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Case report

Growth in Stewart's medium is a simple, rapid and inexpensive screening tool for the identification of *Burkholderia cepacia* complex

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Abstract

Ninety-one percent of *Burkholderia cepacia* complex reference strains (66 out of 72) displayed a yellow slope–green butt colour reaction after growth in Stewart's medium indicating the oxidation of glucose and the absence of an arginine dihydrolase system. This same colour reaction was observed for *Burkholderia gladioli* and several *Ralstonia* species, but not for *Pseudomonas aeruginosa*, *Stenotrophomonas*, *Achromobacter*, *Pandoraea* and several other Gram-negative non-fermenting bacilli. We therefore consider growth in Stewart's medium a useful, simple, rapid and inexpensive screening test to reduce the number of false positive isolates from *B. cepacia* complex selective media.

Keywords: Cystic fibrosis; *Burkholderia cepacia* complex; Isolation; Identification; Stewart's arginine glucose medium

1. Brief report

The accurate identification of *Burkholderia cepacia* complex bacteria presents serious diagnostic difficulties to medical microbiology laboratories. The use of polyphasic taxonomic analyses revealed that organisms previously identified as *B. cepacia* comprise at least nine closely related species, i.e. *B. cepacia*, *Burkholderia multivorans*, *Burkholderia cenocepacia*, *Burkholderia stabilis*, *Burkholderia vietnamiensis*, *Burkholderia dolosa*, *Burkholderia ambifaria*, *Burkholderia anthina* and *Burkholderia pyrrocinia*, known collectively as the *B. cepacia* complex [1–5]. These Gram-negative bacilli can be recovered from soil, water, plants, animals and humans and have also gained attention for their commercial potential as biocontrol agents in both agriculture and bioremediation [1,6]. Although these organisms are generally not pathogenic for healthy persons, they are opportunistic human pathogens and may cause severe, often chronic infections in immunocompromised hosts [1,5]. In the early 1980s, an increasing incidence and prevalence of *B. cepacia* complex isolates was reported in cystic fibrosis (CF) patients. Those CF patients infected with *B. cepacia* complex have an increased morbidity and mortality. Epidemiological studies also demonstrated patient-to-patient spread of outbreak strains [1,5]. We now know that the risk of *B. cepacia* complex cross infection caused by direct or indirect contact is related to a number of factors including patient behaviour, infection control practices and the particular *B. cepacia* complex species and strains involved [5]. Segregating *B. cepacia* complex positive patients has reduced the spread of highly transmissible strains. However, it does not prevent occasional acquisition of *B. cepacia* complex organisms from the natural environment [1,5,7].

Accurate identification is a prerequisite in the management of CF lung disease, in particular for the implementation of infection control measures [5,7], which include segregation, and in CF individuals referred for lung transplantation [8]. Identification of these bacteria from environmental sources is also important in ecological

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studies to determine their habitats and diversity. The use of selective media and appropriate identification procedures is vital for optimum culture and reliable diagnosis [5,9]. Unfortunately, the effectiveness of selective media is hampered by a lack of selectivity and sensitivity and as a consequence they allow growth of other species (e.g. *Burkholderia gladioli*, *Pseudomonas* species, *Chryseobacterium* sp. and others) [1,9]. Identification by traditional and semi-automated biochemical test systems often produces unreliable identification results for these bacteria [1,9] and thus molecular and diagnostic tests and the assistance of referral laboratories have been recommended [10,11].

The aim of our study was to evaluate a simple, rapid and inexpensive culture medium that can be used to identify putative *B. cepacia* complex organisms from selective media, prior to further identification with more complex procedures such as API 20NE or *rec*A-based PCR.

For this purpose, the single tube composite arginine glucose medium, composed by Stewart in 1971, was investigated [12]. Although Stewart's original study did not include *B. cepacia* complex bacteria, the phenotypic reactions of these bacteria were described at a later date [13]. Importantly, both studies pre-date the recent developments in *B. cepacia* complex taxonomy. The Stewart's medium was prepared and inoculated as described before [13]. The constituents were nutrient broth granules, 0.2 g; L-arginine HCl, 0.1 g; glucose, 1.0 g; indicator mixture with bromothymol blue and cresol red, 6 ml; agar, 0.8 g; distilled water, 100 ml (pH 7.4). Metabolisation of glucose and arginine HCl in the medium gives rise to acidic and basic products, which change the colour of the indicator solution in the medium at different levels of pH. Bacterial growth over the surface of the slope allows glucose oxidation, leading to the rapid development of anaerobiosis in the deep butt, conditions under which an arginine dihydrolase system can function and under which bacteria can ferment glucose with the formation of acids throughout the medium [12,13].

The bacterial strains used were all isolates of the *B. cepacia* complex strain panels representing the nine established species [3,4], representative strains of *Pseudomonas aeruginosa* and a selection of species which grow on *B. cepacia* complex selective media and which have been identified in previous polyphasic taxonomic studies [11]: *B. gladioli, Burkholderia fungorum, Stenotrophomonas maltophilia, Ralstonia pickettii, Ralstonia gilardii, Ralstonia mannitolilytica, Ralstonia insidiosa, Ralstonia respiraculi, Pandoraea apista, Pandoraea norimbergensis, Pandoraea pnomenusa, Pandoraea pulmonicola, Pandoraea sputorum, Achromobacter xylosoxidans, Achromobacter insolitus, Herbaspirillum huttiensis, Herbaspirillum* spp., *Advenella incenata, Inquilinus limosus, Elizabethkingia meningoseptica* (*Chryseobacterium meningosepticum*) and *Chryseobacterium* spp. (Table 1). All strains were inoculated by stab culture and incubated at 28 °C. After 24 h and 48 h, colour reactions were noted and compared with the identification schemes described previously [12,13].

Table 1
The organisms inoculated in Stewart's medium with the number of strains tested and the colour reactions observed after 48 h incubation

Organisms	Number of strains tested	Reaction pattern[a] after 48 h
Burkholderia cepacia	4	Y,G
Burkholderia multivorans	8	Y,G
Burkholderia cenocepacia	7	Y,G
	3	Y,Y
Burkholderia stabilis	3	Y,G
	1	Y,Y
Burkholderia vietnamiensis	4	Y,G
Burkholderia dolosa	4	Y,G
Burkholderia ambifaria	4	Y,G
Burkholderia anthina	15	Y,G
	2	Y,Y
Burkholderia pyrrocinia	13	Y,G
Burkholderia ubonensis	1	Y,G
B. cepacia complex[b]	3	Y,G
Burkholderia gladioli	5	Y,G
Burkholderia fungorum	2	V,G
Pandoraea apista	3	V,G
Pandoraea norimbergensis	2	V,G
Pandoraea sputorum	3	V,G
Pandoraea pnomenusa	3	V,G
Pandoraea pulmonicola	3	V,G
Pandoraea sp.	1	V,G
Ralstonia gilardii	3	V,G
Ralstonia pickettii	4	Y,G
Ralstonia mannitolilytica	3	Y,G
Ralstonia insidiosa	3	Y,G
Ralstonia respiraculi	3	V,G
Achromobacter insolitus	2	V,G
Achromobacter xylosoxidans	9	G,G
	2	V,G
Herbaspirillum huttiensis	1	Y,G
	2	G(pale),G
Herbaspirillum sp.	1	G,G
	1	Y,G
	3	G(pale),G
Inquilinus limosus	3	B,G
Elizabethkingia meningoseptica[c]	1	V,G
Chryseobacterium sp.	1	V,G
Chryseobacterium sp.	1	Y,G
Advenella incenata	1	Y,G
	1	G,G
Advenella sp.	1	Y,G
Stenotrophomonas maltophilia	3	B,G
	1	G,G
Pseudomonas aeruginosa	3	Y,V

Y = Yellow (acid production); G = Green (pH unaltered); B = Blue (slight alkali production); V = Violet (strong alkali production).

[a] Reaction pattern: slope, butt (e.g. Y,G).

[b] Genomovar status not determined.

[c] *Chryseobacterium meningosepticum* is transferred to a new genus, *Elizabethkingia* gen. nov. and has as type species the name *Elizabethkingia meningoseptica* comb. nov.

The results of colour changes after 48 h are shown in Table 1. Virtually all *B. cepacia* complex strains (66 out of 72, about 91%) showed the same colour reaction; namely a yellow slope and a green butt. Only six strains showed a yellow-yellow colour reaction (three *B. cenocepacia* strains, two *B. anthina* strains and one *B. stabilis* strain). The

yellow-green colour reaction indicates the oxidation of glucose and the absence of an arginine dihydrolase system. This colour reaction was rarely observed for the other species examined, except for *B. gladioli*, *R. pickettii, R. mannitolilytica, R. insidiosa* and one strain each of *H. huttiensis*, *Herbaspirillum* sp., *A. incenata* and *Advenella* sp.

In a recent study, Stewart's medium was used to identify putative *B. cepacia* complex isolates after growth on a novel selective medium [14]. Thirty-seven out of 170 isolates generated the yellow slope-green butt colour reaction and were therefore considered putative *B. cepacia* complex isolates. Eleven of these were confirmed as *B. cepacia* complex. The remaining isolates (the false positives) included *Herbaspirillum* sp., *B. fungorum*, *Sphingobacterium* sp., *Comamonas acidovorans* and *R. pickettii*. The organisms exhibiting a different colour reaction were further identified by using a polyphasic taxonomic approach and did not include *B. cepacia* complex bacteria. Hence, we did not detect false negatives.

The present data confirm the usefulness of Stewart's medium as a simple, rapid and inexpensive diagnostic test for the presumptive identification of *B. cepacia* complex growing on selective media. Growth on the medium is particularly useful as a screening test when large numbers of isolates require investigation.

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