Peptostreptococcus elsdenii from the Caecum of Pigs

By D. GIESECKE, SOLVEIG WIESMAYR AND M. LEDINEK*

Institut für Tierphysiologie, Universität München, München, Germany

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Large, Gram-negative, anaerobic cocci (LC) fermenting lactate were first isolated by enrichment culture from the sheep rumen (Elsden, Volcani, Gilchrist & Lewis, 1956). High counts (about 10^9 /ml.) were obtained from young calves but not from adult ruminants by Hobson, Mann & Oxford (1958). The cocci were cultured in numbers of 10^6 to 10^3 /ml. from the rumen of cattle fed high-grain rations and named *Peptostreptococcus elsdenii* by Gutierrez, Davis, Lindahl & Warwick (1959). They can be important fermenters of lactate in the rumen and are associated with amylolytic lactate producers such as *Streptococcus bovis* when large amounts of starch are consumed by ruminants. The production of propionic acid by *P. elsdenii* via the acrylate pathway (Ladd, 1959) has attracted the particular interest of biochemists and nutritionists. The types of bacteria active in the rumen are not found only in this organ (Giesecke, 1970), and recent experiments indicate that *P. elsdenii* is also a prominent lactate fermenter in the large intestine of pigs fed on diets containing potatoes.

METHODS

Media. Reinforced clostridial agar (RCM, Oxoid Ltd) in roll-tube cultures with a gas phase of 5 % (v/v) CO_2 + 95 % (v/v) N_2 was used for initial isolation, and a semi-solid lactate medium similar to that of Hobson *et al.* (1958), but incubated under pure N_2 , was used for subculturing, preserving and testing isolates.

Isolation. Samples of caecum contents were obtained from six pigs with caecum cannulae, three of which were fed on raw potatoes and three on steamed potatoes (Ledinek, 1970). Amounts of 10 g. were vigorously shaken with 90 ml. of anaerobic mineral buffer solution (Bryant & Burkey, 1953), and diluted through 10^{-11} before inoculation into RCM.

Biochemical reactions and pH. Biochemical tests were performed according to Hobson *et al.* (1958) with 1 % (w/v) of substrates replacing lactate in the semisolid medium. For growth at different pH values the lactate medium was buffered with tris-maleate (Geigy, 1968). All pH values were measured with a glass electrode, and fermentation acids determined by gas chromatography (Ledinek, 1970).

RESULTS

After 2 to 3 days of incubation in RCM tubes the large Gram-negative cocci formed greenish yellow, circular colonies 1 to 2 mm. in diameter, in numbers corresponding to viable counts of 10^9 to 10^{10} /g. caecum contents. The properties of 13 isolates are listed in Table 1. Viable bacteria observed after incubation (for 24 h.) in semisolid medium

* Present address: Ziuinorejsko Veterinarski Zavod Ptuj, Yugoslavia.

Table 1. *Properties of Peptostreptococcus elsdenii from the caecum of pigs*

	Isolates from pigs fed raw potatoes								Isolates from pigs fed steamed potatoes					Mean final pH†
	R11	R12	R13	R14	R21	R24	R25	R26	R31	G11	G12	G13	G32	
Dilution per ml. at isolation	10 ⁻⁹	10 ⁻⁹	10 ⁻⁸	10 ⁻⁹	10 ⁻⁹	10 ⁻⁸	10 ⁻⁸	10 ⁻⁸	10 ⁻⁹	10 ⁻⁸	10 ⁻⁸	10 ⁻⁸	10 ⁻⁸	
Cell dimensions (μm.)...	1.9 × 1.1		1.8 × 1.4		2.0 × 1.4		2.0 × 1.5		2.0 × 1.5		1.8 × 1.3		1.9 × 1.2	
Fermentation reactions*														
Xylose	+	+	+	+	+	-	-	+	+	+	+	+	-	5.7
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	5.9
Fructose	+	+	+	+	+	+	+	+	+	+	+	+	+	4.8
Maltose	-	+	-	+	-	+	+	+	+	-	+	-	+	5.7
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	5.0
Raffinose	+	+	+	+	+	+	+	+	+	+	+	+	+	5.3
Mannitol	+	+	+	+	+	+	+	+	+	+	+	+	+	5.5
DL-Lactate	+	+	+	+	+	+	+	+	+	+	+	+	+	7.0

Mean final pH†

5.7
5.9
4.8
5.7
5.0
5.3
5.5
7.0

All isolates were Gram-negative cells arranged in diplococci or short chains; all isolates produced H₂S. Gelatine liquefaction, indole production and nitrate reduction were always negative.

* Arabinose, rhamnose, galactose, mannose, trehalose, cellobiose, lactose, starch, inulin, salicin, aesculin, sorbitol, dulcitol, inositol, glycerol, and succinate were not fermented.

† Initial pH 6.7 to 6.8.

showed pronounced flattening of adjacent cell walls and occurred in pairs and short chains of up to 10 cells. In older cultures bacteria of variable size were observed, sometimes arranged in long chains of more than 50 units.

In addition to lactate a limited number of substrates were fermented with the production of gas, which was not analysed. Preliminary observations on five isolates indicated that acetic, propionic, butyric and valeric acids were the fermentation acids from lactate, and caproic acid was formed in addition from glucose. Of seven strains tested at different pH values, all grew at pH 5.2 to 7.2, three at pH 7.8, but none at pH 5.0 or below or pH 8.1 or above. Sodium citrate, which was first tried as a buffer, inhibited growth even at concentrations of 0.01 M. Of five isolates tested, all grew at 37° and 42°, two at 45°, and three at 30°, but no growth was observed at 25° or 50°.

On the basis of these characters it is concluded that the strains of large Gram-negative cocci from the caecum of pigs belong to the species *Peptostreptococcus elsdenii*.

DISCUSSION

Dimensions, Gram-negative staining, anaerobiosis, utilization of lactate, production of H₂S and of fatty acids with 2 to 6 carbon atoms are distinctive features of *Peptostreptococcus elsdenii* (Elsden *et al.* 1956; Gutierrez *et al.* 1959). The isolates from pigs are similar to rumen strains, though some differences appear to exist. The size is smaller than that given for isolates from the rumen ($2.4 \times 2.6 \mu\text{m}$.), but the ages of the latter cultures were not reported. The pig strains differed from those of Elsden *et al.* (1956) and Hobson *et al.* (1958) in fermenting sucrose and raffinose but not sorbitol, and in variable fermentation of maltose and xylose. The strains of Gutierrez *et al.* (1959) failed to attack sorbitol and mannitol but some fermented sucrose and glycerol. *Peptostreptococcus elsdenii* from calves produced growth at a broader range of pH (4.8 to 8.1) than pig strains and seemed less sensitive to citrate (Hobson *et al.* 1958).

Gram-negative, anaerobic cocci resembling *Peptostreptococcus elsdenii* were cultured only occasionally and in very low counts from the gut of young pigs by Fuller & Lev (1964). In the present experiments the pigs were reared, housed, and cared for separately and had thus no contact with ruminants. Microscopic observations showed that Gram-negative cocci accounted for about 25 % of the total bacteria in their caecum contents, and large Gram-negative cocci were 17.4 % of the total anaerobic bacteria (10^{10} to 10^{11} /g. caecum contents) cultured on RCM by Ledinek (1970). On feeding potatoes, considerable amounts of carbohydrate escape pancreatic digestion and are fermented in the large intestine where lactic acid appears to be an important intermediate. It is thus obvious that *P. elsdenii* may occupy an ecological niche in digestive tract habitats other than the rumen.

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