Significance of the detection of beta-galactosidase and of beta-xylosidase in the taxonomic study of the genus *Haemophilus*

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SUMMARY A total of 314 strains of *Haemophilus*, isolated from clinical samples, were studied for the production of beta-galactosidase and beta-xylosidase. None of the *H. influenzae* strains studied (9 beta-lactamase positive strains and 129 beta-lactamase negative strains) possessed these enzymes. Both enzymes were almost constantly observed among strains of *H. paraphrophilus* (10 strains studied) and of *H. paraphrohaemolyticus* (9 strains studied). Among the other species (*H. parainfluenzae*, 55 strains; *H. haemolyticus*, 5 strains; *H. parahaemolyticus*, 97 strains), beta-galactosidase was present in about 30% of the strains studied whereas beta-xylosidase was detected occasionally (3% of the strains studied). Detection of these two enzymes could be a valuable test for the taxonomic study of the genus *Haemophilus*. However, the type of substrate used for the detection of beta-xylosidase is important: use of the para-nitro-phenyl-beta-xylopyranoside yielded more positive results than the use of its ortho-isomer.

The significance of the detection of beta-galactosidase among Enterobacteriaceae and other Gram-negative bacilli has been reported by Le Minor and Ben Hamida (1962) and Lapage *et al.* (1973). The beta-xylosidase activity of different groups of bacteria has previously been examined and its interest outlined by Brisou *et al.* (1972), Hansen *et al.* (1977), Kilian and Bülow (1976), Lajudie and de Barjac (1976), and Richard (1973).

The present study was undertaken to evaluate the taxonomic significance of the detection of these enzymes among the genus *Haemophilus*.

Material and methods

ORGANISMS

During a systematic search conducted in 1976 for the detection of beta-lactamase producing strains, a total of 305 strains of *Haemophilus* were consecutively isolated on a selective medium previously described (Hansen *et al.*, 1973). Strains were collected mainly from specimens from the respiratory tract and the ears of hospital inpatients. They included 129 strains of *H. influenzae*, 55 strains of *H. parainfluenzae*, 5

strains of *H. haemolyticus*, 97 strains of *H. parahaemolyticus*, 10 strains of *H. paraphrophilus*, and 9 strains of *H. paraphrohaemolyticus*. Nine additional beta-lactamase positive strains of *H. influenzae* were kindly provided by Dr R. B. Sykes (Glaxo Research, Greenford: 8 strains) and by Dr P. Piot (Institut de Médecine Tropicale, Antwerp: 1 strain).

METHODS OF EXAMINATION

Identification of the strains was based on morphology, requirement for growth factors (using BBL disks containing either or both of the two X and V growth factors), and haemolytic activity examined after growth on 5% defibrinated horse blood in Heart Infusion Agar (Difco) supplemented with 5% Fildes enrichment (Difco). Additional tests were performed according to the techniques described by Zinnemann *et al.* (1968, 1971) for characterisation of the species described by these authors. The *Haemophilus* strains were assigned to species according to the taxonomy proposed by Zinnemann and Biberstein (1974).

Detection of beta-xyloside xylohydrolase (beta-xylosidase) was performed according to the method of Brisou *et al.* (1972) using, as did these authors, the para-nitro-phenyl-beta-xylopyranoside (PNPX)

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rather than its ortho-isomer, because of its better solubility. A heavy suspension of cells grown on Heart Infusion Agar (Difco) supplemented with 5% Fildes enrichment (Difco) was made in test tubes containing 0.5 ml sterile water and incubated for 24 hours at 37°C in the presence of a PNPX disk prepared in our laboratory as follows. Paper disks (1 cm diameter) were covered with 0.05 ml of a 1% aqueous solution of PNPX and kept cool (+ 4°C) after drying. A yellow colour was indicative of a positive test. Beta-D-galactoside-galactohydrolase (beta-galactosidase) was detected by means of the same technique, an ONPG disk (Bio-Merieux) being substituted for the PNPX disk.

Beta-lactamase production was determined according to the method of Kammer *et al.* (1975), originally described by O'Callaghan *et al.* (1972), by means of the Glaxo chromogenic compound 87/312.

Results

As shown in the Table, two of the 305 wild strains studied produced beta-lactamase; both were typical strains of *H. parainfluenzae*.

All strains of *H. influenzae* lacked beta-galactosidase; this enzyme was often present in strains of *H. parainfluenzae* (42% of the strains tested), of *H. haemolyticus* (3/5) and of *H. parahaemolyticus* (23% of the strains tested). All 10 strains of *H. paraphrophilus* and all nine of *H. paraphrohaemolyticus* were beta-galactosidase positive.

All strains of *H. influenzae* and *H. haemolyticus* lacked beta-xylosidase. Occasional strains of *H. parainfluenzae* (2/55) and of *H. parahaemolyticus* (3/97) possessed this enzyme. In contrast, most strains of *H. paraphrophilus* (9/10) and all strains of *H. paraphrohaemolyticus* were beta-xylosidase positive.

Among the beta-lactamase positive strains, the nine strains of *H. influenzae* lacked the two other enzymes; the two strains of *H. parainfluenzae* possessed beta-galactosidase but lacked beta-xylosidase.

Discussion

Detection of beta-galactosidase and of beta-xylosidase among the genus *Haemophilus* is of considerable taxonomic value. *H. influenzae*, whether producing beta-lactamase or not, always lacked these two enzymes, as already pointed out by Khramova and Andreeva (1975), Kilian (1976), and Lapage *et al.* (1973) for beta-galactosidase, and by Kilian (1976) for beta-xylosidase. However, Khramova and Andreeva (1975) detected beta-galactosidase among strains of *H. aegyptius*, a species considered by Kilian *et al.* (1976) as a haemagglutinating variant of *H. influenzae* biotype III.

Among strains of *H. parainfluenzae*, 23/55 (42%) possessed beta-galactosidase whereas beta-xylosidase was detected in only 2/55 strains tested. These results are not in good agreement with those obtained by other workers. Lapage *et al.* (1973), who tested only three strains, did not detect beta-galactosidase, whereas Khramova and Andreeva (1975) and Kilian (1976) found it, respectively, in 100% and 82% of their strains. However, comparison between our results and those of Kilian is difficult as this author recognises only one species of *H. parainfluenzae*, among which he classes several types of V-dependent species (*H. parainfluenzae*, *H. parahaemolyticus*, and *H. paraphrohaemolyticus*) without taking into account the haemolytic activity and/or the CO₂ requirements of his strains.

In our experience, strains of *H. parahaemolyticus* behave as strains of *H. parainfluenzae*: 23% possessed beta-galactosidase, and 3% beta-xylosidase. We cannot compare these results with those of others: Lapage *et al.* (1973) studied only one strain, and Kilian (1976), as pointed out above, did not distinguish *H. parahaemolyticus* from *H. parainfluenzae*.

H. haemolyticus, a species seldom isolated, was represented by only five strains. All lacked beta-xylosidase; in contrast, three of them showed beta-galactosidase activity. Results of the latter test in the

Table Enzymatic characteristics of 314 *Haemophilus* strains

Species	No. of strains examined	β -lactamase positive	β -galactosidase positive	β -xylosidase positive
<i>H. influenzae</i>				
Wild strains	129	0	0	0
β -lactamase positive	9	9	0	0
Control strains				
<i>H. parainfluenzae</i>	55	2	23	2
<i>H. haemolyticus</i>	5	0	3	0
<i>H. parahaemolyticus</i>	97	0	22	3
<i>H. paraphrophilus</i>	10	0	10	9
<i>H. paraphrohaemolyticus</i>	9	0	9	9

literature are conflicting: Kilian (1976) did not detect this enzyme among his nine strains, Lapage *et al.* (1973) found it in one of two strains studied, and Khramova and Andreeva (1975) were able to demonstrate it in almost all their strains.

Detection of both enzymes has the greatest taxonomic significance among strains of *H. paraphrophilus* and of *H. paraphrohaemolyticus*. As these two species always possess beta-galactosidase and nearly all beta-xylosidase, demonstration of both enzymes in a strain of *Haemophilus* strongly suggests its appurtenance to one of these species.

It is noteworthy that Kilian (1976) could detect only one beta-xylosidase positive strain (a strain of *H. haemoglobinophilus* isolated from a pig) among the 426 strains of *Haemophilus* he studied, whereas we found this enzymatic activity in 23 of the 305 strains we tested. Several factors could explain this discrepancy: use of fresh isolates instead of collection strains, geographic distribution of different strains, and variations in methodology. The latter, in particular, must be kept in mind: we noted that four strains of *H. paraphrophilus*, which were beta-xylosidase positive when tested by the technique used in the present work, lacked this enzyme when the ortho-isomer was used instead of PNPX. We made the same observation among strains of *Acinetobacter* (unpublished data). Moreover, Brisou *et al.* (1972), using the PNPX for detecting beta-xylosidase among Enterobacteriaceae, obtained a higher percentage of positive strains than Kilian and Bülow (1976), who used the ortho-isomer.

This points to the need for uniformity of the techniques applied in the taxonomic study of bacteria, particularly when the bacteria constitute a group as complex as the genus *Haemophilus*.

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