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Peptostreptococcus productus Strain That Grows Rapidly with CO as the Energy Source

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Anaerobic bacteria were enriched with a sewage digestor sludge inoculum and a mineral medium supplemented with B-vitamins and 0.05% yeast extract and with a 50% CO–30% N_2–20% CO_2 (2 atm [202 kPa]) gas phase. Microscopic observation revealed an abundance of gram-positive cocci, 1.0 by 1.4 μm, which occurred in pairs or chains. The coccus, strain U-1, was isolated by using roll tubes with CO as the energy source. Based on morphology, sugars fermented, fermentation products from glucose (H_2, acetate, lactate, and succinate), and other features, strain U-1 was identified as *Peptostreptococcus productus* IIb (similar to the type strain). The doubling time with up to 50% CO was 1.5 h; acetate and CO_2 were the major products. In addition, no significant change in the doubling time was observed with 90% CO. Some stock strains were also able to use CO, although not as well. Strain U-1 produced acetate during growth with H_2-CO_2. Other C_1 compounds did not support growth. Most probable numbers of CO utilizers morphologically identical with strain U-1 were 7.5×10^6 and 1.1×10^5 cells per g for anaerobic digestor sludge and human feces, respectively.

Biological acetogenesis from CO is an interesting phenomenon, with application to studying the biochemistry of anaerobic lithotrophy (15, 28) and the conversion of coal or biomass pyrolysis products, such as H_2 and CO, into useful chemicals (33).

The adventitious oxidation of CO to CO_2 is performed by several anaerobic bacteria, most notably members of the genus *Clostridium* (9, 11, 18, 24, 26). *Methanobacterium thermoautotrophicum* (7, 27) and *Rhodopseudomonas gelatinosa* (8, 29) are able to use CO as an energy source, producing CO_2 and methane or H_2, respectively. It has recently been demonstrated that *Eubacterium limosum* (12), *Acetobacterium woodii* (B. R. S. Genthner, unpublished data), "*Butyribacterium methylotrophicum*" (20), and *Clostridium thermoaceticum* (17) use CO as an energy source, producing CO_2 and acetate:

$$4CO + 4H_2O \rightarrow 2HCO_3^- + CH_3COO^- + 3H^+$$

with a $\Delta G^{0\prime} = -165.41$ kJ per reaction.

The mesophilic CO-utilizing acetogenic bacteria so far studied, which are able to grow relatively well with moderate or high levels of CO (doubling time [T_d] of 7 to 13 h), were isolated with other substrates and later shown to use CO. This paper documents the isolation of a mesophilic, CO-utilizing, acetogenic strain of *Peptostreptococcus productus* which grows at 37°C with a T_d of less than 2 h with as much as 90% CO (2 atm [202 kPa]) as the energy source.

MATERIALS AND METHODS

Source of organisms. *P. productus* B43 (C1 group from calf rumen [6]), 2 (Virginia Polytechnic Institute strain from human feces), SF-50 (rumen [32]), and 1-1 (human feces [30]) were from our culture collection. Strain U-1, discussed in this paper, was isolated from anaerobic sewage digestor sludge.

Media and conditions of cultivation. The anaerobic techniques for the preparation and use of media were essentially those of Hungate (16) as modified (3, 5).

The basal medium was that of Genthner et al. (13), except rumen fluid was replaced by yeast extract (0.05% for enrichment cultures and 0.2% for pure cultures) and the atmosphere of the tubes was 60% N_2–40% CO_2. This medium also contained B-vitamins, minerals, $NaHCO_3$, and Na_2S. Media for growth with CO or H_2-CO_2 were pipetted in 5-ml amounts into serum-stoppered tubes (18 by 150 mm), which were pressurized to 2 atm after inoculation by the method of Balch and Wolfe (3) as modified (13). Media for soluble substrates were pipetted in 3-ml amounts into culture tubes (13 by 100 mm) with an 80% N_2–20% CO_2 gas phase (101 kPa). CO was added to inoculated media with a sterile syringe. Final pressures were 2 atm, and with the exception of the 90% CO mixture, atmospheres were composed of 20% CO_2, the desired concentration of CO, and the balance, N_2. For an atmosphere of 90% CO–10% CO_2, tubes were flushed with CO_2 and partially evacuated before the addition of CO to a final pressure of 2 atm. The atmospheres of control cultures without a gaseous energy source for experiments with a gaseous energy source were exchanged, using a gas manifold (3), to final compositions of 80% N_2–20% CO_2 (2 atm). Gas phases with 50% H_2 or less (2 atm) were provided with a sterile syringe as described above for CO introduction, with H_2 instead of CO. The gas phase of 80% H_2–20% CO_2 (2 atm) was provided by gas exchange as described above for controls. Media for roll tubes (18 by 150 mm; 7 ml in each tube) contained 2% agar. All incubations were performed in the dark at 37°C, and liquid cultures with gaseous energy sources were incubated on a reciprocating shaker.

Energy sources. The utilization of nongaseous energy sources was determined by the growth of duplicate cultures in the basal medium with and without 0.1 ml of stock solution of energy sources. Stocks of energy sources were prepared as 5% (wt/vol) solutions, except for methanol and formate, which were prepared as 3% solutions. Stock solutions of soluble compounds were filter sterilized (0.22 μm), and others were autoclaved for 15 min at 121°C.

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Analytical methods. Growth was determined by measurement of optical density (OD) at 600 nm with a Bausch & Lomb Spectronic 70 spectrophotometer. Gases were analyzed by gas chromatography and manometric pressure determinations as described previously (3, 22). Fermentation acids were determined by gas chromatography of free acids or their butyl esters by methods described previously (12, 25). Fermentation balances were calculated by the method of Barker (4). Cell protein determinations were performed by the method of Lowry et al. (19) after the pelleted cell material was treated with 0.1 M NaOH at 70°C for 15 min.

Most probable number of CO-utilizing bacteria. One-milliliter subsamples of anaerobic sewage digestor sludge and 1 g of fresh human feces were each diluted in 10-fold serial dilutions in basal medium. One-milliliter aliquots of dilutions 10^{-8} to 10^{-4} were used to inoculate triplicate tubes of the 0.2% yeast extract medium with and without 50% CO. The most probable number (1) was calculated from the number of tubes showing complete CO utilization within 7 weeks of incubation.

RESULTS

Isolation. Anaerobic sewage digestor sludge from the municipal waste treatment plant in Urbana, Ill., was used to initiate CO enrichment cultures. The inoculum consisted of 0.5 ml of undiluted sludge and was added to 9.5 ml of medium with 50% CO (2 atm). Initially, the culture required 5 days to deplete the CO present. Subsequent 17% transfers (1 ml of inoculum plus 5 ml of medium) resulted in a T_d of about 6 h. Observation of the culture by phase-contrast microscopy revealed high numbers of a lancet-shaped coccus, often in long chains, along with low numbers of other morphotypes. Roll tubes (0.05% yeast extract) were inoculated, and after only 4 days of incubation, large (2- to 3-mm), convex, iridescent, white colonies with entire edges were present in those containing a highly dilute inoculum (10^{-6}) and 50% CO. These colonies were not present or were less than 0.5 mm in diameter in tubes with N_2 instead of CO. Colonies were transferred to broth with a 50% CO gas phase.

Microscopic examination revealed gram-positive, nonmotile, lancet-shaped cocci (ca. 1.0 by 1.4 μm) that occurred in pairs and chains. Five cultures were maintained, one of which, U-1 (ATCC 35244), was used in further investigations.

Growth with CO as the energy source. Strain U-1 grew well in the 0.2% yeast extract medium with CO as the sole added energy source. T_d decreased as the culture was maintained with 50% CO for several months until it reached ca. 1.5 h. Growth with CO showed a decrease in the CO concentration concomitant with an increase in OD (Fig. 1). In cultures grown with 10 to 50% CO, T_ds were similar, and the maximum growth was proportional to the amount of CO utilized in cultures grown with 40% CO or less. Growth with 90% CO in the 0.2% yeast extract medium was not as consistent; incomplete utilization of CO often occurred. An increase in the concentration of yeast extract to 0.4% allowed complete utilization of CO and a T_d similar to that of cultures grown with 50% CO. Preliminary nutritional investigations indicated that yeast extract was required for growth at all levels of CO examined.

The major products from CO were acetate and CO_2, with ca. 1 mol of acetate recovered for every 4 mol of CO consumed (equation given above; Table 1). Other acids were not produced in significant amounts. The cell protein yield, calculated as the mean of the yields of duplicate cultures grown with 30, 40, and 50% CO and corrected for controls grown without CO, was 1.28 ± 0.09 g/mol of CO consumed.

After several trials with either 50 or 25% CO, strains 1-1 and SF-50 could not use CO; however, strains B43 and 2 grew poorly. Less than half of the original 50% CO was utilized by either strain, and a decrease in the initial concentration of CO to 25% did not result in better growth. The T_d for both cultures was ca. 3 h, and maximum ODs were 0.5 for strain 2 and 0.35 for strain B43.

Adaptation of glucose-grown cells to CO. Results indicated that little selection for cells unable to utilize 50% CO as an energy source occurred during a long-term culture of strain U-1 with 0.1% glucose as the main energy source. The strain was grown with daily transfers of 0.05 ml for 10 weeks in the glucose medium, and then after about two-thirds of maximum growth (OD, 0.91), colony counts in quadruplicate roll tubes containing CO or glucose indicated 3.6×10^8 CO-utilizing colonies per ml and 3.8×10^8 glucose-utilizing colonies per ml; whereas a control culture (OD, 0.84) that had been routinely transferred in 50% CO medium indicated 6.2 and 6.4×10^8 colonies per ml, respectively. The glucose-grown culture had somewhat lower numbers of CFU with either CO or glucose as compared with the CO-grown culture.

Other features of U-1. In addition to CO, growth of strain U-1 occurred with the following compounds: adonitol, arabinose, cellobiose, dextrin, esculin, fructose, galactose, glucose, glycerol, inositol, lactose, maltose, mannitol, melezitose, melibiose, peptone, raffinose, rhamnose, ribose, salicin, sucrose, trehalose, and xylose. Compounds tested which did not support growth were acetate, Casamino Acids, dulcitol, formate, glycogen, lactate, methanol, pectin, pyruvate, starch, succinate, valine, and xylan.

In addition to those compounds listed above, growth of strain U-1 occurred with 80% H_2–20% CO_2. The T_d decreased during continued growth with H_2-CO_2 and was 5 h after many serial transfers. Acetate was the main end product and was recovered in an approximate ratio of 1 mol/4 mol of H_2 consumed (Table 1). The cell protein yield, calculated from duplicate cultures grown with 30, 50, and 70% H_2, was 0.39 ± 0.05 g/mol of H_2 consumed.

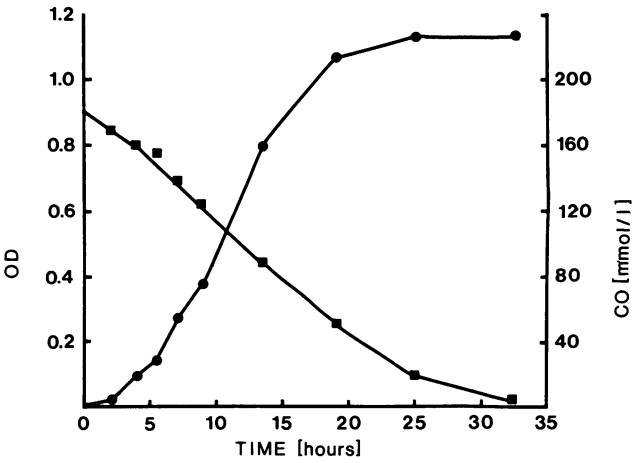

FIG. 1. Time course analysis of strain U-1 in 0.2% yeast extract medium with 50% CO (2 atm) as the energy source. Each point is the mean of triplicate cultures corrected for controls without CO or uninoculated CO medium. Symbols: ●, OD; ■, CO.

TABLE 1. Fermentation balances of strain U-1 in 0.2% yeast extract medium with CO or H_2-CO_2 as the energy source

Energy source (%)	mmol/liter[a]			% Recovery of:	
	Substrate utilized	Fermentation product		Carbon	H
		Acetate	CO_2		
CO (50)	165	35.1	77	89	85
CO (40)	130	31.9	57	93	98
CO (30)	103	26.5	44	94	103
H_2 (70)	222	54.2	ND[b]	ND	98
H_2 (50)	158	37.0	ND	ND	94
H_2 (30)	100	24.5	ND	ND	98

[a] Values shown represent the mean of triplicate (CO) or duplicate (H_2) cultures which have been corrected for controls grown without CO or H_2.

[b] ND, Not determined.

Products from glucose fermentation were H_2, acetate, lactate, and succinate. Growth with CO, glucose, or sucrose did not occur in the presence of oxygen.

Numbers of CO-utilizing bacteria. Most probable numbers for CO-utilizing bacteria were 7.5×10^6 cells per ml and 1.1×10^5 cells per g for anaerobic sewage digestor sludge and human feces, respectively. Microscopic observation of the positive cultures from higher dilutions revealed that the predominant bacteria were morphologically identical to strain U-1.

DISCUSSION

The results describe a mesophilic, anaerobic coccus, strain U-1, which was isolated from anaerobic sewage digestor sludge with CO as the energy source. Based on its morphology, range of energy sources, fermentation products from glucose, and other features, strain U-1 was identified as *P. productus* IIb (similar to the type strain) (14, 23, 32; L. V. Holdeman, *in* P. Sneath, ed., *Bergey's Manual of Systematic Bacteriology*, 9th ed., vol. 2., in press). Before the present work, growth of *P. productus* with C_1 compounds as the main energy source was unknown.

The production of acetate and CO_2 during growth with CO had a stoichiometry (see equation above) similar to that reported for other acetogenic CO utilizers (11, 20).

The T_d of 1.5 h for strain U-1 is much shorter than those previously reported for other species of acetogenic CO utilizers. Strain U-1 was also able to grow rapidly with much higher levels of CO (90%), although this required an increase in the concentration of yeast extract in the medium. As with CO, utilization of H_2-CO_2 by strain U-1 followed a stoichiometry similar to those reported for other acetogens, but with a somewhat shorter T_d (2, 13, 21).

The cell protein yield from growth with CO was significantly greater than that from growth with H_2. This is probably related to the greater change in free energy associated with CO metabolism and is consistent with reports of similar studies with *E. limosum* (Genthner, unpublished data) and "*B. methylotrophicum*" (21).

P. productus is one of the most numerous gram-positive bacterial species in the human bowel, so its presence in anaerobic sewage digestor sludge was not considered unusual. The ability of two other strains, B43 and 2, isolated and routinely maintained for many years in media containing sugars as the energy source, to grow with CO as the energy source suggested that this phenomenon might be widespread.

Our most-probable-number studies indicated that, although bacteria tentatively identified as CO-utilizing *P. productus* occur at significant levels in the human bowel (1.1×10^5 cells per g of feces) and in sewage (7.5×10^6 cells per ml), they are probably only a small portion of the human fecal *P. productus* population (ca. 10^{10} cells per g of feces) (23, 30). However, based on microscopic observations of cultures growing with CO, they appear to be the most numerous species utilizing CO. Although growth with CO as the energy source may occur in natural ecosystems, the relative importance of this ability is probably small. Growth with H_2-CO_2 might be more ecologically significant.

This report documents the first acetogenic anaerobic coccus able to utilize H_2-CO_2 or CO as the energy source at mesophilic temperatures. This ability strongly suggests that *P. productus* belongs in the "clostridium group" of Tanner et al. (28) and is likely to have the nickel-containing CO dehydrogenase as well as other enzymes of the pathway to acetate as seen in *Clostridium thermoaceticum* (31). Other anaerobic cocci in the clostridium group include *Peptostreptococcus micros* (*Peptococcus* [*Diplococcus*] *glycinophilus*), *Peptostreptococcus asaccharolyticus* (*Peptococcus aerogenes*), and *Ruminococcus bromii* (10, 28).

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