Quantitative Aspects of the Intestinal Yeast Flora of Swine

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(Received 5 April 1961)

SUMMARY

Minimum numbers of viable organisms of the yeast species occurring in the digestive tract of 57 pigs were determined in samples from six sites in each animal (stomach, three sites in the small intestine, caecum, rectum). In 27 animals (47.4 %) *Candida slooffii* was found in one or more sites in numbers ranging from less than 800 to 9,000,000 viable organisms/g. wet sample. The trend was an increase in numbers towards the anus, with highest counts in the rectum and next highest counts in the caecum. The food eaten influenced the numbers of *C. slooffii*; food rich in starch apparently increased, while food rich in cellulose and proteins decreased, these numbers. The pig appears to constitute a suitable host for the obligate intestinal saprophyte *C. slooffii*. Other yeast species found at one or more sites in numbers above 100/g. wet sample (in brackets the number of positive animals) were: *Candida krusei* (8); *Saccharomyces cerevisiae* (6); *Pichia membranaefaciens* (6); *C. albicans* (3); *S. telluris* (*C. bovina*) (2); *S. chevalieri* (2); *S. oleaceus* (2); *S. carlsbergensis* (2); *C. parapsilosis* (2); *C. tropicalis* (2); *S. italicus* var. *melibiosi* (1); *S. steineri* (1); *C. utilis* (1); *Torulopsis glabrata* (1); not identified (1). Analysis of the counts of the three most frequent species showed an increase in the anal direction. It is concluded that *C. krusei*, *P. membranaefaciens* and *S. cerevisiae* are facultative intestinal saprophytes of swine.

INTRODUCTION

During the past few years several related yeast species, well adapted to life in the digestive tract of warm-blooded animals, have been described (*Torulopsis pintolopesii*, Van Uden, 1952; *Candida bovina*, Van Uden & Carmo-Sousa, 1957*a*; *Saccharomyces telluris*, Van der Walt, 1957; *C. slooffii*, Van Uden & Carmo-Sousa, 1957*b*). Nutritional fastidiousness and a narrow temperature range with a high minimum are characteristic for this group. *C. slooffii* grows only between 28° and 44° and requires biotin, inositol, nicotinic acid, thiamine and, under certain circumstances (Kreger-van Rij, 1958), pantothenate. Originally isolated from 6 out of 252 horses (Van Uden & Carmo-Sousa, 1957*b*), *C. slooffii* was subsequently found also in the digestive tract of 121 out of 250 pigs (Van Uden, Carmo-Sousa & Farinha, 1958). This high incidence (48.4 %) suggested that the pig rather than the horse might be a natural host for *C. slooffii*. In the present study we have tried to obtain quantitative information about *C. slooffii* and other yeast species which, as previous qualitative work showed (Van Uden *et al.* 1958), occur in the digestive tract of swine.

METHODS

Fifty-seven swine were sampled in the abattoir of Lisbon. Samples were taken from the contents of the digestive tract at the following sites: stomach (S); small intestine at a point one-third of its total length from the pylorus ($I\frac{1}{3}$); small intestine at a point two-thirds of its total length from the pylorus ($I\frac{2}{3}$); small intestine a few cm. oral of the caecum ($I\frac{3}{3}$); caecum (C); rectum (R).

Table 1. *Quantitative occurrence of Candida slooffii in the digestive tract of swine*

Serial number of pig	Site of sample					
	S*	$I\frac{1}{3}$	$I\frac{2}{3}$	$I\frac{3}{3}$	C	R
	Counts of <i>C. slooffii</i> (organisms/g. wet intestinal contents)					
1	< 300	< 300	< 300	830	< 300	< 300
3	380	480	< 300	< 3,000	< 3,000	< 300
4	840	< 300	< 300	18,500	65,000	270,000
6	360	420	370	< 300	< 300	< 300
7	0	0	0	0	< 300	0
11	1,030	< 300	< 300	4,450	< 300	440
12	< 300	0	0	0	< 300	< 3,000
13	< 300	< 300	< 300	< 300	< 300	< 300
14	1,230	0	< 300	< 300	224,000	9,000,000
15	< 300	0	0	< 300	830	1,330
19	0	0	0	0	1,260	98,000
20	0	0	0	0	0	330
21	0	0	0	0	6,750	6,000
22	< 300	< 300	< 300	420	139,000	15,500
23	< 300	< 300	< 300	< 300	1,100	2,490
24	< 300	< 300	< 300	980	3,500	420
25	44,250	2,130	2,060	12,200	329,500	525,000
26	8,450	3,160	3,420	38,500	121,000	80,500
31	< 300	< 300	2,830	520	212,000	660,000
32	< 300	< 300	0	< 300	< 300	470
33	< 300	< 300	< 300	540	< 300	0
34	0	0	0	0	< 300	< 300
35	0	0	0	< 300	< 300	0
43	< 300	0	0	< 300	5,500	21,400
44	6,770	14,160	10,650	4,470	2,640	720
45	1,060	< 300	2,080	19,900	965,000	1,925,000
57	920	1,910	2,220	6,600	7,800	5,750

< 300 = less than 30 colonies on the 1/10 dilution plates.

< 3000 = less than 30 colonies on the 1/100 dilution plates, the 1/10 plates having been lost.

* S = Stomach; $I\frac{1}{3}$ = small intestine at a point one third of its total length from the pylorus; $I\frac{2}{3}$ = small intestine at a point two thirds of its total length from the pylorus; $I\frac{3}{3}$ = small intestine a few cm. oral of the caecum; C = caecum; R = rectum.

Portions (1 g.) of the samples were suspended in 10 ml. sterile distilled water, shaken during 10 min. and serial dilutions plated in 1 ml. amounts in a medium of the following composition: glucose, 2 % (w/v); yeast extract (Difco), 0.5 % (w/v); peptone, 1 % (w/v); agar, 2 % (w/v); penicillin, 60 units/ml.; streptomycin, 100 units/ml. After incubation at 37° for 48–72 hr., the numbers of yeast colonies were recorded according to macroscopic and microscopic morphology. Representatives of each type were subcultured for identification. The yeast isolates were identified by the methods of Lodder & Kreger-van Rij (1952), Wickerham (1951) and Van Uden & Farinha (1958).

RESULTS

We found *Candida slooffii* in the digestive tract of 27 of the 57 pigs studied, an incidence of 47.4%, which is near the value 48.4% found during a previous qualitative survey (van Uden *et al.* 1958). Thus *C. slooffii* appears to occur in about 50% of the digestive tracts of the pigs examined. This is an unusually high incidence for a single yeast species in the digestive tract of healthy unselected individuals of a warm-blooded host species and is inferior only to the incidences found for *Saccharo-*

Table 2. *Yeasts other than Candida slooffii, occurring in the digestive tract of swine in numbers above 100/g. wet intestinal contents*

Serial number of pig	Site of sample					
	S*	I _{1/3}	I _{2/3}	I _{3/4}	C	R
	Counts of yeast (organisms/g. wet intestinal contents)					
2	0	0	0	0	0	A < 300
3	0	0	0	H 9,400	H 7,400	H < 300
8	0	0	0	0	0	A 3,000 C 4,000
9	0	0	0	0	0	A 5,100 C 2,040
13	0	0	0	0	0	A < 300
16	0	0	0	0	0	F < 300
17	A† < 300 L < 300	0	0	0	A < 300	A 12,100 F 15,800
19	C 340	C < 300	C < 300	C < 300	0	0
20	A < 300	0	0	A 2,340 M 700	A 2,790 N 350	0
27	A 2,310	A < 300 C < 300 J < 300	A < 300 D < 300	A < 300 G < 300 D 330	A < 300	C 2,160 B < 300 E < 300
34	D 330	D < 300	D < 300	D < 300	0	C < 300 B < 300
35	A < 300	0	0	B < 300	B < 300	J 12,000
39	0	0	0	0	0	E 52,500
40	B 20,300	B 450	B 3,300	B 4,500	B 300,000	B 1,855,000
41	0	0	0	0	0	B < 300 C < 300 I < 300 P < 300
42	B 30,000	0	B 30,000	B 30,000	B 435,000	B 2,148,000 R 201,000
44	D 8,270	D 7,720 G 3,860	0	D 7,270	D 1,760	D < 300 Q < 300
47	0	0	0	0	0	P 16,500

* S = Stomach; I_{1/3} = small intestine at a point one-third of its total length from the pylorus; I_{2/3}; small intestine at a point two-thirds of its total length from the pylorus; I_{3/4} = small intestine a few cm. oral of the caecum; C = caecum; R = rectum.

† Key to organisms:

A = *Candida krusei*

B = *Pichia membranaefaciens*

C = *Saccharomyces cerevisiae*

D = *C. albicans*

E = *S. chevalieri*

F = *C. parapsilosis*

G = *C. tropicalis*

H = *S. telluris*

I = *S. italicus* var. *melibiosi*

J = *S. carlsbergensis*

L = *S. steineri*

M = *C. utilis*

N = *Torulopsis glabrata*

P = *S. oleaceus*

Q = *C. bovina*

R = Not identified

< 300 = less than 30 but more than 10 colonies on the 1/10 dilution plates.

mycopsis guttulata in rabbits (Shifrine & Phaff, 1958) and *Torulopsis pintolopesii* in mice and rats (Mackinnon, 1959). This indicates a high suitability of swine as hosts for *C. slooffii*.

The minimum numbers of viable organisms of *Candida slooffii*/g. wet intestinal contents differed from animal to animal and from site to site in the same animal, between extremes of 9,000,000 and less than 800 (Table 1). The counts of *C. slooffii* in the digestive tract showed a trend to increase towards the anus; the second highest counts were most frequent in caecal contents, the highest counts in rectal contents (Fig. 1). This shows that *C. slooffii* multiplies while moving through the digestive tract. The *C. slooffii* population reaches its highest density when leaving the digestive tract. This implies some drainage at the pig's expense of the growth factors on which *C. slooffii* is dependent (see introduction).

Table 8. Incidences in swine digestive tract of yeast species other than *Candida slooffii*, showing more than 100 organisms/g. wet intestinal contents, compared with the incidences of yeast species isolated during a qualitative survey

The figures give percentage of animals which were positive.

Quantitative survey	(%)	Qualitative survey*	(%)
<i>Candida krusei</i>	14.0	<i>C. krusei</i>	14.8
<i>Saccharomyces cerevisiae</i>	10.5	<i>S. tellustris</i> (<i>C. bovina</i>)	14.0
<i>Pichia membranaefaciens</i>	10.5	<i>C. albicans</i>	9.2
<i>C. albicans</i>	5.8	<i>S. cerevisiae</i>	8.8
<i>S. tellustris</i> (<i>C. bovina</i>)	3.5	<i>P. membranaefaciens</i> (<i>C. mycoderma</i>)	7.6
<i>S. chevalieri</i>	3.5	<i>C. tropicalis</i>	6.0
<i>S. oleaceus</i>	3.5	<i>T. glabrata</i>	3.2
<i>S. carlsbergensis</i>	3.5	<i>S. italicus</i>	0.8
<i>C. parapsilosis</i>	3.5	<i>C. parapsilosis</i>	0.8
<i>C. tropicalis</i>	3.5	<i>S. carlsbergensis</i>	0.4
<i>S. italicus</i> var. <i>melibiosi</i>	1.8	<i>P. farinosa</i>	0.4
<i>S. steineri</i>	1.8	<i>Hansenula angusta</i>	0.4
<i>C. utilis</i>	1.8	<i>T. famata</i>	0.4
<i>Torulopsis glabrata</i>	1.8	<i>Trichosporon cutaneum</i>	0.4
Not identified	1.8		

* After Van Uden, Carmo Sousa & Farinha (1958).

Though swine are suitable hosts for *Candida slooffii*, not every animal harbours this yeast at a given time and the numbers or organisms in the positive animals may vary greatly. One factor which interferes with individual host suitability seems to be the diet fed. From our not wholly complete records about the diets of the pigs sampled, the following can be said: (1) most pigs which did not reveal the presence of *C. slooffii* had been kept on a green food diet; (2) most pigs with low numbers of *C. slooffii* throughout the digestive tract had been on a mixed diet, including kitchen refuse; (3) most pigs with high counts for *C. slooffii* had been kept on a grain diet, chiefly maize. These observations suggest that food rich in cellulose (1) and proteins (2) had an adverse effect on intestinal *C. slooffii*, and food rich in starch (3) a stimulatory effect.

In 18 of the 57 pigs, yeast species other than *Candida slooffii* were found in numbers exceeding 100 viable organisms/g. intestinal contents (Table 2). Most of these species had also been found in an earlier qualitative survey (Table 8). *C. krusei*,

Fig. 1. Frequency of highest and second highest counts for *Candida slooffii* in six sites of the digestive tract of 57 swine. S = stomach; I_{1/3} = small intestine at a point one-third of its total length from the pylorus; I_{2/3} = small intestine at a point two-thirds of its total length from the pylorus; I_{3/4} = small intestine a few cm. oral of the caecum; C = caecum; R = rectum. + = highest counts; O = second highest counts.

Fig. 2. Frequency of highest counts for *Candida krusei*, *Saccharomyces cerevisiae* and *Pichia membranaefaciens* in six sites of the digestive tract of 57 swine: S = stomach; I_{1/3} = small intestine at a point one-third of its total length from the pylorus; I_{2/3} = small intestine at a point two thirds of its total length from the pylorus; I_{3/4} = small intestine a few cm. oral of the caecum; C = caecum; R = rectum. O = *Candida krusei*; + = *Saccharomyces cerevisiae*; ∇ = *Pichia membranaefaciens*.

Pichia membranaefaciens and *Saccharomyces cerevisiae* were the most frequent species with numbers ranging from more than 100 to more than 2,000,000 organisms/g. wet intestinal contents. These three species are widespread in nature. When they occur in the digestive tract they are therefore either passers-by or facultative saprophytes. The latter possibility is the case in the pig's digestive tract as is shown in Fig. 2; the highest counts for those three species were more frequent in the rectum than in other parts of the digestive tract, which shows their capacity of multiplication in this environment. *S. tellustris* (*C. bovina*), *C. albicans* and *Torulopsis glabrata*, well known as obligate saprophytes of warm-blooded animals, do not seem to be particularly well adapted to healthy pigs, as is suggested by their low incidences and low cell numbers (Table 2).

This work was subsidized by the Direcção Geral dos Serviços Pecuários, Secretariat of Agriculture, Lisbon, and by the Instituto de Alta Cultura, Lisbon.

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