HYDROGEN SULPHIDE-POSITIVE STRAINS OF *ESCHERICHIA COLI* FROM SWINE

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SINCE the introduction into use of Hektoen Enteric Agar in this laboratory a few years ago, hydrogen sulphide (H₂S)-producing variants of *Escherichia coli* have been isolated from approximately 1% of human faecal specimens sent for routine culture (unpublished data). Because it is probable that an important reservoir for many bacteria that may colonise the human gut is afforded by the intestines of swine, a trial was devised to determine the occurrence of H₂S-positive strains of *E. coli* in these animals. Many were found, and this paper describes their properties and those of their plasmids.

MATERIALS AND METHODS

Bacteria, plasmids and phages. The 32 wild-type H₂S-positive strains of *E. coli* included in this study were recovered from the caecal contents or mesenteric lymph nodes or both of 60 healthy pigs slaughtered at Recife abattoirs. The Hys plasmids were transferred into *E. coli* 20R764 Fi⁻ rif (K12). In plasmid interaction studies *E. coli* 27R207 HfrH nal and *E. coli* 14R525 F⁻ nal, respectively, were used to test for the Fi character and the physical independence of plasmids. Reference plasmids of some Fi⁺ incompatibility groups (R386, R538*drd1*, R124, and H1y212) were transferred into *E. coli* J53. Donor-specific and female-specific phages used were R17 and ϕ 2.

Isolation and characterisation of cultures. Specimens were homogenised in saline and directly streaked on Hektoen Enteric Agar (HEA; Difco Laboratories). The plates were incubated at 37°C for 24 h and any pale yellow or black colonies were screened by culturing on Triple Sugar-Iron Agar (TSI; Difco) and Lysine-Iron Agar (LIA; Merck). Confirmatory identification was made by standard biochemical procedures (Edwards and Ewing, 1972). Determination of the O and H antigens was carried out by Ida Ørskov at the WHO Collaborative Centre for Reference and Research on Escherichia, Statens Seruminstitut, Copenhagen, Denmark. Colicinogeny was studied by the agar-overlay method (Fredericq, 1957), and haemolysin production by culturing on sheep blood agar. The ability to produce heat-labile enterotoxin was tested on Y1 adrenal cells in tissue miniculture (Sack and Sack, 1975). Drug sensitivity testing was carried out by the single disk technique (Bauer *et al.*, 1966) with the following drugs: ampicillin (10 µg), chloramphenicol (30 µg), streptomycin (10 µg), tetracycline (30 µg), sulphamethoxazole (300 µg), nalidixic acid (30 µg), and gentamicin (10 µg).

Matings and plasmid interactions. These were carried out as described by Smith *et al.* (1973). As Hys may sometimes be transferred linked to Tc (Stolteru *et al.*, 1972), or associated with Raf (Ørskov and Ørskov, 1973), cultures that fermented raffinose or were tetracycline-resistant were selected for transfer study.

RESULTS

Frequency of strains

Of 60 pigs examined, 28 (46.6%) yielded H₂S-positive strains of *E. coli*. Usually, a small number of suspect colonies were observed on HEA plates. Fourteen isolates were obtained from caecal contents only, seven from lymph nodes only, and 14 from both sources. In four animals strains of different biotype and serotype were isolated from the caecal contents and lymph nodes. Thus, in all, 32 strains were included in the study.

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TABLE
Fermentation types and serotypes of H₂S-positive strains of E. coli

Strains	Adonitol	Dulcitol	Raffinose	Rhamnose	Sucrose	Salicin	Sorbitol	Serotypes	
								O	H
PE1643	—	+	+	+	+	—	+	8	21
PE1669	—	—	+	+	+	—	+	8	...
PE1631	—	—	—	+	—	—	+	9	...
PE1647	—	—	—	+	—	—	+	9	...
PE1674	—	+	—	+	—	—	+	9	...
PE1651	+	—	—	+	—	+	—	11	...
PE1644	+	—	+	+	—	+	+	32	19
PE1687	—	+	+	+	+	—	+	37	8
PE1673	—	+	—	+	—	+	+	64	...
PE1672	—	+	—	+	—	—	+	65	11
PE1670	—	—	—	—	—	—	+	68	...
PE1684	—	+	+	+	+	—	+	81	...
PE1657	+	—	—	+	—	—	—	88	11
PE1652	—	+	+	+	+	+	+	112	35
PE1646	+	—	+	+	—	+	+	113	4
PE1685	—	—	+	+	+	+	+	132	52
PE1693	—	+	—	+	—	—	+	155	45
PE1653	+	—	+	+	+	+	+	159	...
PE1688	—	—	+	+	+	+	+	u	16
PE1690	—	—	—	+	+	+	+	u	16
PE1649	—	—	—	+	—	+	+	u	...
PE1656	—	—	—	—	—	—	+	u	...
PE1675	—	+	—	+	—	—	+	u	...
PE1676	—	—	—	+	—	+	+	u	...
PE1677	—	+	—	+	—	—	+	u	...
PE1692	—	—	—	+	—	—	+	...	11
PE1630	—	+	+	+	—	+	+	...	...
PE1632	—	+	—	+	—	—	+	...	...
PE1633	—	+	—	+	—	—	+	...	...
PE1648	—	+	+	+	+	+	+	...	...
PE1682	—	+	+	+	+	+	+	...	...
PE1689	—	+	—	+	—	+	+	...	...

u = Untypable culture.

... = Rough or non-motile culture.

Properties of strains

All strains produced H₂S in LIA, were methyl red and indole positive, and fermented lactose and mannitol promptly. On the other hand, they were uniformly negative in malonate, inositol, cellobiose, Simmons' citrate and KCN media. The results of other tests (H₂S in TSI; gas from glucose, lysine, arginine, ornithine, adonitol, dulcitol, raffinose, rhamnose, sucrose, salicin and sorbitol; motility) were variable. Sugar fermentation permitted the grouping of the strains into 18 different biotypes (table). Serotyping showed that 21 strains belonged to 18 O : H types, five were untypable, and the remainder were rough (table). No strain possessed the K88 antigen, only one was colicinogenic and none produced haemolysin or heat-labile enterotoxin. Fourteen isolates were drug-sensitive, 16 were resistant to tetracycline and two were resistant to tetracycline, streptomycin, and sulphonamide.

Properties of Hys plasmids

Of 13 raffinose- and H₂S-positive strains, four transferred their Hys-Raf plasmids into K12 at the very low efficiency of approximately 10⁻⁶ per donor cell. All Hys-Raf plasmids

were Fi^+ and did not inhibit the phage $\phi 2$. They co-existed stably with a number of Fi^+ plasmids but not with R386 (incompatibility group FI). Of 18 tetracycline-resistant and H_2S -positive strains, two transmitted the plasmid Tc alone while another transferred Tc jointly with Hys. The Tc-Hys plasmid, in contrast to Hys-Raf, is Fi^- .

DISCUSSION

The investigation showed that H_2S -positive strains of *E. coli* are common in swine. This finding is relevant to the question (Ørskov and Ørskov, 1973) of whether these variants occur more frequently in man or in animals.

The discovery of a large number of H_2S -positive isolates in Recife cannot be considered fortuitous. It is attributable to the recognition of the morphological features of the H_2S -positive colonies on HEA. On this medium, H_2S -positive strains of *E. coli*, like some strains of *Citrobacter*, produce pale yellow colonies with or without black centres; H_2S -negative strains produce salmon-coloured colonies. The lactose-positive strains of *Salmonella typhimurium* that are common in São Paulo, Brazil (Pessôa, 1973) are easily distinguished from H_2S -positive *E. coli* because their colonies on HEA are salmon-coloured with small black centres.

The use of LIA for screening purposes was also helpful in detecting H_2S -positive *E. coli* strains. Véron and Gasser (1963) showed that many H_2S -positive and lactose-fermenting enterobacteria fail to blacken TSI because of the acid reaction of the medium.

With regard to other biochemical properties, the behaviour of H_2S -positive porcine strains was typical of *E. coli*, except that a relatively large number of isolates (15.6%) fermented adonitol. However, attempts to transfer the genes responsible for adonitol utilisation into K12 were unsuccessful.

Considered as a group, porcine H_2S -positive *E. coli* strains lack many properties that are encoded by plasmids. As H_2S production is also located on an extrachromosomal genetic element, a possible explanation is that mutual plasmid exclusion plays a role in the natural environment.

The variety of the biotypes and serotypes found shows that H_2S production in *E. coli* is not a characteristic of unique clones within the species. A number of these biotypes and serotypes are frequently found in man, and this, as well as the fact that swine Hys-Raf plasmids belong to incompatibility group FI, as do the human ones (Magalhães and Vêras, 1977), suggests that human and porcine strains of *E. coli* might have a common origin.

SUMMARY

Thirty-two H_2S -positive strains of *Escherichia coli*, isolated from the caecal contents or mesenteric lymph nodes or both of 60 apparently healthy pigs, were characterised. Eighteen different serotypes and 18 different fermentative types were found. During conjugation with *E. coli* K12, four strains transferred their Hys plasmids linked to Raf, and another transferred Hys associated with the Tc determinant. All Hys-Raf plasmids belong to incompatibility group FI, whilst Tc-Hys is an Fi^- plasmid.

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