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HYDROGEN SULPHIDE PRODUCING VARIANTS OF ESCHERICHIA COLI

WIDESPREAD OCCURRENCE IN ANIMALS AND HUMANS WITHIN A CONFINED ENVIRONMENT

By

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SØGAARD, HENRY: *Hydrogen sulphide producing variants of Escherichia coli. Widespread occurrence in animals and humans within a confined environment.* Acta vet. scand. 1975, *16*, 31—38. — Of 178 strains of E. coli isolated on non-selective medium from faeces of pigs and humans living in the same farm 14 produced hydrogen sulphide. The H_2S producing variants all belonged to different clones suggesting that the H_2S character was plasmid-mediated. Attempts to transfer the H_2S character were not successful nor were attempts to motilize the gene by introduction of a transmissible R factor.

H_2S production; Escherichia coli.

Until recent years the inability of Escherichia coli to produce hydrogen sulphide in media routinely used for differentiation of Enterobacteriaceae was considered a key characteristic of that species (*Edwards & Ewing* 1972). In screening for Salmonellae, H_2S production in presumptive tests is an important reaction as a starting point for more detailed examinations. The existence of variants of E. coli producing H_2S, which has recently been reported (*Darland & Davis* 1973, *Lautrop et al.* 1971, *Layne et al.* 1971, *Stoleru et al.* 1972, *Ørskov & Ørskov* 1973) from several countries, is a disturbing factor which should be borne in mind by microbiologists dealing with differentiation of Enterobacteriaceae. This is especially true if the variants at the same time do not ferment lactose. Several of the H_2S producing strains of

E. coli so far described were of animal origin (*Stoleru et al., Ørskov & Ørskov*). In this paper a report is given on the isolation in the same farm of 14 different clones of H_2S producing E. coli, 12 from pigs and 2 from humans in contact with the animals.

MATERIAL AND METHODS

Isolation and identification of strains

The herd of pigs examined consisted of 350 animals. Rectal swabs were taken from 19 sows and 42 piglets (0—14 weeks). Faecal specimens collected from 5 persons on the farm were examined as well. Altogether 178 strains were isolated and identified as E. coli by the following reactions: Facultatively anaerobic in semi-solid agar, reduction of nitrate to nitrite, negative Voges-Proskauer test, indol production, acid and gas production from glucose, lysine decarboxylase positive, no fermentation of malonate and no liquefication of gelatin.

H_2S producing strains

All strains were tested for H_2S production by stab inoculation in tubed ferrous chloride gelatin medium as described by *Kristensen et al.* (1935). The 14 strains were plated on a medium containing thiosulphate and ferric citrate (*Lautrop et al.* 1971). The medium is designated TFPL. On this medium H_2S production of individual colonies can be demonstrated by the formation of a central black precipitate appearing after 24 hrs. aerobic incubation at 37°C.

Biochemical characterization of H_2S producing variants

In order to characterize the strains biochemically more in details they were submitted to the following tests: lactose fermentation on Drigalski agar, urease test, fermentation in liquid media of the following sugars: mannitol, sucrose, sorbitol, rhamnose, trehalose, inositol, arabinose, xylose, adonitol, and dulcitol, growth in KCN, ornithine decarboxylase test, and growth in a fluid mineral medium with ammonium salt and citrate as sole carbon source.

Sensitivity to antibiotics

A disc diffusion technique was applied. The method has been described previously (*Søgaard* 1973).

Genetic transfer experiments

A nalidixic acid resistant mutant of E. coli K12, W 3132, which is a methionine requiring auxotroph strain, was used as prospective recipient in crosses. Antibiotic resistant strains were tested for transmissible R factors. The technique has been described in an earlier report (*Søgaard*). In order to determine whether the ability to produce H_2S was plasmid-mediated, 2 methods were applied. In attempting to demonstrate transfer in direct crosses with W 3132 the procedure described by *Lautrop et al.* (1971) was used. Of the mating cultures 5×0.1 ml of tenfold serial dilutions on TFPL medium were plated, and dilutions yielding 100—200 colonies/0.1 ml were examined for colonies with black precipitates. As control a H_2S producing E. coli strain, D 1148, received from the international Escherichia Centre, Copenhagen, was included in the experiments. This strain transfers H_2S at a frequency of $6—7 \times 10^{-1}$ (*Ørskov & Ørskov* 1973). It was next attempted to mobilize H_2S determinants not transferred in direct crosses by introducing an R factor into the strains (*Anderson & Lewis* 1965). Broth cultures of the H_2S producing strains were centrifuged, and 0.1 ml aliquots of the deposit were streaked on Drigalski agar plates containing 20 µg/ml of streptomycin. Streptomycin-resistant mutants were picked and used as prospective recipients in crosses with a transmissible R factor carrying E. coli strain, resistant to tetracycline ($H_2S + S^r \times R^T$). Selection was done on TFPL plates with streptomycin (20 µg/ml) and tetracycline (10 µg/ml) incorporated. Recombinant strains which had received R^T were then used as donors in secondary crosses with W 3132, Nal^r and selection was done on TFPL medium with nalidixic acid (25 µg/ml) and tetracycline.

Serological typing

The H_2S+ strains were serologically typed at the International Escherichia Centre (Drs I. and F. Ørskov).

RESULTS AND DISCUSSION

The H_2S producing variants were detected by their ability to form a heavy black precipitate in the ferrous chloride gelatin medium. Thirteen strains were positive after overnight incubation, 1 strain became positive after 2 days. All 14 strains produced H_2S from thiosulphate when plated onto TFPL medium.

Table 1. Frequency of isolation of H_2S producing E. coli strains.

Origin	Number of individuals examined	Number of individuals with H_2S+ E. coli	Number of strains examined	% H_2S+ strains
Piglets	42	3 (7 %)	103	3
Sows	19	9 (47 %)	49	18
Human	7	2 (29 %)	26	7
Total	68	14 (21 %)	178	8

The frequency with which H_2S positive E. coli strains could be isolated from animals and humans in the farm was surprisingly high considering the small number of these variants so far described (Table 1). In 12/61 of the animals such strains were detected, and 2/7 humans living on the premises excreted H_2S positive strains as well. It is noteworthy that the frequency was higher among the sows (47 %) than among the piglets (7 %). Eleven strains belonged to different serological types (Table 2). Three strains which could not be typed (rough: H—) differed

Table 2. H_2S producing E. coli strains. Origin, serotypes, and resistance patterns.

Strains	Origin	Serotype	Resistance pattern
S 50 I	sow	025:H30	FS
S 52 II	sow	02:H—	FS
S 53 II	sow	0?:H42	FS
S 54 II	sow	0120:H—	FS
S 55 II	sow	0101:H—	FS
S 59 I	sow	rough:H—	S
S 61 II	sow	051:H20	FS
S 64 III	sow	08:H—	FS
S 66 IV	sow	0113:H—	FS
S 79 III	piglet	rough:H—	SuS
S 92 II	piglet	089:H—	FS
S 101 IV	piglet	08,016:H—	FS
H 3 S	man	015:H—	SuS
H 2 T	man	rough:H—	T

Abbreviations: Su: sulphonamides, S: streptomycin, T: tetracyclines, FS: sensitive to sulphonamides, tetracyclines, streptomycin, chloramphenicol, and ampicillin.

Table 3. Fermentation types of 14 H_2S producing E. coli strains.

Number of strains	Acid produced from				
	lactose	sorbitol	rhamnose	adonitol	dulcitol
1	—	+	+	+	—
2	+	+	+	—	—
4	+	+	+	—	+
1	+	+	—	—	—
1	+	—	+	+	—
2	—	+	+	—	+
2	+	+	+	+	—
1	+	+	—	—	—

with regard to fermentation type (Table 3). The strains thus were all different clones. This fact strongly suggested that dissemination of a transmissible plasmid mediating H_2S production had occurred in this particular environment. Attempts to substantiate this suggestion, however, were not successful. In direct crosses with an E. coli K 12 which was known to be a competent recipient of plasmids none of the 14 strains transferred H_2S production, at least not at a frequency which could be detected by screening 1000—2000 colonies of the recipient strain. In the same experiments, the control strain D 1148 regularly transferred the H_2S marker at a frequency of $6—7 \times 10^{-1}$. *Lautrop et al.* (1971) examined 26 H_2S positive strains isolated from hospital patients. From only 1 of these strains, transfer was demonstrated. *Stoleru et al.* (1972) demonstrated transfer from 6 of 12 strains. *Ørskov & Ørskov* (1973) tested 32 strains of which 11 transferred the H_2S character.

It has been demonstrated that resistance transfer factors can carry out transfer of genetic information other than that of drug resistance (*Anderson & Lewis* 1965). Since direct crosses of the H_2S positive strains were not successful it was attempted whether the H_2S gene could be mobilized by a tetracycline R factor. This factor could be transferred to 3 of the 14 strains (S 50 I, S 66 IV, S 92 II). Transfer of the R factor from these recombinant strains to the final recipient, W 3132, took place from all 3 strains at a frequency of 10^{-2}, but no H_2S+ recombinants were detected among 1000—2000 colonies examined in each experiment.

Darland & Davis (1973) analyzed 144 H_2S positive E. coli isolates biochemically, serologically and with respect to antibiotic

susceptibility and compared the characteristics of this population with those of 144 H_2S negative strains selected at random. They found that the H_2S positive group was distinguishable from the reference group, one interesting difference being the much greater frequency with which multiple resistance was encountered among H_2S positive variants. This fact suggests that resistance transfer factors may be involved. Of the strains described in this report 4 were resistant, but in no case could transmissible R factors be demonstrated. It has been reported that plasmids exist in E. coli which simultaneously transfer genes for H_2S production and tetracycline resistance and that both genes may belong to one single replicon (*Stoleru et al.*).

Layne et al. (1971) on fractionating DNA from a H_2S positive E. coli strain demonstrated a satellite band with a percentage of guanine plus cytosine very close to the value of DNA from Proteus mirabilis and P. vulgaris. *Ørskov & Ørskov* demonstrated linkage between raffinose production and H_2S production in the same plasmid whereas tetracycline resistance co-transferred with H_2S production was mediated by another plasmid.

The widespread occurrence within a limited environment of H_2S producing variants of E. coli indicates that some selective force may have been involved. Any conclusive answers to this question cannot be given. It is interesting, however, to note that the variants were isolated most frequently among sows considering that this study was undertaken 6 months after the time when use of tetracyclines as feed stuff supplement had been prohibited. According to information from the owner such supplemented feeding stuffs had been used at the time when the sows were born. Presuming that plasmids had occurred which mediated at the same time H_2S production and tetracycline resistance, dissemination of the former character might have resulted from selection of tetracycline resistant bowel bacteria. The isolation of H_2S positive E. coli variants in humans living in contact with the animals raises the question whether domestic animals constitute a reservoir of such organisms from which spreading takes place to the human population.

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SAMMENDRAG

Svovlbrintedannende varianter af Echerichia coli. Udbredt forekomst hos dyr og mennesker inden for et afgrænset miljø.

Escherichia coli's manglende evne til at danne svovlbrinte i substrater, der anvendes til rutinemæssig differentiering af Enterobacteriacea, har indtil for nylig været anset for et vigtigt karakteristikum for denne art. Gennem de seneste år er imidlertid isoleret en del stammer, hvoraf mange har været af animal oprindelse, der med sikkerhed har kunnet karakteriseres som E. coli til trods for, at de var svovlbrintedannende. I det foreliggende arbejde gives en beskrivelse af 14 svovlbrintedannende E. coli stammer, der blev fundet blandt 178 stammer isoleret fra dyr og mennesker på den samme landbrugsejendom.

Samtlige 14 stammer kunne på grundlag af biokemiske og serologiske undersøgelser vises at tilhøre forskellige kloner.

Dette faktum, sammenholdt med den store hyppighed, hvormed de optrådte i miljøet, tydede på, at H_2S-genet spredtes blandt E. coli populationen via et plasmid. Overførsel af egenskaben til E. coli K12 W 3132 ved direkte krydsning kunne imidlertid ikke påvises, muligvis som følge af mangel på egnede selektionsmetoder. Det blev endvidere forsøgt at mobilisere genet ved introduktion af en R factor. Heller ikke ved denne procedure kunne antagelsen om plasmidbetinget spredning endeligt verificeres.

Til trods for disse negative resultater forekommer det usandsynligt, at uafhængige kromosomale mutationer skulle optræde med så stor hyppighed som beskrevet.

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