Profiles of amino-acid utilisation and production amongst strains of *Branhamella catarrhalis*

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Summary. Utilisation and production of amino acids by isolates of *Branhamella catarrhalis* was studied by ion exchange chromatography after cells had been grown in nutrient broth and Mueller-Hinton broth. The profiles of amino acids used and produced by each strain were compared by a single linkage cluster algorithm. The results of this study reflect the biochemical and physiological heterogeneity amongst strains of *B. catarrhalis*.

Introduction

Gram-negative aerobic cocci belonging to the species *Branhamella catarrhalis* form part of the normal nasopharyngeal flora of man but are also frequently associated with upper respiratory tract infections (Coffey *et al.*, 1967; Ninane *et al.*, 1978; Bøvre and Hagen, 1981). Since the first report of penicillin resistance amongst strains of *B. catarrhalis* (Malmvall *et al.*, 1977), β -lactamase-producing strains have been isolated with increasing frequency (Kamme *et al.*, 1983). These organisms are characterised by their inability to utilise carbohydrates as a source of energy. Consequently, many chemotaxonomic markers, such as the presence of long chain fatty acids (Jantzen *et al.*, 1974; Jantzen and Bryn, 1985), and genetic studies (Kingsbury, 1967) have been valuable in defining species. The DNA base composition of all strains so far examined is 40–43 mols % G + C (Bøvre, 1984).

Early observations on the metabolism of *B. catarrhalis* highlighted the importance of amino-acid catabolism (Fitting and Scherp, 1950). In particular, glutamic acid and alanine were metabolised by washed cells with the liberation of ammonia, CO₂ and keto acids. Attempts were subsequently made to use amino acids as nutritional markers (Otero, 1972). We have investigated several closely-related strains of *B. catarrhalis* to evaluate amino-acid uptake and production profiles as possible biochemical markers for studying strain variation that is not readily detected by chemotaxonomic methods.

Materials and methods

Bacteria

The following bacterial strains isolated by Dr C. Kamme (Department of Medical Microbiology, University of Lund, Sweden) were used: *B. catarrhalis* strains 1, 3, 4, 5 and 6 (penicillin-resistant, plasmid-mediated) and 65C and R-235 (penicillin-sensitive). All strains were cultured on blood-agar plates (Oxoid No. 2), then incubated at 37°C overnight and stored at 4°C for 7–10 days when fresh subcultures were made.

Growth in liquid media for amino-acid profiles

Nutrient broth (NB) (Oxoid) and Mueller-Hinton broth (MH) (Oxoid) were used to assess the uptake or production of amino acids. From an overnight blood-agar culture a suspension was made in nutrient broth to a density of c. $(2-4) \times 10^{12}$ cells/L; 1 ml of bacterial suspension was inoculated into 100-ml volumes of NB and MH, mixed, and a 2-ml sample (0 h) withdrawn. Thereafter, flasks were incubated with shaking at 37°C for 24 h. Further 2-ml samples were withdrawn after 5 h and at the end of incubation (24 h). Viable cell counts were made by the luminescence ATP technique as described by Dupont Instruments with a Lumac NRB (cell content releasing agent) extraction procedure (Chappelle and Levin, 1968). The samples were membrane-filtered to remove bacteria and stored at –25°C until amino-acid analysis.

Amino-acid analysis

Amino-acid analysis was performed by ion exchange chromatography with a Beckman 119 cl amino-acid analyser. A single column was used with lithium citrate elution buffers of pH 2.83, 3.70 and 3.75 respectively. Before analysis, samples were diluted with equal volumes

Table I. Results of amino-acid analyses of *B. catarrhalis* strains in relation to culture medium

Strain no.	Amino acids			
	utilised in		produced in	
	NB	MH	NB	MH
1	C	none	H	D, N
3	B, C, D, E, F	A, B, C, D, E, F	none	H, I, K, M, N
4	C, I, J	A, D, I, J	none	N
5	B, C, E, F	B, C, D, E	G, H	H, I, K
6	C	A, B, C, D, E	G, H	H, K, L, N
65C	A, B, C, E, F	C, D, E	G, H, N	G, H, I, J, K, L, M, N
R-235	C	B, C, D, E	G, H, N	H, K, L, N

A=aspartic acid, B=serine, C=glutamic acid, D=proline, E=glycine, F=histidine, G=threonine, H=valine, I=cystine, J=methionine, K=isoleucine, L=phenylalanine, M=ornithine, N=lysine.

of Beckman dilution buffer, pH 2.2, and 100- μ l samples were applied to the column. The calibration standard mixture was supplied by the Pierce Chemical Company.

Samples analysed represented cell-free filtrates of each culture after incubation for 5 h and 24 h, and of inoculated media before incubation; two additional samples—uninoculated NB and MH, which served as blanks—were also analysed. All the results of amino-acid determinations were expressed in nmol/ml. To evaluate these and to determine which amino acids were utilised or produced, a value of 50% above or below the control was arbitrarily taken as production or uptake, respectively, for each amino acid.

Computer analysis

The similarities between strains were assessed by a simple single linkage programme which derived the amalgamation distances between strains from all the data from the amino-acid utilisation and production profiles. The biomedical package (Dixon, 1985) was used for these analyses, as described by Shah *et al.* (1987).

Results

Since there were only minor quantitative differences in amino-acid utilisation or production between 0-h and 5-h samples, the results obtained with only 24-h samples were recorded. The profile of amino-acid uptake or production was constant for each strain in a particular medium. The results of the analyses of the 24-h samples are shown in table I and quantitative data for one amino acid are given in table II. Strains of *B. catarrhalis* utilised up to six amino acids and produced up to eight amino acids when grown in two different media (table I). Glutamic acid was used by all strains

grown in NB whereas proline, glycine and glutamic acid were used by most strains grown in MH.

Generally, more amino acids were utilised in MH than in NB (table I). Strain 6, for example, assimilated only glutamic acid in NB whereas aspartic acid, serine, glutamic acid, proline and glycine were taken up in MH (table I). Glutamic acid was the most readily utilised amino acid. In NB, all strains used this amino acid and all but strains 1 and 4 utilised it in MH. For strains 1, 6, and R-235 incubated in NB, glutamic acid was the only amino acid utilised (table I). Threonine, valine, isoleucine, lysine and phenylalanine were not taken up from either medium. Cystine and methionine were taken up from both media by strain 4. Thus a preference for assimilating sulphur-containing amino acids clearly characterises this strain.

All isolates except strain 4 produced valine in

Table II. Quantitative utilisation of serine as an example of an amino acid used to different extents in NB and MH by strains of *B. catarrhalis*

Strain no.	Medium	Serine utilised (nmols/ml) at		Percentage reduction
		0 h	24 h	
3	MH	3121	635	80
	NB	440	149	66
5	MH	3819	1246	67
	NB	392	131	67
6	MH	4086	886	77
65C	NB	472	107	77
R235	MH	3369	0	100

Table III. Profile of amino-acid uptake and production by a selected strain (no. 3) of *B. catarrhalis* grown in NB and MH for 24 h

Amino acid	Amount of amino acid (nmols/ml) at		Change in amount
	0 h	24 h	
<i>NB</i>			
phosphoserine	94.18	83.50	...
threonine	298.40	441.46	...
serine	440.50	149.72	decrease
proline	209.80	0.0	decrease
glycine	1209.64	404.90	decrease
alanine	1216.40	820.24	...
valine	331.38	673.62	...
cysteine	46.20	38.74	...
methionine	153.70	170.50	...
isoleucine	226.20	304.04	...
leucine	667.14	722.12	...
tyrosine	143.74	157.62	...
phenylalanine	229.78	253.40	...
ornithine	159.10	137.56	...
lysine	377.18	476.70	...
histidine	59.56	27.08	decrease
arginine	NR	292.10	...
glutamic acid	458.14	tr	decrease
aspartic acid	337.74	182.62	...
<i>MH</i>			
phosphoserine	360.0	344.16	...
threonine	1910.1	2537.0	...
serine	3121.9	635.64	decrease
proline	4823.2	206.16	decrease
glycine	1968.0	143.94	decrease
alanine	2923.2	1860.54	...
valine	1914.8	4432.4	increase
cysteine	36.6	68.7	increase
methionine	994.2	1489.96	...
isoleucine	1065.66	2445.8	increase
leucine	3554.0	5232.0	...
tyrosine	957.0	1303.94	...
phenylalanine	1540.58	2118.40	...
ornithine	229.08	454.90	increase
lysine	3188.0	4866.0	increase
histidine	520.58	148.80	decrease
arginine	957.88	712.0	...
glutamic acid	7896.4	1198.56	decrease
aspartic acid	4116.02	1778.50	decrease

NR = not resolved; tr = trace.

NB or MH or both. Strain 65C had the most complex profile in MH; it produced threonine, valine, cysteine, methionine, isoleucine, phenylalanine, ornithine and lysine. The profile of amino acids produced by each strain was distinct; nutritionally, strain 4 was the least related to the

remaining strains (table I). Most strains used five or six amino acids; strain 65C was characterised by an abnormally high level of amino acid production. An example of a detailed profile of uptake and production in both media is shown in table III. The inter-relationships between strains by comparison of the profiles of amino acids used and produced were ascertained by a single linkage dendrogram (not shown). Strains 6 and R-235 were almost identical and were related to each other at the 98% similarity level. Strains 3 and 5 were related to each other at the 63% level of similarity and were more closely related to strains 6 and R-235 than to the other strains. Strains 1, 4, and 65C had only a low affinity with the other strains, and amongst themselves, and must represent different nutritional biotypes within the species.

Discussion

It is evident that there were significant differences between cultures of the same strain grown in NB and MH, indicating that peptides in the medium may suppress the uptake or production of certain amino acids. *B. catarrhalis* utilises a simple range of free amino acids but the profile obtained for each strain is a distinctive feature. The common occurrence of glutamic acid uptake by the test strains is in agreement with the data of Fitting and Scherp (1950) who reported the rapid uptake of glutamic acid by cell suspensions. Furthermore, Dent (1982) reported four types of glutamate dehydrogenase electrophoretic patterns amongst seven strains. The present data lend further support for heterogeneity amongst strains of *B. catarrhalis*.

Our method of identifying nutritional variants of *B. catarrhalis* is reliable and could be valuable in biochemical and physiological studies. Similar studies in which identical techniques were used have been reported with amino acid-fermenting clostridia (Mead, 1971). Recent advances in high performance liquid chromatographic techniques (see for example, Harpold *et al.*, 1985) would make nutritional biotyping of strains of *B. catarrhalis* more feasible for future studies.

We thank Dr C. Kamme, Department of Medical Microbiology, University of Lund, for providing us with strains of *B. catarrhalis* obtained during his mutational studies. The help of Mr A. Nemeth, Consultant of the Computer Center at the Faculty of Medicine, Kuwait, in constructing the programme to analyse amino-acid data is greatly appreciated and we are also grateful to Dr Senthilselvan for his help with similarity analyses.

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