Identification of *Brevibacterium* from clinical sources

DG PITCHER, H MALNICK

From the National Collection of Type Cultures, Central Public Health Laboratories, London NW9 5HT

SUMMARY Coryneform bacteria of the genus *Brevibacterium* occur on the normal skin surface, but reports of human infection with this genus are lacking. A number of cultures of coryneform bacteria sent to the National Collection of Type Cultures for identification have been identified as *Brevibacterium* spp on the basis of their cell wall composition and ability to produce methanethiol from L-methionine. We describe a rapid method for the detection of methane-thiol and confirmatory tests which differentiate *Brevibacterium* from morphologically similar genera.

Of 27 isolates of coryneform bacteria which were sent to the National Collection of Type Cultures for identification but which could not be identified by conventional tests, 13 conformed to the genus *Brevibacterium* in the rapid production of methane-thiol from L-methionine and possessing the cell wall component meso-diaminopimelic acid but not arabinose. Two strains were of animal origin. One was a tissue culture contaminant and eight were from a variety of sites in the human body. These were compared with 11 reference cultures of *Brevibacterium* spp, eight *Brevibacterium* strains isolated from human skin,¹ 41 reference *Corynebacterium* spp, and seven other Gram positive bacteria. The results show that on the basis of four tests, *Brevibacterium* isolates can be clearly differentiated from *Corynebacterium* spp, which may be encountered in clinical specimens.

Material and methods

Cultures were maintained in the freeze dried state and plated on to nutrient agar (Oxoid) as required. Bacterial strains and their sources are listed in Table 1.

BIOCHEMICAL TESTS

Test media were inoculated by transferring a loopful of cells from a 24 h growth on nutrient agar. All tests were carried out at 30°C and 37°C and were examined at 24 h, 7 days, and 14 days. Milk casein hydrolysis was tested on litmus milk agar.² Degradation of tyrosine and xanthine was tested on nutrient agar containing well dispersed suspensions of 4%

(wt/vol) L-tyrosine and xanthine respectively. Positive results were indicated by the presence of zones of clearing around areas of growth.

DETECTION OF METHANE-THIOL PRODUCTION

Milk-DTNB medium

A modification of the method of Laakso³ was used to detect methane-thiol production on a solid medium. Low fat skimmed milk (Oxoid), 30g, was dispersed in distilled water to 500 ml and heated to 100°C with constant mixing. A separate solution (500 ml) contained agar 10g, L-methionine 1g, K₂HPO₄ 1g, and 5 5'-dithiobis-2-nitrobenzoic acid (DTNB) 0.2g (Sigma).

Both solutions were sterilised by autoclaving at 15 psi for 10 min, after which the temperature was lowered to 50°C. The solutions were mixed and distributed as 2ml slopes in bijoux bottles. Duplicate slopes were heavily inoculated with a loopful of culture from a nutrient agar plate; the bottles were tightly capped and incubated at 30°C and 37°C. The slopes were inspected at 4 h and 20 h. The presence of a bright yellow colour due to the formation of an aryl mercaptan diffusing through the medium was accepted as a positive result.

DETECTION OF CELL WALL COMPONENTS

Samples for cell wall analysis were prepared as described by Bousfield *et al.*⁴ meso-diaminopimelic acid and neutral sugars were identified in hydrolysates by thin layer chromatography as described by Pitcher.⁵

Results

Table 2 lists the results of biochemical tests on *Bre-*

Table 1 Sources of strains

Original designation	NCTC number	Source	Isolated from	Reference
L1	A288/80	D Pitcher, London	Normal human skin	
L29	A290/80	D Pitcher, London	Normal human skin	
L55	A289/80	D Pitcher, London	Normal human skin	
L65	A291/90	D Pitcher, London	Normal human skin	
P151	A293/80	D Pitcher, London	Normal human skin	11
D69	A286/80	R van Furth, Netherlands	Normal human skin	11
K656	A100/80	JJ Leyden, USA	Toe clefts—tinea pedis	11
K673	A287/80	JJ Leyden, USA	Toe clefts—tinea pedis	11
—	A37/73	JA Vale and GW Scott, London	Ear—bilateral ororrhea	10
—	A76/79	R Terry, Burnley	Ear (same strain, several patients)	
—	A76/84	MR Miller, Leeds	Blood culture	
—	A232/83	J Kensit, London	Blood culture (possible contaminant)	
49584	A12/79	RJC Hart, Exeter	Blood culture	
—	A40/71	JA Vale and GW Scott, London	Cerebrospinal fluid	10
—	A127/80	DI Annear, Australia	Hip sinus	
391/M/1974	A119/74	Tanzania	Discharging sinus of foot	
—	A128/84	PL Hood, Brunel University	Mouse—liver	
—	A220/80	K Mohan, Nigeria	Poultry—bumblefoot disease	13
—	A2/73	SD Elek, London	Tissue culture	

vibacterium spp and reference bacteria. *B. linens* and *B. iodinum* grew on all media at 30°C but not at 37°C and results at 30°C only are recorded. For other reference bacteria and *Brevibacterium* strains the results at 30°C and 37°C did not differ.

GROWTH AND REACTION ON MILK-DTNB AGAR
With the exception of *Corynebacterium renale* and *Mycobacterium smegmatis*, species of *Corynebacterium* and Gram positive reference bacteria grew poorly or not at all on milk-DTNB agar.

All strains of *Brevibacterium* produced a pronounced yellowing of the milk-DTNB in 4 h, which was not observed in other species, although *Mycobacterium smegmatis* developed this colour in 20 h.

CONFIRMATORY BIOCHEMICAL TESTS

Caseinolysis could not be observed on milk-DTNB agar except in a small number of strains after prolonged incubation and was probably due to the thickness of the slopes. On litmus-milk-agar plates, however, zones of clearing were apparent at seven days in all *Brevibacterium* except two strains of *B. linens* and one of the human isolates (A76/84). Among the reference strains, only *C. renale* exhibited caseinolysis.

Tyrosine was degraded by all *Brevibacterium* strains, by *C. minutissimum* (four strains), by one strain of *C. xerosis* and three of 20 clinical isolates of *Corynebacterium*. Xanthine was degraded by all culture collection strains of *Brevibacterium*. Results for skin and test *Brevibacterium* strains varied, however; 11 of 19 strains were positive. All other coryneform and reference cultures failed to degrade xanthine.

Results for casein, tyrosine, and xanthine hydrolysis were recorded after seven days' incubation.

Further incubation to 14 days did not show any late activity.

CELL WALLS

All strains designated *Brevibacterium* possessed meso-diaminopimelic acid and galactose but not arabinose in their cell walls. All strains described as *Corynebacterium* spp contained meso-diaminopimelic acid and arabinose.

Discussion

The genus *Brevibacterium* was previously considered *incertae sedis*⁶ but has now been amended to describe species with chemotaxonomic characters closely resembling *B. linens*.⁷ Four species have been described which comply with the generic criteria: *B. linens* (amended description) and *B. iodinum*,⁷ *B. casei*, and *B. epidermidis*.⁸ *B. linens* and *B. iodinum* grow poorly or not at all at 37°C. The former species exhibits a yellow to orange pigment particularly in the light, and the latter produces blue extracellular crystals of iodine under suitable conditions. All four species of *Brevibacterium* are respiratory aerobes and do not ferment sugars. This, together with their coryneform morphology, may in the past have led to their misidentification as *C. bovis*.⁹ Two strains, A37/73 and A40/71, were so described by Vale and Scott.¹⁰ *C. bovis*, however, is not proteolytic and does not produce methane-thiol from methionine.

Sharpe *et al.*¹¹ studied the properties of methane-thiol producing coryneform bacteria from cheese, milk, and human skin and found them to possess the *Brevibacterium* cell wall type. Although *Brevibacterium* spp have been reported as occurring on the human skin surface¹ and may produce antibiotics which affect the distribution of the normal flora,¹²

Table 2 Cell wall type and biochemical results

Reference strains	Source	Cell wall		Production of methane-thiol		Tyrosine degraded	Xanthine degraded	Casein hydrolysed
		mDAP	Arabinose					
				4h	20h			
Corynebacterium spp								
C bovis	NCTC 3224	+	+	—	—	—	—	—
C diphtheriae	NCTC 3984	+	+	—	—	—	—	—
C kütcheri	NCTC 949	+	+	—	—	—	—	—
C minutissimum	NCTC 10284	+	+	—	—	+	—	—
C minutissimum	NCTC 10285	+	+	—	—	+	—	—
C minutissimum	NCTC 10288	+	+	—	—	+	—	—
C minutissimum	NCTC 10289	+	+	—	—	+	—	—
C mycetoides	NCTC 9864	+	+	—	—	—	—	—
C pilosum	ATCC 29592	+	+	—	—	—	—	—
C pseudodiphtheriticum	NCTC 231	+	+	—	—	—	—	—
C pseudodiphtheriticum	NCTC 8633	+	+	—	—	—	—	—
C pseudodiphtheriticum	NCTC 8634	+	+	—	—	—	—	—
C renale	NCTC 7448	+	+	—	—	—	—	+
C striatum	NCTC 764	+	+	—	—	—	—	—
C ulcerans	NCTC 7910	+	+	—	—	—	—	—
C xerosis	ATCC 373	+	+	—	—	—	—	—
C xerosis	ATCC 7094	+	+	—	—	+	—	—
C xerosis	NCTC 7243	+	+	—	—	—	—	—
C xerosis	NCTC 7883	+	+	—	—	—	—	—
C xerosis	NCTC 7929	+	+	—	—	—	—	—
C xerosis	NCTC 9755	+	+	—	—	—	—	—
Corynebacterium spp (clinical isolates)	20 strains	20/20	20/20	0/20	0/20	3/20	0/20	0/20
Other Gram positive bacteria								
Actinomyces pyogenes	NCTC 5224	—	—	—	—	—	—	—
Caseobacter polymorpha	ATCC 33010	+	+	—	—	—	—	—
Erysipelothrix rhusiopathiae	NCTC 8163	—	—	—	—	—	—	—
Listeria monocytogenes	NCTC 7973	+	—	—	—	—	—	—
Micrococcus luteus	NCTC 2665	—	—	—	—	—	—	—
Mycobacterium smegmatis	NCTC 8159	+	+	—	+	—	—	—
Rhodococcus equi	NCTC 1621	+	+	—	—	—	—	—
Brevibacterium spp								
B linens	NCIB 8546	+	—	+	+	+	+	+
B linens	NCIB 8377	+	—	+	+	+	+	—
B linens	ATCC 19391	+	—	+	+	+	+	+
B linens	ATCC 9172	+	—	+	+	+	+	—
B linens	NCDO 1002	+	—	+	+	+	+	+
B casei	NCDO 2051	+	—	+	+	+	+	+
B casei	NCDO 2048	+	—	+	+	+	+	+
B iodinum	NCDO 613	+	—	+	+	+	+	+
B iodinum	NCTC 9742	+	—	+	+	+	+	+
B epidermidis	NCTC 11083	+	—	+	+	+	+	+
B epidermidis	NCTC 11084	+	—	+	+	+	+	+
Isolates (see Table 1)								
	A288/80	+	—	+	+	+	+	+
	A290/80	+	—	+	+	+	+	+
	A289/80	+	—	+	+	+	—	+
	A291/80	+	—	+	+	+	—	+
	A293/80	+	—	+	+	+	+	+
	A286/80	+	—	+	+	+	+	+
	A100/80	+	—	+	+	+	—	+
	A287/80	+	—	+	+	+	+	+
	A37/73	+	—	+	+	+	—	+
	A76/79	+	—	+	+	+	—	+
	A76/84	+	—	+	+	+	+	—
	A232/83	+	—	+	+	+	+	+
	A12/79	+	—	+	+	+	+	+
	A40/71	+	—	+	+	+	—	+
	A127/80	+	—	+	+	+	—	+
	A119/74	+	—	+	+	+	+	+
	A128/84	+	—	+	+	+	—	+
	A220/80	+	—	+	+	+	—	+
	A2/73	+	—	+	+	+	+	+

B. linens and *B. iodinum*—poor or no growth at 37°C; results are for 30°C. All other results are for 30°C and 37°C. mDAP = meso-diaminopimelic acid.

reports of human or animal infection with *Brevibacterium* isolates are lacking, with the exception of one description of bumblefoot disease in poultry from which coryneform bacteria closely resembling *Brevibacterium* were isolated.¹³ One of these (A220/80) is included in our study.

B. linens produces a variety of volatile sulphur products, including compounds which are important in the flavour and aroma of cheeses, while enrichment of the growth medium with L-methionine increases the concentration of methane-thiol produced.¹⁴

All the *Brevibacterium* strains included in this study had a potent cheese like smell on incubation and gave a positive milk-DTNB test. Thus it is likely that the production of volatile sulphur compounds is a shared characteristic of other species in the genus. Volatile sulphides including methane-thiol are produced by Gram negative bacteria of several genera associated with stored fish and poultry spoilage. These have been detected by the yellowing of filter papers impregnated with DTNB suspended above cultures of food spoilage bacteria grown in medium supplemented with cysteine and methionine, to detect both hydrogen sulphide and volatile organic sulphides.¹⁵ Utilising the ability of *Brevibacterium* to grow on milk agar, a medium on which *Corynebacterium* species grow poorly, and incorporating DTNB and L-methionine enable these two genera to be clearly separated. The confirmation tests of casein, tyrosine, and xanthine hydrolysis serve to enhance the distinction.

Brevibacterium isolates in clinical material may be contaminants derived from human skin or from the environment or they may be secondary invaders. The possibility remains, however, that because the genus has not been considered as a source of potential pathogens, isolates have in the past been misidentified as *Corynebacterium* species. None of the clinical isolates of *Brevibacterium* that we have studied produced the characteristic pigments of *B. linens* and *B. iodinum* and all grew well at 37°C. At present, however, a distinction between *B. casei* and *B. epidermidis* cannot be made on the basis of morphology, colonial appearance, or biochemical tests. The essential difference is one of habitat: *B. casei* isolates are from dairy products and *B. epidermidis*

are from human skin. In the absence of adequate species differentiation we can only describe the clinical isolates as *Brevibacterium* sp.

We are grateful to Dr LR Hill for his advice.

References

- Pitcher DG. Rapid identification of cell wall components as a guide to the classification of aerobic coryneform bacteria from human skin. *J Med Microbiol* 1977;10:439-46.
- Webster GF, McGinley KJ. Use of litmus milk agar for presumptive identification of cutaneous propionibacteria. *J Clin Microbiol* 1977;5:661-2.
- Laakso S. The relationship between methionine uptake and demethiolation in a methionine utilizing mutant of *Pseudomonas fluorescens* UK 1. *J Gen Microbiol* 1976;95:391-4.
- Bousfield IJ, Smith GL, Dando TR, Hobbs G. Numerical analysis of total fatty acid profiles in the identification of coryneform, nocardioform and some other bacteria. *J Gen Microbiol* 1983;129:375-94.
- Pitcher DG. Deoxyribonucleic acid base-composition of *Corynebacterium diphtheriae* and other corynebacteria with cell wall type IV. *FEMS Microbiol Lett* 1983;16:291-5.
- Buchanan RE, Gibbons NE, eds. *Bergey's manual of determinative bacteriology*, 8th ed. Baltimore: Williams and Wilkins, 1974.
- Collins MD, Jones D, Keddie RM, Sneath PHA. Reclassification of *Chromobacterium iodinum* (Davis) in a redefined genus *Brevibacterium* (Breed) as *Brevibacterium iodinum* nom. rev., comb. nov. *J Gen Microbiol* 1980;120:1-10.
- Collins MD, Farrow JAE, Goodfellow M, Minnikin DE. *Brevibacterium casei* sp nov and *Brevibacterium epidermidis* sp nov. *System Appl Microbiol* 1983;4:388-95.
- Hine JE, Hill LR, Lapage SP. *Corynebacterium* spp in human disease. *Lancet* 1978;ii:376.
- Vale JA, Scott GW. *Corynebacterium bovis* as a cause of human disease. *Lancet* 1977;iii:682.
- Sharpe ME, Law BA, Phillips BA, Pitcher DG. Methane-thiol production by coryneform bacteria: strains from dairy and human skin sources and *Brevibacterium linens*. *J Gen Microbiol* 1977;101:345-9.
- Al-Admawy AM, Noble WC. Antibiotic production by cutaneous *Brevibacterium* sp. *J Appl Bacteriol* 1981;51:535-40.
- Mohan K. *Brevibacterium* sp from poultry. *Antonie van Leeuwenhoek* 1981;47:449-53.
- Cuer A, Dauphin G, Kergomard A, Dumont JP, Adda J. Production of S-methylthioacetate by *Brevibacterium linens*. *Appl Environ Microbiol* 1979;38:332-4.
- McMeekin TA, Gibbs PA, Patterson JT. Detection of volatile sulphide-producing bacteria isolated from poultry processing plants. *Appl Environ Microbiol* 1978;35:1216-8.

Requests for reprints to: Dr DG Pitcher, National Collection of Type Cultures, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT, England.