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Evaluation of a novel chromogenic agar for the detection of *Burkholderia cepacia* complex from pharmaceutical and cosmetic samples

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Background

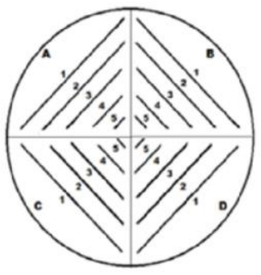
Burkholderia cepacia complex (Bcc) bacteria are opportunistic pathogens that specially affect people with cystic fibrosis and other immunocomprised patients.
These bacteria are also frequent contaminants of industrial products like pharmaceuticals and cosmetics, implying a risk to patients and a concern for Public Health.
Since numerous outbreaks due to contaminated products have been reported worldwide, Bcc absence in drugs has been considered mandatory by different codifications.
For example, the United States Pharmacopoeia recently incorporated testing for detection of Bcc in non-sterile products, USP <60>. Methodology for detecting Bcc usually includes the use of the *Burkholderia cepacia* selective agar (BCSA), a selective but non-chromogenic culture media.
In this work, we evaluate the performance of a novel chromogenic agar, CHROMagar™ B.cepacia (ChromBC) in the detection of Bcc on pharmaceutical and cosmetic samples.

Methods

The performance of ChromBC and BCSA media was compared by evaluating their ability of recovering Bcc and inhibiting other bacteria by means of the Mossel's ecometric method.
A comparison on pure strains was carried out on 25 Bcc and 8 non-Bcc isolates recovered from man-made products and industrial settings.
Recovery of Bcc in industrial products was tested in a cough syrup, a cosmetic cream and purified water. Products were spiked either at low levels of the Bcc strains (*B. contaminans* LMG 23361, *B. cenocepacia* J2315 and *B. cepacia* ATCC® 25416), or at high levels of *Pseudomonas aeruginosa* (*P. aeruginosa* ATCC® 9027), or with a combination of both. After spiking, products were enriched in Trypticase Soy Broth (TSB) for 24h at 35°C and isolated in parallel on BCSA and ChromBC plates. Both media were incubated at 35°C for 24-48 h and observed according to the manufacturer's recommendations.

Echometric method

A score is assigned in order to the maximun growth achieved according to the following scheme (absolute growth index)

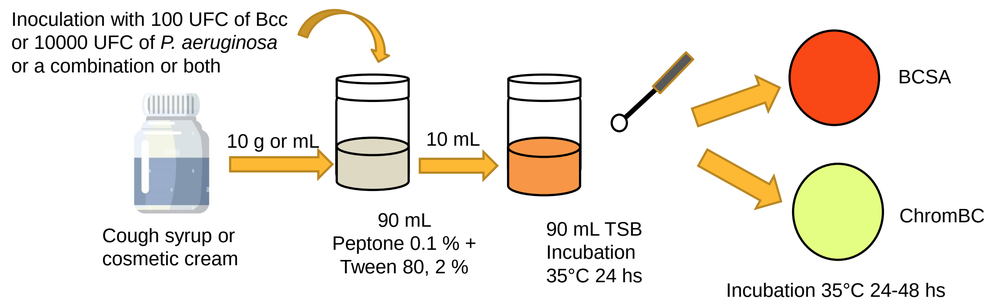


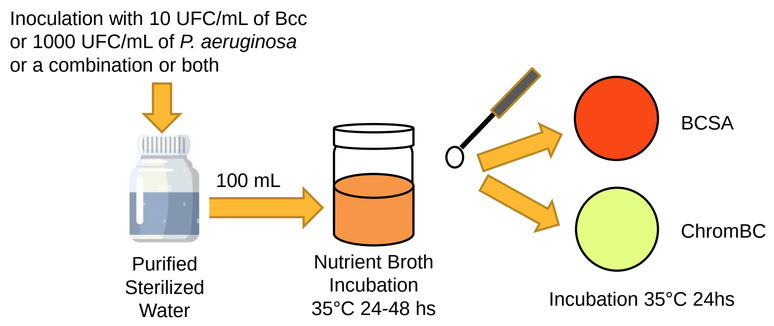
Sector	AGI	Sector	AGI	Sector	AGI	Sector	AGI
A1	5	B1	10	C1	15	D1	20
A2	25	B2	30	C2	35	D2	40
A3	45	B3	50	C3	55	D3	60
A4	65	B4	70	C4	75	D4	80
A5	85	B5	90	C5	95	D5	100

A relative growth index is obtained by comparing the absolute growth index in the studied media with the absolute growth index in Trypticase Soy Agar

Relative growth index: absolute growth index (studied media) X 100
absolute growth media (TSA)

Recovery of Bcc in spiked samples





Results

Ecometric method

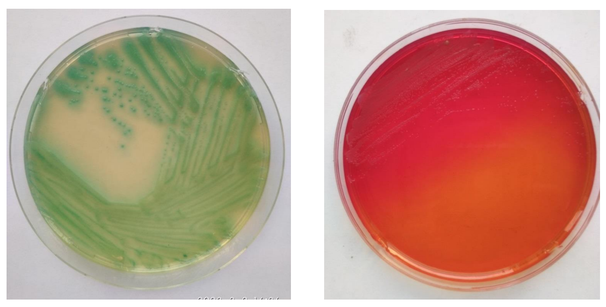
Relative growth Index in Bcc isolates

Microorganism	ChromBC		BCSA	
	24 hs	48 hs	24 hs	48 hs
B contaminans LMG 23361	72,7	100	71.8	100
B cenocepacia J2315	71.2	100	72.5	100
B. cepacia ATCC 25416	38,46	96.7	42.6	97.5
B contaminans FFI6	66.7	100	63.8	100
B. contaminans FFI10	71.7	88.3	85.0	100
B contaminans FFI15	62.8	92.5	58.3	90.0
B contaminans FFI28	24.1	90,3	25.0	93.8
B contaminans FFI29	59.7	100	36.7	98.3
B contaminans FFI30	90.7	100	90.7	100
B contaminans FFI33	50.2	96.2	62.5	92.3
B contaminans FFI34	83.1	100	77.2	96.7
B contaminans FFI36	85.4	100	80.1	100
B contaminans FFI41	71,13	100	52.9	100
B. aenigmatica FFI 4	88,9	98.3	52.8	98.3
B. aenigmatica FFI 17	94.3	100	95.1	100
B. aenigmatica FFI 18	88.86	98.3	67.3	96,5
B. aenigmatica FFI 20	94,3	100	96,7	100
B. aenigmatica FFI 21	63.9	98.3	77.2	100
B cepacia FFI 14	61.1	96.7	67.3	95
B cepacia FFI 38	35,4	100	52.9	96,7
B cepacia FFI 39	71.2	96.8	71.2	96.1
B cepacia FFI 40	69.2	100	61.5	98.3
B vietnamiensis FFI25	37.7	91.7	46.7	96.7
B vietnamiensis FFI26	52.7	90.3	58.3	94.7
B vietnamiensis FFI27	58.9	93.3	53.8	91.7
B arboris FFI 33	71.2	90.0	77.7	90.0
B cenocepacia FFI11	62.5	95	59.8	95
B cenocepacia FFI32	73.8	96.7	66.8	98.3

Relative growth Index in non- Bcc isolates

Microorganism	ChromBC		BCSA	
	24 hs	48 hs	24 hs	48 hs
P aeruginosa FFI101	0.0	0.0	0.0	0.0
P aeruginosa FFI 102	0.0	0.0	0.0	31.7
P. fluorescens FFI110	7.1	26.7	2.9	28.3
P. fluorescens FFI111	22.9	33.3	8,3	16.7
P. putida FFI119	0.0	15.0	0.0	16.7
B gladioli FFI87i	61.3	81.6	41.7	91.6
S. aureus FFI150	0.0	0.0	0.0	0.0
Achromobacter spp. FFI130	0.0	5.0	16.7	57.4

Recovery of *B. contaminans* from cough syrup



ChromBC BCSA

Recovery of Bcc in spiked samples

Cough syrup and cosmetic cream

Cough syrup						Cosmetic cream					
B contaminans LMG 23361		*P aeruginosa*		*P aeruginosa* + *B contaminans*		*B contaminans* LMG 23361		*P aeruginosa*		*P aeruginosa* + *B contaminans*	
Chrom BC	BCSA	Chrom BC	BCSA	Chrom BC	BCSA	Chrom BC	BCSA	Chrom BC	BCSA	Chrom BC	BCSA
+	+	-	-	+	+	+	+	-	-	+	+
B cenocepacia J2315		*P aeruginosa*		*P aeruginosa* + *B cenocepacia*		*B cenocepacia J2315*		*P aeruginosa*		*P aeruginosa* + *B cenocepacia*	
Chrom BC	BCSA	Chrom BC	BCSA	Chrom BC	BCSA	Chrom BC	BCSA	Chrom BC	BCSA	Chrom BC	BCSA
+	+	-	-	+	+	+	+	-	-	+	+
B cepacia ATCC 25416		*P aeruginosa*		*P aeruginosa* + *Bcepacia*		*B cepacia* ATCC 25416		*P aeruginosa*		*P aeruginosa* + *B contaminans*	
Chrom BC	BCSA	Chrom BC	BCSA	Chrom BC	BCSA	Chrom BC	BCSA	Chrom BC	BCSA	Chrom BC	BCSA
+	+	-	-	+	+	+	+	-	-	+	+

Purified water

B contaminans LMG 23361											
Incubation of Nutrient Broth 24 hs						Incubation of Nutrient Broth 48hs					
B contaminans		*P aeruginosa*		*P aeruginosa* + *B contaminans*		*B contaminans*		*P aeruginosa*		*P aeruginosa* + *B contaminans*	
Chrom BC	BCSA	Chrom BC	BCSA	Chrom BC	BCSA	Chrom BC	BCSA	Chrom BC	BCSA	Chrom BC	BCSA
+	+	-	-	+	+	+	+	-	-	+	+
B ccenocepacia J2315											
Incubation of Nutrient Broth 24 hs						Incubation of Nutrient Broth 48hs					
B cenocepacia		*P aeruginosa*		*P aeruginosa* + *B cenocepacia*		*B cenocepacia*		*P aeruginosa*		*P aeruginosa* + *B cenocepacia*	
Chrom BC	BCSA	Chrom BC	BCSA	Chrom BC	BCSA	Chrom BC	BCSA	Chrom BC	BCSA	Chrom BC	BCSA
-	-	-	-	-	-	+	+	-	-	+	+
B cepacia ATCC 25416											
Incubation of Nutrient Broth 24 hs						Incubation of Nutrient Broth 48hs					
B cepacia		*P aeruginosa*		*P aeruginosa* + *B cepacia*		*B contaminans*		*P aeruginosa*		*P aeruginosa* + *B cepacia*	
Chrom BC	BCSA	Chrom BC	BCSA	Chrom BC	BCSA	Chrom BC	BCSA	Chrom BC	BCSA	Chrom BC	BCSA
+	+	-	-	+	+	+	+	-	-	+	+

Conclusions

ChromBC resulted a useful tool for Bcc detection in industrial samples with a better selectivity on non-Bcc strains.

33rd ECCMID EUROPEAN CONGRESS OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES
Copenhagen, Denmark
15–18 April 2023