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BACTERIOLOGY

CHARACTERISATION OF INACTIVE *ESCHERICHIA COLI* ISOLATES FROM SUCKLING PIGS WITH DIARRHOEIC SYNDROME

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ABSTRACT. *Five strains of inactive* Escherichia coli *were isolated, and partially characterised, from the intestinal content of suckling piglets with diarrhoeic syndrome originating from 2 large-scale swine breeding units. These strains are immobile, do not produce gas through glucose fermentation, are lysinedecarboxylase-negative, and ferment lactose late (within 5-7 days). They do not have antigens K88 ab, K88 ac and K99, are Sereny's test-negative, do not synthetise the thermostable enterotoxin, although two of them synthesise the thermolabile enterotoxin. Based on this character, the strains of inactive* E. coli *may be involved in the breakout of the diarrhoeic syndrome in suckling piglets.*

KEYWORDS: Inactive *Escherichia coli*; Thermolabile enterotoxin; suckling pigs with diarrhoeic syndrome

INTRODUCTION

Pig colibacillosis is known to be possibly induced by several pathogenic *E. coli* types such as: enterotoxigenic (ETEC), verocytotoxigenic (VTEC), enteropathogenic (EPEC) and septicaemic (SEPEC) *E. coli*. The *E. coli* type mainly associated with the sucklers diarrhoea is ETEC [2].

At the same time, the existence of the atypical strains described under various names such as inactive [4] and *alkalescens-dispar* [5] *E. coli* [Kauffmann, 1996, in 1] is known and accepted. It should be noted that the pathogenic enteroinvasive *E. coli* (EIEC) type comprises atypic strains [5]. Thus, the evaluation of the possibilities to involve the atypical, non-enteroinvasive strains of *E. coli* in the diarrhoeic syndrome triggering in suckling pigs is necessary the more so as there exist scanty literature data on the virulence of this strains type.

Considering the high frequency of the bacterial diarrhoeic syndrome in the suckling piglets in large-scale breeding compounds, along with the isolation - in some cases - of *E. coli* strains that are both atypic (immobile, agasogenic, lysinedecarboxylase-

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negative and that ferment lactose late (*i.e.* within 5-7 days) and non-enteroinvasive, and that fall - in keeping with Bergey's Manual of Determinative Bacteriology, ed.IX, 1994 [4] – into the biogroup of inactive *E. coli*, the characterisation of these strains was thought useful in order to evaluate the possibilities of their involvement in this syndrome onset.

MATERIAL AND METHOD

Material

Five strains were studied of inactive *E. coli* isolated from the intestinal content of suckling piglets with diarrhoeic syndrome originating from two large scale swine breeding farms.

Methods

1. Negative contrast staining electron microscopy (20,000 x).
2. Biochemical tests on the following media: TSI, mobility-indole-urea (MIU), mannite-mobility, MILF, Simmons' gelose, peptone water with 1% lactose. Cultivation on gelose with 5% ram blood for the haemolytical activity.
3. Biochemical characters analysis by API 20-E (Bio, Mérieux).
4. Determination of sensitivity to antibiotics (Sanofi disks).
5. Rapid agglutination test with sera against *E. coli* K88ab, *E. coli* K88ac and *E. coli* K99.
6. The ligated intestinal loop test in suckling pigs for the thermolabile (LT) enterotoxin [Taylor *et al.*, 1958, Taylor *et al.*, 1961, Nielsen, 1963 in l].
7. The test for the thermostable (ST) enterotoxin in suckling mice [Dean *et al.*, 1972 in 3].
8. Sereny's test [Sereny, 1957 in 3] for the evaluation of the *E. coli* strains enteroinvasive ability.
9. The test for the Shiga-like (verotoxin) toxin conducted on Vero cells [Konowalchuck *et al.*, 1977, Strockbine *et al.*, 1986, in 3].

RESULTS AND DISCUSSIONS

1. Electron microscopy evidenced that all the 5 strains of inactive *E. coli* has pili, but not flagella as well (Figure 1).
2. The biochemical characters of these strains, investigated by cultivation on various media, are presented in Table 1 that evidences all the test strains to be agasogenic, lysinedecarboxylase-negative, immobile, and late (5-7 days) lactose-fermenters.

   Additionally, all the strains are non-haemolytic.

3. The results of the biochemical properties investigations by the API 20-E system are presented in Table 2. The system did not ensure - in the case of these strains - the differentiation between the typic and inactive *E. coli* (see the Bio Mérieux identification catalogue). Noteworthily, all the strains have the same biochemical characters.
4. The antibiograms results (Table 3) show the strains resistance to tetracycline, streptomycin, and chloramphenicol.
5. The fives strains of inactive *E. coli* do not possess antigens K88ab, K88ac and K99.

6-9. The results of the four pathogenicity tests presented in Table 4 evidence that 2 strains synthesise the LT enterotoxin, that none of them synthetises either the ST enterotoxin or verotoxin, and that none is enteroinvasive.

Table 1 Inactive *E. coli* biochemical characters investigated on polytropic media, Simmons' gelose, peptone water with 1% lactose

	Inactive *E. coli* 37 III	**Inactive *E. coli* 1318 / 1**	**Inactive *E. coli* 1318 / 2**	**Inactive *E. coli* 1386 / 2**	**Inactive *E. coli* 1386 / 6**
Glucose fermentation	+	+	+	+	+
Glucose fermentation with gas release	-	-	-	-	-
Glucose fermentation					
- 48 hours/TSI	-	-	-	-	-
- 4 days/peptone added water	-	-	-	-	-
- 5-7 days/peptone added water	+	+	+	+	+
Saccharose fermentation (TSI)	-	-	-	-	-
Mannitol fermentation	+	+	+	+	+
H_2S production	-	-	-	-	-
Indole production	+	+	+	+	+
Urea decomposition	-	-	-	-	-
Use of sodium citrate as a unique sourse	-	-	-	-	-
Lysine decarboxylase	-	-	-	-	-
Phenylalaninedesaminase	-	-	-	-	-
Mobility (MIU, MILF, mannite-mobility)	-	-	-	-	-

Table 2 Inactive *E. coli* biochemical characters investigated by the API 20-E system

	Inactive *E. coli* 37 III	**Inactive *E. coli* 1318 / 1**	**Inactive *E. coli* 1318 / 2**	**Inactive *E. coli* 1386 / 2**	**Inactive *E. coli* 1386 / 6**
Beta-galactosidase	+	+	+	+	+
Arginine dehydrolase	-	-	-	-	-
Lysine-decarboxylase	-	-	-	-	-
Ornithine decarboxylase	-	-	-	-	-
Citrate use	-	-	-	-	-
H_2S production	-	-	-	-	-
Urease	-	-	-	-	-
Tryptophan desaminase	-	-	-	-	-
Indole production	+	+	+	+	+
Acetoin production	-	-	-	-	-
Gelatinase	-	-	-	-	-
Glucose fermentation	+	+	+	+	+
Mannitol fermentation	+	+	+	+	+
Inositol fermentation	-	-	-	-	-
Sorbitol fermentation	+	+	+	+	+
Rhamnose fermentation	+	+	+	+	+
Sucrose fermentation	-	-	-	-	-
Melibiose fermentation	+	+	+	+	+
Amigdalin fermentation	-	-	-	-	-
Arabinosis fermentation	+	+	+	+	+
Cytochrome-oxidase	-	-	-	-	-
NO_2^- production	+	+	+	+	+
Mobility	-	-	-	-	-

Table 3 Antibiosensitivity of 3 inactive *E. coli* strains

	Ampicil-lin	**Chloram-phenicol**	**Genta-mycin**	**Kana-mycin**	**Flume-quine**	**Nalidixic acid**	**Strep-tomycin**	**Tetracy-cline**
Inactive *E. coli* 37 III	S	R	S	R	MS	S	R	R
Inactive *E. coli* 1318/1	S	R	S	S	S	S	R	R
Inactive *E. coli* 1318/2	S	R	S	S	S	S	R	R

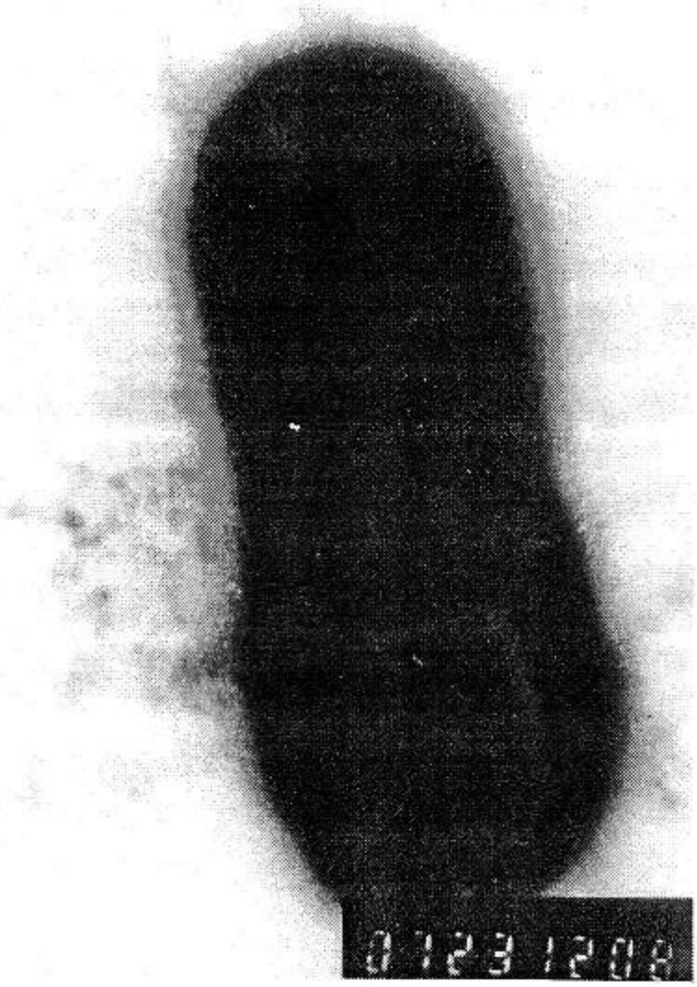
S = Sensitive R = Resistent MS = Moderately sensitive

Table 4 Results of pathogenicity tests on inactive *E. coli* strains

	Inactive *E. coli* 37 III	**Inactive *E. coli* 1318 / 1**	**Inactive *E. coli* 1318 / 2**	**Inactive *E. coli* 1386 / 2**	**Inactive *E. coli* 1386 / 6**
Thermolabile enterotoxine (LT)	+	-	+	-	-
Thermostable enterotoxin (ST)	-	-	-	-	-
Sereny's test (enteroinvasiveness)	-	-	-	-	-
Shiga-like toxin (verotoxin)	-	-	-	NI	NI

NI = Not investigated

Figure 1 Inactive *E. coli* strain 1386/2 possesses pili, not flagella as well (TEM, negative contrast, 30,000 x).



CONCLUSIONS

Five strains of inactive, Sereny's test negative (non-enteroinvasive) *E. coli* strains - 2 of which are enterotoxigenic (LT synthesisers) - were isolated from the intestinal content of suckling pigs with diarrheic syndrome from 2 large-scale swine breeding units, and partially characterised. These strains differ from the atypic, virulent *E. coli* strains described in the literature [5] inasmuch as they do not belong to the pathogenic EIEC type, but to the ETEC one.

As some inactive *E. coli* strains were found to be enterotoxigenic, the conclusion is that they may be involved in the diarrhoeic syndrome breakout in suckling piglets. This finding is primarily useful in the diagnosing activity, as it demonstrates the necessity of also investigating - from the virulence factors viewpoint - the strains falling into the inactive *E. coli* biogroup.

REFERENCES

1. **Decun M., 1986.** Infecţiile colibacilare la animale. *Ed. Ceres, Bucureşti,* 10, 154-157.
2. **Fairbrother J.M., 1993.** Les colibacilloses du porc. *Ann. Méd. Vét.*, **137:** 369-375.
3. **Germany Y., 1995.** Méthodes de laboratoire; Pouvoir entéropathogène des bactéries (*Escherichia coli* agents d'entérites). *Institut Pasteur, Paris,* 204.
4. **Holt J.G., Krieg N.R., Sneath P.H.A., Staley J.T., Williams S.T., 1994.** *Bergey's Manual of Determinative Bacteriology, ninth edition, Williams & Wilkins, Baltimore,* 175-222.
5. **Le Minor L., Richard C., 1993.** Méthodes de laboratoire pour l'identification des entérobactéries. *Institut Pasteur, Paris,* 55-72.