Letters in Applied Microbiology, 2026, 79(1), ovaf141
https://doi.org/10.1093/lambio/ovaf141
Advance access publication date: 22 December 2025
Research Article

LETTERS IN APPLIED MICROBIOLOGY
OXFORD UNIVERSITY PRESS

Evaluation of a chromogenic agar medium for the detection of *Burkholderia cepacia* complex from industrial samples

Tomás Herrera Durigneux[1], Julián Mantovano[2], José Degrossi[2,3,*]

[1]Facultad de Ciencias Exactas y Naturales. Universidad de Buenos Aires, Ciudad Autónoma de Buenos Aires, 1428, Argentina
[2]Cátedra de Salud Pública e Higiene Ambiental. Facultad de Farmacia y Bioquímica. Universidad de Buenos Aires, Ciudad Autónoma de Buenos Aires, 1113, Argentina
[3]Instituto de Investigaciones en Bacteriología y Virología Molecular (IBaViM). Facultad de Farmacia y Bioquímica. Universidad de Buenos Aires, Ciudad Autónoma de Buenos Aires 1113, Argentina.

*Corresponding author. Cátedra de Salud Pública e Higiene Ambiental. Facultad de Farmacia y Bioquímica, UBA. Junín 956 4° Piso (CP 1113), C.A.B.A., Argentina. E-mail: jdegross@ffyb.uba.ar

Abstract

Burkholderia cepacia complex (Bcc) bacteria are opportunistic pathogens that particularly affect people with cystic fibrosis and other immunosuppressed patients. These bacteria are also frequent contaminants of industrial products such as medicines and cosmetics, posing a risk to patients and raising public health concerns. Due to numerous outbreaks caused by contaminated products reported worldwide, the absence of Bcc in drugs has become mandatory under various codifications. For example, the US Pharmacopoeia recently incorporated testing for Bcc detection in non-sterile products (USP < 60 >). Detection of Bcc usually involves the use of the *Burkholderia cepacia* selective agar (BCSA), a selective but non-differential culture medium. In this study, we compared a recently developed chromogenic agar, CHROMagar™ B.cepacia, with BCSA for the recovery of Bcc and inhibition of non-Bcc species. We also evaluated both media for the detection of Bcc in artificially contaminated pharmaceutical and cosmetic samples. Both media exhibited similar inhibitory properties. Notably, all Bcc isolates in this study developed blue–green colonies on CHROMagar™ B.cepacia, enabling rapid and presumptive identification. These results suggest that CHROMagar™ B.cepacia could be a valuable tool for the rapid detection of Bcc in industrial samples.

Impact Statement

Rapid and accurate detection of *Burkholderia cepacia* complex (Bcc) bacteria may be challenging for industrial microbiology laboratories, as the usually employed culture medium, *Burkholderia cepacia* selective agar, has selective but not differential properties. In this study, we demonstrate that the recently developed CHROMagar™ B.cepacia medium allows a rapid and differential recovery of Bcc species, which grow with a characteristic blue–green color. This chromogenic feature reduces false positives and minimizes the need of additional confirmatory tests. The incorporation of this chromogenic medium into industrial microbiology laboratories could facilitate and accelerate the detection of Bcc in contaminated samples.

Keywords: *Burkholderia cepacia* complex; chromogenic agar; industrial microbiology; microbial contamination; quality control

Introduction

The *Burkholderia cepacia* complex (Bcc) is a group of Gram-negative non-fermenting bacteria that are widely distributed in natural environments such as soil and water. These bacteria can cause severe infections in immunocompromised patients, particularly affecting people with cystic fibrosis (CF), and chronic granulomatous disease (Mahenthiralingam et al. 2008). Furthermore, an increasing number of Bcc infections in other susceptible patients have been reported all around the world due to the use of contaminated products, including disinfectants, cosmetics, medical devices, diagnostic reagents, personal-care products, and medicines (Martin et al. 2011, Song et al. 2018, Seth-Smith et al. 2019, Rhee et al. 2022). The contamination of these products with Bcc poses a threat to public health and raises concerns for sanitary authorities. This situation has prompted the development of various regulations to incorporate assays for Bcc detection. The USA is a representative example where Bcc has been reported as the most frequent bacteria causative of recalls in non-sterile pharmaceutical products (Jimenez 2007). A notable case involved the contamination of oral liquid docusate that caused multi-state outbreaks between 2016 and 2017 and resulted in a massive recall of this product (Glowicz et al. 2018). The United States Pharmacopeia (USP) incorporated Chapter < 60 >; Microbiological Examination of non-sterile products-Tests for *Burkholderia cepacia* complex, effective in December 2019 (United States Pharmacopoeia 2019). Other countries are also incorporating limits for Bcc. For instance in Argentina, testing for Bcc is mandatory in non-sterile pharmaceutical products intended for inhalation, oromucosal, gingival, nasal, auricular/otic routes, and aqueous preparations for oral use (National Administration of Drugs, Food and Medical Devices, 2022).

Although tests for Bcc are being incorporated in pharmaceutical codifications, the recovery and identification of these bacteria can be challenging for microbiologic pharmaceutical laboratories due to the lack of standardized methodologies. Bcc detection usually involves an isolation step using a selective agar medium followed by identification through molecular probes. The *Burkholderia cepacia* selective agar (BCSA) is the most commonly used selective agar for clinical samples and is the medium recommended by USP (Henry et al.

Received 13 September 2025; **revised** 8 December 2025; **accepted** 9 December 2025

1997; United States Pharmacopoeia 2019). Selectiveness of BCSA is based upon a combination of antibiotics: gentamicin, vancomycin and polymyxin B with colorants: phenol red and crystal violet. Non-Bcc microorganisms with resistance to these compounds can grow on BCSA (Burns, Rolain 2014). Moreover, Bcc colonies that grow on BCSA do not exhibit specific differential characteristics. USP chapter < 60 > indicates that greenish-brown colonies with a yellow halo or white colonies surrounded by a pink-red zone are possible Bcc colonies and should be confirmed through identification tests.

Bcc identification is usually performed by molecular tools (Mahenthiralingam et al. 2008, Devanga Ragupathi, Veeraraghavan 2019). PCR amplification of the *recA* gene is widely employed for this purpose (Mahenthiralingam et al. 2000). Other identification techniques such as matrix-assisted laser desorption/ionization-time-of-flight have been increasingly used due to the short time required for obtaining results (Vanlaere et al. 2008). Several molecular tools have been developed with the aim of recovering Bcc from contaminated industrial products. In this sense, a real-time PCR and a Loop-mediated Isothermal Amplification (LAMP) must be mentioned (Jimenez et al. 2018, Daddy Gaoh et al. 2021).

In this study, we evaluate a recently developed and commercialized chromogenic selective Bcc agar (CHROMagar™ B.cepacia). Chromogenic media have been developed for the detection and identification of different microorganisms. These media utilize a specific reaction that produces the development of colonies exhibiting a differential color corresponding to the microorganism of interest. Chromogenic media have been employed in clinical, environmental and industrial laboratories for the rapid detection of *Staphylococcus aureus*, *Enterococcus*, Shiga toxin-producing *Escherichia coli*, *Serratia marcescens,* and *Acinetobacter baumannii* among others (Chihara et al. 2009, Cho et al. 2020, Jenkins et al. 2020, Pérez-Viso et al. 2021, Yusuf et al. 2023). In a recent study, Mauri-Aransolo et al. demonstrated that CHROMagar™ B.cepacia exhibits high sensitivity and specificity for detecting clinical Bcc isolates (Maruri-Aransolo et al. 2024). Based on these findings, we hypothesized that CHROMagar™ B.cepacia could also be effective in the analysis of industrial samples. With this aim, we evaluated the utility of this medium in the field of industrial microbiology. Specifically, CHROMagar™ B.cepacia was compared to BCSA regarding its ability to recover Bcc isolates and inhibit the growth of non-Bcc bacteria obtained from industrial samples. Additionally, we assessed the recovery of Bcc from artificially contaminated industrial products and water.

Materials and methods

Bacterial strains and isolates

Six reference strains were employed in this study: four Bcc (*B. cepacia* ATCC 25416, *B. cenocepacia* J2315, *B. contaminans* LMG23361, and *B. multivorans* LMG13010) and two non-Bcc strains (*Pseudomonas aeruginosa* ATCC 9027 and *Staphylococcus aureus* ATCC 6538). In addition, 34 isolates obtained from industrial products, processes, and water were included (25 Bcc isolates and nine non-Bcc isolates). Detailed information on all strains and isolates is provided in Table 1.

Culture media

CHROMagar™ B.cepacia was provided by the manufacturer (CHROMagar, France). BCSA was prepared from compounds as described by Henry et al. (1997). Soybean-Casein Digest Agar (TSA) and Trypticase Soy Broth (TSB) were obtained from Britania Labs. (Buenos Aires, Argentina). All media were prepared following the manufacturer instructions or literature protocols (Henry et al. 1997).

Comparative evaluation of culture media

Three approaches were applied to compare the performance of CHROMagar™ B.cepacia and BCSA: the Mossel's ecometric method, and two tests described in USP: the growth-promoting and inhibitory property of the media test, and the suitability of test for absence/presence of Bcc (United States Pharmacopoeia 2025, Mossel et al. 1980).

The ecometric method was performed to evaluate productivity and selectivity of both culture media employing all isolates mentioned above. Briefly, each isolate was subcultured on TSA and incubated at 35°C for 24 h. Colonies were then transferred to 10 ml of TSB and incubated at 35°C for 4 h. Aliquots were streaked onto CHROMagar™ B.cepacia, BCSA, and TSA plates following the ecometric pattern (Fig. 1): sequentially from A1 to A5; B1 to B5; C1 to C5 and D1 to D5. Plates were incubated at 35°C, and the highest sector exhibiting visible bacterial growth at 24 and 48 h was recorded. Based on the highest growth sector, an absolute growth index (AGI) was assigned according to values displayed in Table 2. A relative growth index (RGI) was calculated as (AGI of test medium/AGI of TSA) × 100. Each strain or isolate was evaluated in triplicate in each media. The experiments were conducted on different days by at least two different operators to ensure reproducibility and to evaluate inter-operator variability. Productivity was considered satisfactory for Bcc isolates with RGI > 70, while selectivity was deemed acceptable for non-Bcc isolates with RGI < 30. To compare productivity of BCSA and CHROMagar™ B.cepacia, a paired *t*-test was used. A significance level of $P < 0.005$ was set to determine the existence of significant differences. Data were analyzed using GraphPad Prism (GraphPad Software, California, USA).

USP growth promotion and inhibitory assays were carried out using the six reference strains only. The growth promotion test was performed individually with each Bcc strain applying the Surface-Spread Method described in USP Chapter < 61> (United Stetes Pharmacopoeia 2019). Briefly, 0.1 ml of a suspension containing $\sim 1 \times 10^3$ CFU ml^{-1} of each Bcc strain in phosphate buffer pH 7.2, was spread onto plates of CHROMagar™ B.cepacia, BCSA and TSA. Plates were incubated at 35°C and growth was evaluated after 24 and 48 h.

For the inhibition assay, non-Bcc reference strains were individually tested applying the Surface Spread Method described in USP Chapter < 61 > . Plates of CHROMagar™ B.cepacia, BCSA and TSA were inoculated with 0.1 ml of, respectively, *P. aeruginosa* or *S. aureus* suspensions ($\sim 1 \times 10^4$ CFU ml^{-1} in phosphate buffer pH 7.2). Plates were incubated at 35°C and growth was evaluated after 48 and 72 h.

The suitability test for absence/presence of Bcc was evaluated using a commercial cough syrup, a cosmetic cream and purified water For cough syrup and cosmetic cream, 10 ml or 10 g of product were mixed with 90 ml of buffered sodium

Table 1. Comparison of CHROMagar™ B.cepacia and BCSA employing the Mossel's ecometric method.

Microorganism	Origin	Mossel ecometric method (RGI)			
		CHROMagar™ B.cepacia		BCSA	
		24 h	48 h	24 h	48 h
B contaminans LMG 23 361	Sheep mastitis	72,7	100	71.8	100
B cenocepacia J2315	CF patient	71.2	100	72.5	100
B. cepacia ATCC 25 416	Onion	38,46	96.7	42.6	97.5
B. multivorans LMG 13 010	CF patient	62.9	91.2	40.0	88,3
B contaminans FFI 6	Purified water. Pharmaceutical industry	66.7	100	63.8	100
B. contaminans FFI 10	Distilled water. Water supplier	71.7	88.3	85.0	100
B contaminans FFI 15	Disinfectant (pharmaceutical product)	62.8	92.5	58.3	90.0
B contaminans FFI 28	Pharmaceutical product	24.1	90,3	25.0	93.8
B contaminans FFI 29	Pharmaceutical product	59.7	100	36.7	98.3
B contaminans FFI 30	Pharmaceutical product	90.7	100	90.7	100
B contaminans FFI 33	Cosmetic cream	50.2	96.2	62.5	92.3
B contaminans FFI 34	Purified water. Beverage industry	83.1	100	77.2	96.7
B contaminans FFI 36	Pharmaceutical product	85.4	100	80.1	100
B contaminans FFI 41	Purified water	71,13	100	52.9	100
B. aenigmatica FFI 4	Purified water. Pharmaceutical industry	88,9	98.3	52.8	98.3
B. aenigmatica FFI 17	Floor cleaner	94.3	100	95.1	100
B. aenigmatica FFI 18	Floor cleaner	88.86	98.3	67.3	96,5
B. aenigmatica FFI 20	Floor cleaner	94,3	100	96,7	100
B. aenigmatica FFI 21	Cosmetic cream	63.9	98.3	77.2	100
B cepacia FFI 14	Cosmetic cream	61.1	96.7	67.3	95
B cepacia FFI 38	Cosmetic cream	35,4	100	52.9	96,7
B cepacia FFI 39	Cosmetic cream	71.2	96.8	71.2	96.1
B cepacia FFI 40	Cosmetic cream	69.2	100	61.5	98.3
B vietnamiensis FFI 25	Clothes conditioner	37.7	91.7	46.7	96.7
B vietnamiensis FFI 26	Clothes conditioner	52.7	90.3	58.3	94.7
B vietnamiensis FFI 27	Clothes conditioner	58.9	93.3	53.8	91.7
B arboris FFI 33	Moist washcloths	71.2	90.0	77.7	90.0
B cenocepacia FFI 11	Purified water. Household cleaning products industry	62.5	95	59.8	95
B cenocepacia FFI 32	Clothes conditioner	73.8	96.7	66.8	98.3
P aeruginosa ATCC 9027	Outer ear infection	0.0	0.0	0.0	0.0
S. aureus ATCC 6538	Human lesion	0.0	0.0	0.0	0.0
P aeruginosa FFI 101	Disinfectant	0.0	0.0	0.0	31.7
P aeruginosa FFI 102	Purified water. Pharmaceutical industry	0.0	0.0	0.0	0.0
P. fluorescens FFI 110	Disinfectant	7.1	26.7	2.9	28.3
P. fluorescens FFI 111	Floor cleaner	22.9	33.3	8,3	16.7
P. putida FFI 109	Drinking water	0.0	15.0	0.0	16.7
B gladioli FFI 181	Purified water. Pharmaceutical industry	61.3	81.6	41.7	91.6
S. aureus FFI 150	Floor cleaner	0.0	0.0	0.0	0.0
Achromobacter spp. FFI 130	Industrial water	0.0	5.0	16.7	57.4
Stenotrophomonas maltophilia FFI 191	Water from reverse osmosis	0.0	0.0	0.0	0.0

RGI: relative growth index
RGI values represent the averages of RGI obtained from individual assays performed in triplicate.

chloride–peptone solution (pH 7) supplemented with 1% (v/v) of Polysorbate 80. After shaking for 1 min, 10 ml of the suspension were transferred into 90 ml of TSB. Each Bcc reference strain was cultured on TSA for 24 h at 35°C, and then suspended in phosphate buffer (pH 7.2) to a final concentration of ≤100 CFU ml^{-1}. Similarly, a suspension of *P. aeruginosa* with $\sim 1 \times 10^3$ CFU ml^{-1} was prepared. Flasks with TSB added with the dilutions of the products were inoculated with 1 ml of Bcc suspension. At the same time another set of flasks with TSB and the dilutions of the products were inoculated with 1 ml of *P. aeruginosa* suspension. A third set of broth media with the dilutions of the products was inoculated with 1 ml of both suspensions. For the absence/presence assay with purified water, aliquots of 100 ml of sterile purified water were added to 100 ml of double-concentrated TSB. Inoculation of individual flasks with Bcc suspensions, *P. aeruginosa* suspension and a mixture of both suspensions was performed as above. All inoculated broth media were incubated at 35°C during 24 and 48 h. After those periods of time, samples with grown bacteria were subcultured on BCSA and CHROMagar™ B.cepacia agar plates by streaking and incubated at 35°C. Plates were evaluated for Bcc growth after 24 and 48 h of incubation.

Results and discussion

Rapid and accurate detection of Bcc in mass-consumption industrial products is a key factor for preventing contamination and potential infections associated with these bacteria (Tavares et al. 2020).

Regulatory standards such as the USP which recently incorporated Bcc testing in non-sterile products, specify the use of BCSA and subsequent confirmation by identification tests. Given that the Bcc comprises at least 24 species, the use of biochemical tests is not enough, and molecular probes are required for identification (Devanga Ragupathi et al. 2019).

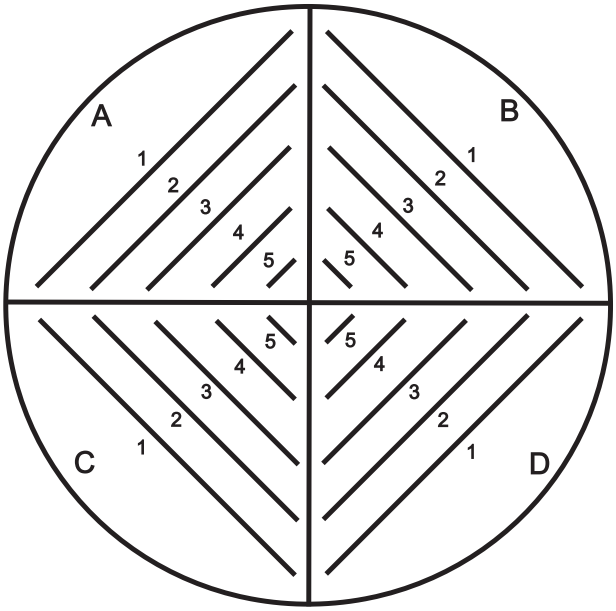

Figure 1. Mossel´s ecometric method. Scheme of streaked plates

While several studies focused on a rapid, direct detection of Bcc from industrial products, primarily through molecular techniques, few researches examined the classical methodology for recovering microorganisms: enrichment in a nutrient broth and isolation in a selective media (Jimenez et al. 2018, Ahn et al. 2020, Daddy Gaoh et al. 2021). This classical approach is included in pharmaceutical codifications and remains accessible to all industrial microbiology laboratories. A previous research compared the use of oligotrophic culture media with TSA or TSB emphasizing the enrichment step (Ahn et al. 2019). In contrast, our study focused on the isolation step, comparing a chromogenic agar medium with the widely employed BCSA. This comparison was performed using assays usually conducted in pharmaceutical microbiology laboratories.

The ecometric method, a semi-quantitative technique employed for a rapid quality control and for the comparison of different lots of culture media, was employed to assess productivity and selectivity (Sandle 2016). Four Bcc collection strains and 25 industrial isolates were tested. Bcc species isolated from industrial sources were reported in a previous research and belonged to *B. contaminans* (n:10), *B. aenigmatica* (n:5), *B. cepacia* (n:4), *B. vietnamiensis* (n:3), *B. cenocepacia* (n:2), and *B. arboris* (n:1) species (Table 1) (De Volder et al. 2021). This diversity allowed us to evaluate the performance of both culture media with isolates of different Bcc species recovered from industrial origin. It was of particular interest given that several works suggest differences between clinical and industrial isolates (Baldwin et al. 2007, Coulon et al. 2021).

Acceptable productivity (RGI > 70) was observed with all tested isolates in both media after 48 h of incubation at 35°C (Table 1). Within 24 h, productivity on CHROMagar™ B.cepacia was slightly superior than on BCSA (50% and 46.43% respectively). However, statistically analysis showed no significant differences between two media (*P*: 0.33 at 24 h of incubation; *P*: 0.83 at 48 h of incubation).

All Bcc isolates grew on CHROMagar™ B.cepacia developing characteristic blue–green colonies which facilitated their detection and subsequent identification. In contrast, Bcc colonies on BCSA lacked of a distinctive coloration. Regarding selectivity, three non Bcc isolates grew on BCSA with RGI > 30 at 48 h of incubation (*P. aeruginosa*, *B. gladioli*, and *Achromobacter* sp) and two isolates grew on CHROMagar™ B.cepacia under the same incubation conditions. Both non-Bcc isolates that grew on CHROMagar™ B.cepacia with RGI > 30 (*B. gladioli* and *P. fluorescens*), developed a blue–green color similar to that described for Bcc. Isolates from other non-Bcc species (*P. putida* and *Achromobacter* spp) grew on CHROMagar™ B.cepacia but colonies did not develop the characteristic color observed in Bcc colonies.

Regarding codified assays, the promotion and inhibition tests are required to evaluate the performance and inhibitory capability of culture media. Furthermore, we employed the suitability test for absence/presence of microorganisms, to compare the efficacy of both media in recovering Bcc from artificially contaminated samples. To obtain a comprehensive comparison of both media, this test was carried out in three different matrices. Additionally, all suitability assays were repeated in presence of a high number of *P. aeruginosa* cells to evaluate the interference of background flora. For these assays, all Bcc strains mentioned in USP chapter < 60 > as well as *B. contaminans* type strain were employed. We included *B. contaminans* since it has been reported as a frequent contaminant of industrial products (De Volder 2021). Adequate recovery of these Bcc strains was verified in both CHROMagar™ B.cepacia and BCSA, with incubation of 48 h at 35°C (Table 3). All Bcc strains developed on CHROMagar™ B.cepacia colonies with a characteristic blue–green pigment and a larger size than those observed on BCSA. These differences were evidenced even at 24 h of incubation (Fig. 2). Inhibition tests were satisfactory on both media as the non-Bcc strains evaluated did not grow on either on CHROMagar™ B.cepacia or BCSA within 72 h of incubation. Regarding the suitability test for absence/presence, all Bcc strains employed were recovered from all the artificially contaminated samples using both media. In most cases the strains were recovered with 24 h broth enrichment and 24 h incubation of the isolation

Table 2. Mossel´s ecometric method.

Sector	AGI	Sector	AGI	Sector	AGI	Sector	AGI
A1	5	B1	10	C1	15	D1	20
A2	25	B2	30	C2	35	D2	40
A3	45	B3	50	C3	55	D3	60
A4	65	B4	70	C4	75	D4	80
A5	85	B5	90	C5	95	D5	100

Sector: last streaked line in which bacterial growth is observed
AGI: number assigned according to the sector achieved
Values of Absolute Growth Index (AGI) assigned according to the maximum growth obtained in each plate after incubation.

Table 3. Growth promotion and inhibition tests according USP < 61 >.

Strain	TSA		CHROMagar™ B.cepacia		BCSA	
	24 h	**48 h**	**24 h**	**48 h**	**24 h**	**48 h**
B. cepacia ATCC 26 416	60	89	57	94	32	83
B. cenocepacia J2315	35	94	42	98	45	77
B. multivorans LMG 13 010	59	105	27	98	41 UFC	92
B. conraminans LMG 23 361	39	92	59	103	34	88
	TSA		CHROMagar™ B.cepacia		BCSA	
	48 h	72 h	48 h	72 h	48 h	72 h
P. aeruginosa ATCC 9027	788	$1,3 \times 10^3$	< 1	< 1	< 1	< 1
S. aureus ATCC 6538	825	942	< 1	< 1	< 1	< 1

Values are expressed in Colony Forming Units (CFU).

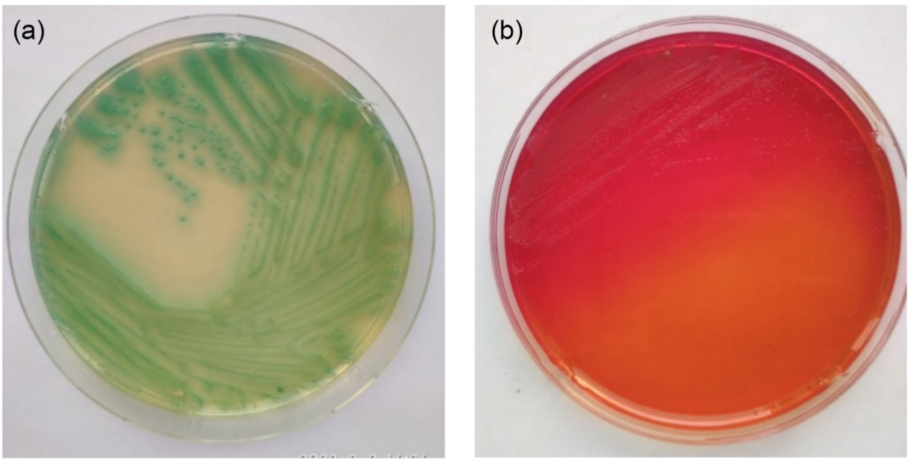

Figure 2. Recovery of *B. contaminans* from cough syrup (24 h of incubation). (a) CHROMagar™ B.cepacia. (b) BCSA.

media (CHROMagar™ B.cepacia or BCSA irrespective of the medium) (Table 4). Only *B. cenocepacia* in purified water and *B. multivorans* in cosmetic cream required 24 h of broth enrichment incubation followed by 48 h of incubation on both BCSA and CHROMagar™ B.cepacia. Recovery of Bcc strains was verified in both culture media even in the presence of a high number of other microorganisms like *P. aeruginosa*.

In summary, our results demonstrate similar recovery of industrial-origin Bcc with both media in all assays. Selectivity is a desirable characteristic of isolation culture media to minimize false positive results and reduce confirmatory identification probes. As observed with BCSA, the chromogenic media is not completely selective and allows the growth of other species such as *B. gladioli*. Since BCSA is a non-differential culture medium, all colonies which grow on it require identity confirmation by PCR or another suitable method. This time and resource-consuming process was reduced with the use of CHROMagar™ B.cepacia, as only a few non-Bcc isolates grew on this medium exhibiting the same color of Bcc colonies. This differential colony development represents an important advantage of CHROMagar™ B.cepacia. The characteristic blue–green color produced by Bcc colonies on this chromogenic medium improves visualization and differentiation, thereby reducing false positives and minimizing the number of confirmatory tests necessary to verify the presence or absence of Bcc in a sample. Furthermore, this reduction in confirmatory testing translates into economic benefits, by decreasing reagents consumption and labor costs. However, it should be noted that CHROMagar™ B.cepacia does not completely exclude the growth of certain non-Bcc species. Therefore, this medium should be considered primarily as a presumptive identification tool. A recent report suggests the incorporation of CHROMagar™ B.cepacia in clinical laboratories (Mauri-Aransolo et al. 2024). Our results are consistent with this research and support the use of this chromogenic medium in industrial microbiology laboratories. Particularly in the pharmaceutical field, the use of chromogenic media is considered an alternative assay that must be validated and proven to be superior or at least equivalent to codified assays. Although we demonstrated faster and more specific results obtained with CHROMagar™ B.cepacia than with BCSA, validation must be performed in each product in which chromogenic agar will be applied for microbiological control. A limitation of this study is the sample size, particularly regarding the diversity of Bcc species and non-Bcc isolates. Future studies including a broader range of environmental and industrial contaminants would further validate the productivity and selectivity of CHROMagar™ B.cepacia.

Conclusion

We conclude that both CHROMagar™ B.cepacia and BCSA are suitable for the detection of Bcc in industrial samples. Although it is not completely selective, the chromogenic medium offers clear practical advantages in terms of rapid and straightforward identification of Bcc colonies. These findings support the incorporation of CHROMagar™ B.cepacia as a suitable alternative to BCSA for presumptive identification in the enrichment and isolation scheme for Bcc detection in industrial samples.

Table 4. Recovery of Bcc in spiked samples.

Microorganism	Cough Syrup				Cosmetic cream				Purified water			
	BCSA		CHROMBC*		BCSA		CHROMBC*		BCSA		CHROMBC*	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
P. aeruginosa ATCC 9027	−	−	−	−	−	−	−	−	−	−	−	−
B. cepacia ATCC 25416	+	+	+	+	+	+	+	+	+	+	+	+
B. cepacia ATCC 25416 + *P. aeruginosa* ATCC 9027	+	+	+	+	+	+	+	+	+	+	+	+
B. cenocepacia J2315	+	+	+	+	+	+	+	+	−	+	−	+
B. cenocepacia J2315 + *P. aeruginosa* ATCC 9027	+	+	+	+	+	+	+	+	−	+	−	+
B. contaminans LMG 23 361	+	+	+	+	+	+	+	+	+	+	+	+
B. contaminans LMG 23361 + *P. aeruginosa* ATCC 9027	+	+	+	+	+	+	+	+	+	+	+	+
B. multivorans LMG 13 010	+	+	+	+	−	+	−	+	+	+	+	+
B. multivorans LMG 13010 + *P. aeruginosa* ATCC 9027	+	+	+	+	−	+	−	+	+	+	+	+

*CHROMBC: CHROMagar™ B.cepacia.

Acknowledgments

The authors express their gratitude to Agustina Lopez de Volder and Noelia Breglia for assistance with isolates collection and all microbiologists from industrial labs that kindly provided isolates. Artificial intelligence was used as a supporting tool for the review of style and grammatical correction of the manuscript.

Author contributions

Tomás Herrera Durigneux (Conceptualization [supporting], Formal Analysis [equal], Investigation [lead], Writing – original draft [lead], Writing – review & editing [supporting]), Julián Mantovano (Investigation [supporting], Validation [equal], Writing – review & editing [supporting]), Degrossi José (Conceptualization [lead], Formal Analysis [equal], Funding acquisition [lead], Investigation [supporting], Supervision [lead], Validation [equal], Writing – original draft [supporting], Writing – review & editing [lead]).

Conflict of interest: The authors declare no competing interests.

Funding

This research has been funded by the University of Buenos Aires, School of Pharmacy and Biochemistry and the own authors through the Account "Microbial Methods."

Data availability

The data underlying this article are available in the article

References

Ahn Y, Gibson B, Williams A *et al*. A comparison of culture-based, real-time PCR, droplet digital PCR and flow cytometric methods for the detection *of Burkholderia cepacia* complex in nuclease-free water and antiseptics. *J Ind Microbiol Biotechnol* 2020;**47**:475–84. https://doi.org/10.1007/s10295-020-02287-3

Ahn Y, Lee U, Lee Y *et al*. Oligotrophic media compared with a tryptic soy agar or broth for the recovery of *Burkholderia cepacia* complex from different storage temperatures and culture conditions. *J Microbiol Biotechnol* 2019;**10**:1495–05. https://doi.org/10.4014/jmb.1906.06024

Baldwin A, Mahenthiralingam E, Drevinek P *et al*. Environmental *Burkholderia cepacia* complex isolates in human infections. *Emerg Infect Dis* 2007;**13**:458–61. https://doi.org/10.3201/eid1303.060403

Burns JL, Rolain JM. Culture-based diagnostic microbiology in cystic fibrosis: can we simplify the complexity? *J Cyst Fibros* 2014;**13**:1–9. https://doi.org/10.1016/j.jcf.2013.09.004

Chihara S, Hayden MK, Minogue-Corbett E *et al*. Shortened time to identify *Staphylococcus* species from blood cultures and methicillin resistance testing using CHROMAgar. *Int J Microbiol* 2009;**2009**:636502. https://doi.org/10.1155/2009/636502

Cho S, Hiott LM, Woodley TA *et al*. Evaluation of a new chromogenic agar for the detection of environmental *Enterococcus*. *J Microbiol Methods* 2020;**178**:106082. https://doi.org/10.1016/j.mimet.2020.106082

Coulon PML, Zlosnik JEA, Déziel E. Presence of the Hmq system and production of 4-hydroxy-3-Methyl-2-alkylquinolines are heterogeneously distributed between *Burkholderia cepacia* complex species and more prevalent among environmental than clinical isolates. *Microbiol Spectr* 2021;**9**:e0012721. https://doi.org/10.1128/Spectrum.00127-21

Daddy Gaoh S, Kweon O, Lee YJ *et al*. Loop-Mediated Isothermal Amplification (LAMP) assay for detecting *Burkholderia cepacia* complex in non-sterile pharmaceutical products. *Pathogens* 2021;**10**:1071. https://doi.org/10.3390/pathogens10091071

De Volder A, Teves S, Isasmendi A *et al*. Distribution of *Burkholderia cepacia* complex species isolated from industrial processes and contaminated products in Argentina. *Int Microbiol* 2021;**24**:157–67. https://doi.org/10.1007/s10123-020-00151-z

Devanga Ragupathi NK, Veeraraghavan B. Accurate identification and epidemiological characterization of Burkholderia cepacia complex: an update. *Ann Clin Microbiol Antimicrob* 2019;**18**:7. https://doi.org/10.1186/s12941-019-0306-0

Glowicz J, Crist M, Gould C *et al*. A multistate investigation of health care-associated *Burkholderia cepacia* complex infections related to liquid docusate sodium contamination, January-October 2016. *Am J Infect Control* 2018;**46**:649–55. https://doi.org/10.1016/j.ajic.2017.11

Henry DA, Campbell ME, LiPuma JJ *et al*. Identification of *Burkholderia cepacia* isolates from patients with cystic fibrosis and use of a simple new selective medium. *J Clin Microbiol* 1997;**35**:614–9. https://doi.org/10.1128/jcm.35.3.614-619.1997

Jenkins C, Perry NT, Godbole G *et al*. Evaluation of chromogenic selective agar (CHROMagar STEC) for the direct detection of Shiga toxin-producing *Escherichia coli* from faecal specimens. *J Med Microbiol* 2020;**69**:487–91. https://doi.org/10.1099/jmm.0.001136

Jimenez L, Jashari T, Vasquez J *et al*. Real-time PCR detection of *Burkholderia cepacia* in pharmaceutical products contaminated with low levels of bacterial contamination. *PDA J Pharm Sci Technol* 2018;**72**:73–80. https://doi.org/10.5731/pdajpst.2017.007971

Jimenez L. Microbial diversity in pharmaceutical product recalls and environments. *PDA J Pharm Sci Technol* 2007;**61**:383–99.

Mahenthiralingam E, Baldwin A, Dowson CG. *Burkholderia cepacia* Complex bacteria: opportunistic pathogens with important natural biology. *J App Microbiol* 2008;**104**:1539–51. https://doi.org/10.1111/j.1365-2672.2007.03706.x

Mahenthiralingam E, Bischof J, Byrne SK *et al*. DNA-based diagnostic approaches for identification of *Burkholderia cepacia* complex, *Burkholderia vietnamiensis*, *Burkholderia multivorans*, *Burkholderia stabilis*, and *Burkholderia cepacia* genomovars I and III. *J Clin Microbiol* 2000;**38**:3165–73. https://doi.org/10.1128/JCM.38.9.3165-3173.2000

Martin M, Christiansen B, Caspari G *et al*. Hospital-wide outbreak of *Burkholderia contaminans* caused by prefabricated moist washcloths. *J Hosp Infect* 2011;**77**:267–70. https://doi.org/10.1016/j.jhin.2010.10.004

Maruri-Aransolo A, de Dios Caballero J, Michelena M *et al*. Evaluation of CHROMagar™ B.cepacia agar for the detection of *Burkholderia cepacia* complex species from sputum samples of patients with cystic fibrosis. *Eur J Clin Microbiol Infect Dis* 2024;**43**:1349–53. https://doi.org/10.1007/s10096-024-04845-4

Mossel DA, van Rossem F, Koopmans M *et al*. Quality control of solid culture media: a comparison of the classic and the so-called ecometric technique. *J Appl Bacteriol* 1980;**49**:439–54. https://doi.org/10.1111/j.1365-2672.1980.tb04719.x

National Administration of Drugs, Food and Medical Devices (ANMAT). Disposition 6967/2022 Ministry of Health. *Official Bulletin of the Argentine Republic*. Buenos Aires: Legal and Technical Secretariat of Argentina , 2022. https://www.boletinoficial.gob.ar/detalleAviso/primera/270610/20220831(18 November 2025, date last accessed).

Pérez-Viso B, Aracil-Gisbert S, Coque TM *et al*. Evaluation of CHROMagar™-Serratia agar, a new chromogenic medium for the detection and isolation of *Serratia marcescens*. *Eur J Clin Microbiol Infect Dis* 2021;**40**:2593–96. https://doi.org/10.1007/s10096-021-04328-w

Rhee C, Baker MA, Tucker R *et al*. Cluster of *Burkholderia cepacia* complex infections associated with extracorporeal membrane oxygenation water heater devices. *Clin Infect Dis* 2022;**29**:1610–17. https://doi.org/10.1093/cid/ciac200

Sandle T. Microbiological culture media. In: Sandle T (ed.), *Pharmaceutical Microbiology: Essentials for Quality Assurance and Quality Control*. United Kingdom: Woodhead Publishing. Elsevier LTD, 2016;47–59. https://doi.org/10.1016/B978-0-08-100022-9.00010-4

Seth-Smith HMB, Casanova C, Sommerstein R *et al*. Phenotypic and genomic analyses of *Burkholderia stabilis* clinical contamination, Switzerland. *Emerg Infect Dis* 2019;**25**:1084–92. https://doi.org/10.3201/eid2506.172119

Song JE, Kwak YG, Um TH *et al*. Outbreak of *Burkholderia cepacia* pseudobacteraemia caused by intrinsically contaminated commercial 0.5% chlorhexidine solution in neonatal intensive care units. *J Hosp Infect* 2018;**98**:295–99. https://doi.org/10.1016/j.jhin.2017.09.012

Tavares M, Kozak M, Balola A *et al*. *Burkholderia cepacia* complex bacteria: a feared contamination risk in water-based pharmaceutical products. *Clin Microbiol Rev* 2020;**33**:e00139–19. https://doi.org/10.1128/CMR.00139-19

United States Pharmacopeia. General Chapter (60) Microbiological Examination of Nonsterile Products-tests for Burkholderia cepacia complex.In: US Pharmacopeia Convention(ed.), *USP-NF* Rockville, MD: US Pharmacopoeia Convention, 2019. https://doi.org/10.31003/USPNF_M12455_02_01

United States Pharmacopeia. General Chapter (61) Microbiological examination of nonsterile products: microbial enumeration tests. In: US Pharmacopeia Convention (ed.), *USP-NF*. Rockville, MD: US Pharmacopoeia Convention 2025. https://doi.org/10.31003/USPNF_M98800_02_01

Vanlaere E, Sergeant K, Dawyndt P *et al*. Matrix-assisted laser desorption ionization-time-of-flight mass spectrometry of intact cells allows rapid identification of *Burkholderia cepacia* complex. *J Microbiol Methods* 2008;**75**:279–86. https://doi.org/10.1016/j.mimet.2008.06.016

Yusuf I, Skiebe E, Wilharm G. Evaluation of CHROMagar Acinetobacter and MacConkey media for the recovery of *Acinetobacter baumannii* from soil samples. *Lett Appl Microbiol* 2023;**76**:ovac051. https://doi.org/10.1093/lambio/ovac051

Received 13 September 2025; **revised** 8 December 2025; **accepted** 9 December 2025