SHORT ARTICLE

A modified scheme for biotyping *Gardnerella vaginalis*

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Summary. A scheme is proposed for biotyping *Gardnerella vaginalis*, based on detection of hippurate hydrolysis, β -galactosidase (ONPG) and lipase, and fermentation of arabinose, galactose and xylose. Seventeen biotypes were found among 197 strains from asymptomatic women and patients with bacterial vaginosis (non-specific vaginitis). The distribution of biotypes was similar in both populations but some biotypes were found more frequently in patients. The proposed scheme is compared with those previously described.

Introduction

Several simple schemes have been described for the rapid identification of isolates of *Gardnerella vaginalis* (Greenwood and Pickett, 1979; Shaw *et al.*, 1981; Piot *et al.*, 1982; Taylor and Phillips, 1983; Benito *et al.*, 1985). In some of these studies strains exhibited considerable phenotypic variation. A preliminary scheme utilising five biochemical tests differentiated 15 biotypes in 100 strains (Benito *et al.*, 1985). In the present study we have evaluated a modification of this scheme incorporating the lipase test (Piot *et al.*, 1984).

Materials and methods

Collection of specimens

Vaginal fluid from asymptomatic women and from patients with bacterial vaginosis was collected on calcium alginate swabs and transferred directly on to plates of HBT selective medium (Totten *et al.*, 1982). The latter consists of a basal layer of 7 ml of Columbia agar base, containing colistin 10 μ g/ml and nalidixic acid 15 μ g/ml (BBL) with amphotericin B 2 μ g/ml added after autoclaving, and a 13-ml overlay of the same medium plus 5% human blood. Proteose Peptone No. 3 (Difco) 1% was added to both layers before autoclaving and 0.0075% Tween 80 (BBL) was added to both layers after autoclaving. Inoculated plates were incubated immediately at 36°C in CO₂ 5% for 48 h.

Characterisations of strains

Isolates were characterised according to the following criteria (Benito *et al.*, 1985): haemolysis in human-blood agar; absence of haemolysis in sheep-blood agar; hydrolysis of starch; production of α and β glucosidase; and sensitivity to trimethoprim (5- μ g disk) and to metronidazole (50- μ g disk).

The 197 strains included in the study came from patients in clinics for the treatment and control of

sexually transmitted diseases and family planning centres. The strains were isolated from 84 asymptomatic women and from 113 women with bacterial vaginosis.

Biotyping scheme

Hippurate hydrolysis was tested by the method of Hwang and Ederer (1975) and the lipase test by the method of Piot *et al.* (1984); 5 mM *o*-nitrophenyl- β -D-galactopyranoside (ONPG) (Sigma) in 1 M PBS was used for the β -galactosidase test. The reagent was distributed in 0.3 ml portions and inoculated with a loopful (6 mm diameter) of the 48 h cultures of *G. vaginalis*. These were incubated in a water bath at 37°C and read at 3 h and 18 h.

Carbohydrate-fermentation tests were performed in Purple Broth Base (Difco) supplemented with horse serum 5%. The medium was distributed in 5-ml volumes before autoclaving, inoculated with two loops of a 48-h culture and incubated up to 72 h at 37°C in air. Tubes with doubtful reactions were re-incubated and examined daily for 5 days.

Results

The biochemical tests used to define the different biotypes were chosen for their simplicity of performance and interpretation, reproducibility and discriminatory capacity. Twenty strains repeatedly tested after numerous subcultures, before and after storage at –70°C in a medium previously described (Benito *et al.*, 1985), gave consistent results.

The biotypes delineated by the six tests are shown in table I. With respect to hippurate hydrolysis and β -galactosidase activity, the strains were distributed into four groups. Each group was subdivided into biotypes by their fermentation reactions; 15 biotypes were obtained in this way. All biotypes gave a positive reaction with the lipase test and two additional biotypes, IIa₁ and IVa₁ gave a negative lipase reaction.

As shown in table I, 45.7% of the strains belonged to group I (hippurate- and ONPG-positive), 24.9% to group II (hippurate-positive, ONPG-negative), 16.2% to group

III (hippurate-negative, ONPG-positive) and 13.2% of the strains to group IV (negative results in both tests). According to the different fermentation patterns and the lipase test, four biotypes were obtained in group I, five in group II, four in group III and four in group IV. The most frequent biotypes were Ia (61 strains), IIa (28), Ib (25) and IIa₁ (17).

The distribution of biotypes according to source is shown in table II. Biotypes IIa, IIa₁, IIb, IIIa, IIIc and IVa₁ were more prevalent in women with vaginosis, whereas biotype IVa was commoner in asymptomatic women. In the populations studied there were no significant differences between the prevalence of different biotypes.

Discussion

Phenotypic variation in *G. vaginalis* has been described previously (Greenwood and Pickett, 1979; Shaw *et al.*, 1981; Piot *et al.*, 1982; Taylor and Phillips, 1983; Benito *et al.*, 1985). This led us to consider establishing a biotyping scheme for strain characterisation and epidemiological studies.

In 1984 Piot *et al.*, using a scheme of biotyping of *G. vaginalis* based on the hippurate hydrolysis, ONPG and lipase tests, found eight different biotypes. Previously we described a biotyping scheme using hippurate hydrolysis, ONPG and fermentation of xylose, arabinose and galactose (Benito *et al.*, 1985). In the present study a modified scheme is described in which we have incorporated the lipase test (Piot *et al.*, 1984).

The commonest biotypes encountered amongst our 197 strains were Ia (61), IIa (28) and Ib (25) accounting for 58% of all isolates (table I). Strains from all groups were

isolated more frequently from women with bacterial vaginosis (table II). Although for the majority of biotypes the figures are similar in both groups, biotypes IIa, IIa₁, IIb, IIIa, IIIc and IVa₁ predominated in vaginosis isolates. Only biotype IVa was very frequent in asymptomatic women; the only two isolates of biotype Id were isolated from this last group. In the populations studied we have not encountered significant differences between the occurrence of the different biotypes.

A comparison of the biotypes defined by our scheme and those obtained by Piot *et al.* (1984) is shown in table III. Considering only the three biochemical tests employed by these authors: biotypes in group I would correspond to the biotype 1 of Piot, the strains of group II to their biotype 2 and biotype IIa₁ to biotype 5; the strains of group III to biotype 4; those of group IV to biotype 3; and biotype IVa₁ to Piot's biotype 7. We have not found combinations corresponding to biotypes 6 and 8.

Although the results of the present study are similar to those found in the other scheme, they differ in the geographical distribution of strains (Piot *et al.*, 1984). Thus, in group I and II the percentages amongst our strains approach those in strains from the USA, except in the case of biotype III₁ (Piot biotype 5) in which the percentage of our isolates is lower than that found in strains from the USA, Africa and Belgium. In group III, the percentage of biotype 4 found by Piot *et al.* in Belgian strains is doubled in our strains. Group IV was isolated in a slightly higher proportion than in isolates from the three other geographical locations (Piot *et al.*, 1984).

The use of three additional sugar fermentation tests in our scheme defined a greater number of biotypes thus increasing the discriminatory capacity. In the absence of reliable methods for serotyping *G. vaginalis*, present

Table I. Biotypes of 197 strains of *G. vaginalis*

Biotype	Number of strains	Percentage of total (197)	Biochemical tests					
			hippurate	ONPG	lipase	arabinose	galactose	xylose
Ia	61	45.7	+	+	+	+	+	+
Ib	25		+	+	+	+	+	—
Ic	2		+	+	+	—	—	+
Id	2		+	+	+	—	—	—
IIa	28	24.9	+	—	+	—	—	—
IIa ₁	13		+	—	—	—	—	—
IIb	4		+	—	+	—	—	+
IIc	2		+	—	+	+	+	+
IId	2		+	—	+	+	+	—
IIIa	17	16.2	—	+	+	+	+	+
IIIb	7		—	+	+	+	+	—
IIIc	3		—	+	+	—	—	—
IIId	5		—	+	+	—	—	+
IVa	11	13.2	—	—	+	—	—	—
IVa ₁	8		—	—	—	—	—	—
IVb	5		—	—	+	+	+	—
IVc	2		—	—	+	+	+	+

Table II. Distribution of biotypes of *G. vaginalis* by source

Biotype	Number of strains (%) in subjects		
	total	asymptomatics	bacterial vaginosis
Ia	61	28 (46)	33 (54)
Ib	25	11 (44)	14 (56)
Ic	2	1 (50)	1 (50)
Id	2	2 (100)	
Group I	90	42 (47)	48 (53)
IIa	28	8 (29)	20 (71)
IIa ₁	13	5 (38)	8 (62)
IIb	4	1 (25)	3 (75)
IIc	2	1 (50)	1 (50)
IId	2	1 (50)	1 (50)
Group II	49	16 (33)	33 (67)
IIIa	17	6 (35)	11 (65)
IIIb	7	4 (57)	3 (43)
IIIc	3	1 (33)	2 (67)
IIId	5	3 (60)	2 (40)
Group III	32	14 (44)	18 (56)
IVa	11	7 (64)	4 (36)
IVa ₁	8	2 (25)	6 (75)
IVb	5	2 (40)	3 (60)
IVc	2	1 (50)	1 (50)
Group IV	26	12 (46)	14 (54)

Table III. Comparison of two schemes for biotyping *G. vaginalis*

Proposed scheme	Biochemical tests			Piot's scheme†	
	hippurate	ONPG	lipase		
Group I (45·7)* (Ia, Ib, Ic, Id)	+	+	+	1	(26–60)*
Group II (18·3) (IIa, IIb, IIc, IId)	+	—	+	2	(8–24)
IIa ₁ (6·6)	+	—	—	5	(16–31)
Group III (16·2)	—	+	+	4	(8)
Group IV (9·1)	—	—	+	3	(4–5)
IVa ₁ (4·1)	—	—	—	7	(2–8)

* Percentage of strains.

† Piot *et al.* (1984)

epidemiological studies will depend on the use of biotyping. To evaluate the usefulness of such schemes, more extensive studies must be performed in various populations to determine the possible association of particular biotypes with bacterial vaginosis.

In view of considerable phenotypic variation between strains of *G. vaginalis* it is possible that some biotypes may constitute taxons distinct from *G. vaginalis*. Resolution of this problem must await appropriate genetic studies of such strains.

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