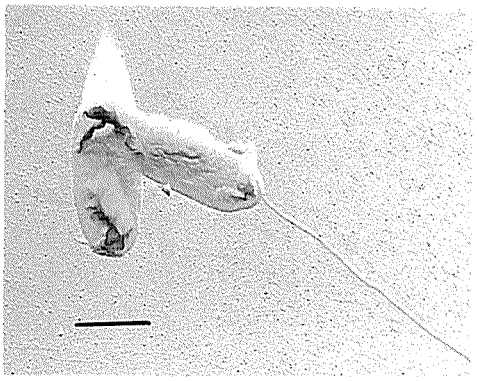

Fig. 1. Electron micrograph of an isolate V-16. Magnification was 6,000. The bar indicates 1 μm.

Table 2. Utilization of rare and natural sugars by V-16 in 100-fold diluted NB.

Sugars	Relative growth (%)	Sugar consumption (%)	pH
None	100	−	7.4
D-Arabinose	210	17	7.2
L-Arabinose	210	26	7.1
D-Ribose	170	10	7.1
D-Xylose	240	29	7.0
D-Lyxose	160	31	7.0
D-Glucose	190	21	7.1
D-Mannose	140	17	7.2
D-Galactose	200	16	7.1
L-Galactose	220	14	7.2
D-Fructose	200	17	7.2
D-Sorbose	190	23	7.2
L-Sorbose	150	10	7.2
D-Psicose	210	44	6.5
D-Tagatose	150	1	7.1
L-Tagatose	200	43	7.1
Maltose	110	18	7.3
Sucrose	110	−	7.4
Lactose	100	21	7.2
Melibiose	120	6	7.4

The sugars itaricized are rare sugars.

Growth stimulation by D-psicose and L-tagatose.

Figure 2 shows the growth curves of V-16 in NB／100 supplemented with 0.1% of D-psicose and L-tagatose. The growth was stimulated by the supplement of these rare koto-hexoses, and the peaks of growth were observed after 15～18 days. Only when D-psicose was supplemented, pH value of the culture fluid was significantly depressed at latter term of the cultivation.

Growth inhibition by D-psicose in higher concentrations of basal media.

The isolate, V-16, grew well still in 2.5-fold diluted of NB medium. Then the effect of the supplement of 0.1% D-psicose on the growth of this organism was examined when concentrations of the basal medium were higher than NB／100. In addition, the effect of the D-psicose was also examined using the synthetic media as basal in which the concentrations of amino acids, vitamins, and nucleic acids were higher than those

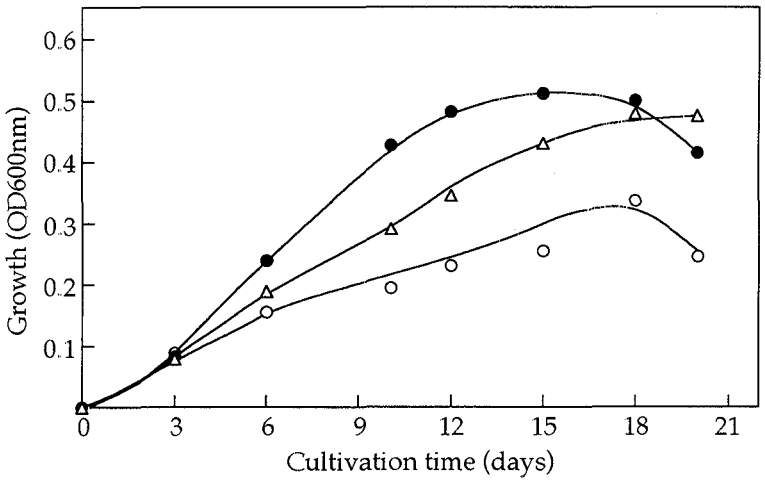

Fig. 2. Growth of V-16 in 1/100 nutrient broth (NB) supplemented with D-psicose and L-tagatose.
The organism, V-16, was grown in 1/100 NB supplemented with 0.1% of D-psicose (●), L-tagatose (△) and none (○).

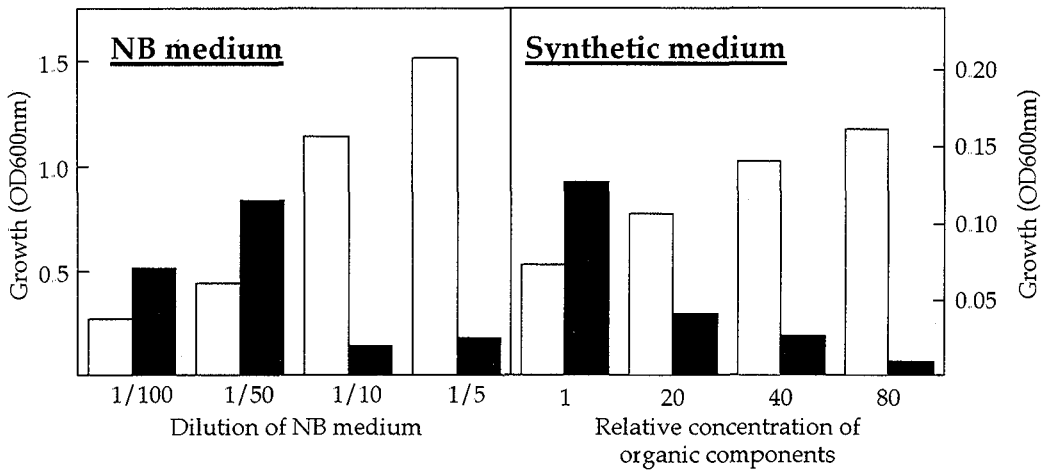

Fig. 3. Effect of D-psicose on growth of V-16 in various concentrations of basal media.
The organism, V-16, was grown in various concentrations of NB medium and the synthetic medium supplemented with 0.1% of D-psicose (■) or without it (□) for 10 days.

of the standard medium. As shown in Fig. 3, D-psicose stimulated the growth in NB／50, NB／100 and in the standard synthetic medium, but significantly inhibited that in 20～80-fold higher concentrations of these organic nutrient mixtures in the synthetic medium as well as in NB／10 and NB／5.

Recovery from the growth inhibition by D-psicose.

When the isolate was incubated in NB／10 supplemented with 0.1% D-psicose, the growth was inhibited during the earlyer cultivation and began after about 10 days, and after 25 days the relative growth was 160% to that in non-supplemented NB／10 (Fig. 4). The cells of 11 days age in the first culture sup-

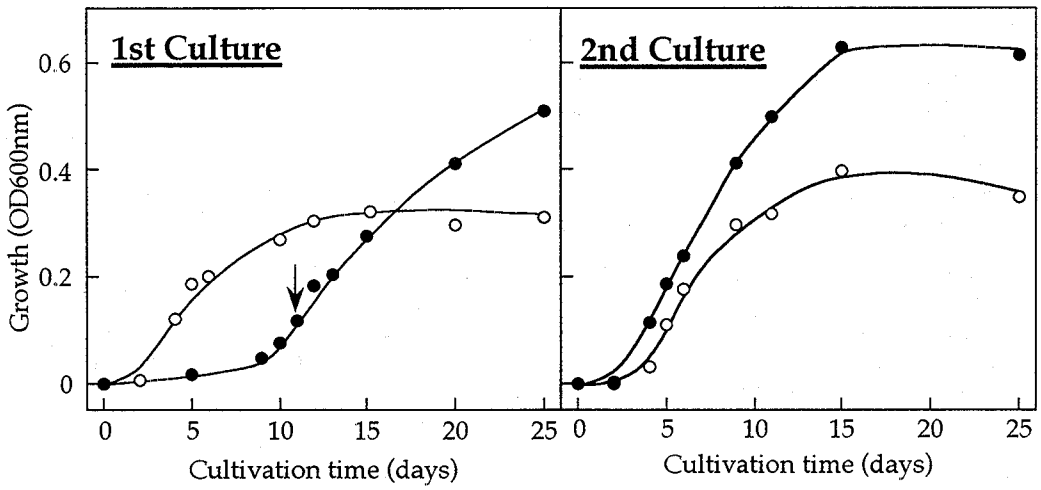

Fig. 4. Growth inhibition of V-16 by D-psicose and recovery from it.
The organism, V-16, was grown in 1/10 NB supplemented with 0.1% of D-psicose (●) or without it (○). The cells (→) of 11 days age in the 1st D-psicose culture were inoculated into the fresh media for the 2nd culture.

plemented with D-psicose were inoculated in the fresh media for the second cultures. In the second cultures, the cells grew more in the medium supplemented with D-psicose than in the non-supplemented medium (Fig. 4).

Utilization of D-psicose by resting cells reaction

In order to obtain efficiently much cells utilizing D-psicose with as small amount of it as possible, we tested utilization of D-psicose by resting cells reaction. But under the reaction conditions tested the resting cells of V-16 consumed only a few percent of initial amount of D-psicose.

Discussion

The isolate, V-16, is an interesting bacteria with regard to utilization of rare keto-hexoses such as D-psicose, L-tagatose and D-sorbose (Table 2), because these rare sugars have never been reported to be utilized for bacterial growth. In rat, D-psicose metabolism was reported.[13] In order to study the metabolic enzymes of these rare sugars in bacteria, it is preferable that cells capable of utilizing such sugars are prepared enough in mass. Then we tried to obtain much growth of V-16 in higher concentrations of the basal medium. However, the growth of V-16 was remarkably inhibited by the supplement with D-psicose to the nutrient rich basal media (Fig. 3). And after the about ten days cultivation the growth of V-16 recovered from the inhibition by D-psicose (Fig. 4). The mechanism of the growth inhibition by D-psicose and the recovery from it will be future subjects.

We wish to investigate the bacterial metabolic enzymes of D-psicose, L-tagatose and D-sorbose. However, the oligotrophic isolate, V-16, is not useful for a bacterial source of these enzymes, because it is difficult to obtain abundantly cells to induce these enzymes with as small amount of the substrates as possible.

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（received May 31, 1995）

低栄養細菌V－16の生育におよぼす希少糖Dープシコースの影響

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土壌から分離された低栄養細菌V－16は1／100NB培地中では，希少糖であるDープシコース，Lータガトースを0.1％添加することにより生育が促進された。またこの時これらの糖は炭素源として40％以上消費された。従来これらの希少糖が細菌によって利用されるという報告はなかった。しかし本菌は基本培地として，より高濃度のNB培地あるいはアミノ酸，核酸塩基やビタミンなど有機成分の濃度を高くした合成培地を使った時，逆にDープシコース添加によって生育が著しく阻害された。この生育阻害は培養前期に起こり，10日目を過ぎる頃生育が回復した。ただし，Dープシコース代謝酵素を誘導できる細胞を大量に得ることが困難であったことから，本菌はDープシコース代謝の研究材料としては不都合であった。