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Original Article

Biochemical identification techniques and antibiotic susceptibility profile of lipolytic ambiental bacteria from effluents

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Abstract

Different methodologies have been developed throughout the years to identify environmental microorganisms to improve bioremediation techniques, determine susceptibility profiles of bacteria in contaminated environments, and reduce the impact of microorganisms in ecosystems. Two methods of bacterial biochemical identification are compared and the susceptibility profile of bacteria, isolated from residential and industrial wastewater, is determined. Twenty-four bacteria were retrieved from the bacteria bank of the Environmental Microbiology Laboratory at the Institute of Biology (IB) of the Universidade Federal de Pelotas, Pelotas, Brazil. Bacteria were identified by conventional biochemical tests and by the VITEK®2 automated system. Further, the susceptibility profile to antibiotics was also determined by the automated system. Six species of bacteria (*Raoutella planticola*, *K. pneumoniae* ssp. *pneumoniae*, *Serratia marcescens*, *Raoutella* sp., *E. cloacae* and *Klebsiella oxytoca*) were identified by conventional biochemical tests, while three species of bacteria (*K. pneumoniae* ssp. *pneumoniae*, *S. marcescens* and *K. oxytoca*) were identified by VITEK®2 automated system. VITEK®2 indicated agreement in 19 (79.17%) isolates and difference in five (20.83%) isolates when compared to results from conventional biochemical tests. Further, antibiotic susceptibility profile results showed that all isolates (100%) were resistant to at least one out of the 18 antibiotics tested by VITEK®2. Thus, no multi-resistant bacteria that may be used in effluent treatment systems or in bioremediation processes have been reported. Results indicate VITEK ® 2 automated system as a potential methodology in the determination of susceptibility profile and identification of environmental bacteria.

Keywords: environmental bactéria, VITEK®2, *S. marcescens*, microorganisms, biochemistry, multi-resistant bacteria.

Técnicas de identificação bioquímica e perfil de suscetibilidade antibiótica de bactérias ambientais lipolíticas de efluentes

Resumo

Diferentes metodologias foram desenvolvidas ao longo dos anos para identificar microrganismos ambientais para melhorar as técnicas de biorremediação, determinar perfis de suscetibilidade de bactérias em ambientes contaminados e reduzir o impacto de microrganismos nos ecossistemas. Dois métodos de identificação bioquímica bacteriana são comparados e o perfil de susceptibilidade de bactérias, isoladas de efluentes residenciais e industriais, é determinado. Vinte e quatro bactérias foram coletadas do banco de bactérias do Laboratório de Microbiologia Ambiental do Instituto de Biologia (IB) da Universidade Federal de Pelotas, Pelotas, Brasil. As bactérias foram identificadas por testes bioquímicos convencionais e pelo sistema automatizado VITEK®2. Além disso, o perfil de suscetibilidade aos antibióticos também foi determinado pelo sistema automatizado. Seis espécies de bactérias (*Raoutella planticola*, *K. pneumoniae* ssp. *pneumoniae*,

Serratia marcescens, *Raoutella* sp., *E. cloacae* e *Klebsiella oxytoca*) foram identificadas por testes bioquímicos convencionais, enquanto três espécies de bactérias (*K. pneumoniae* ssp. *pneumoniae*, *S. marcescens* e *K. oxytoca*) foram identificados pelo sistema automatizado VITEK®2. VITEK®2 indicou concordância em 19 (79,17%) isolados e diferença em cinco (20,83%) isolados quando comparados aos resultados de testes bioquímicos convencionais. Além disso, os resultados do perfil de suscetibilidade aos antibióticos mostraram que todos os isolados (100%) foram resistentes a pelo menos um dos 18 antibióticos testados pelo VITEK®2. Assim, não foram relatadas bactérias multirresistentes que possam ser usadas em sistemas de tratamento de efluentes ou em processos de biorremediação. Os resultados indicam que o sistema automatizado VITEK ® 2 é uma metodologia potencial na determinação do perfil de suscetibilidade e identificação de bactérias ambientais.

Palavras-chave: bactérias ambientais, VITEK®2, *S. marcescens*, microorganismos, bioquímica, bactérias multirresistentes.

1. Introduction

Several anthropic activities are increasing residual levels worldwide. There is great concern on soil and water due to increasing pollution as a result of different industrial, mineralization, agriculture and domestic processes everywhere (Ren et al., 2009). Main environmental impacts by organic solid wastes originate from fermentation of waste. The fermentation produces organic acid formation, contributing towards unpleasant odors and decrease of oxygen in the water, with damages to water animals (Graminha et al., 2008).

Further, other types of wastes, such as oil and fat effluents from residences, restaurants and food industries, are on the increase due to the human feeding processes. The lipids, indissoluble in water and highly stable, in the effluents are the result of industrial activities and are compounds that greatly impact the environment (Hasan et al., 2006; Odeyemi et al., 2013). These contaminants remain on the water surface in the form of foams and bubbles (Olivo et al., 2010; Polonio et al.,2014).

The microorganisms' metabolic process is responsible for the removal of contaminants (Mandri and Lin, 2007) by reducing their concentration (Bello, 2007) or by transforming pollutants into compounds with low toxicity (Colla and Costa, 2003; Mongkolthanaruk and Dharmsthiti, 2002).

During decades, bacteria have been routinely identified in laboratories by biochemical tests and by microscopic identification, which, as a rule, need time for their execution, ranging from a couple of hours to several days (Martiny et al., 2012). Since bacteria do not have enough morphological characteristics to pinpoint their peculiarities, several methodologies have been developed to test their metabolic or enzymatic reactions, enabling grouping and identification at genus or species level (Busch and Nitschko, 1999; Romeu et al., 2010).

Conventional analyses are time-consuming techniques and semi-automatic methods require great amounts of biologic material. In fact, the above constitute significant disadvantages for the identification of microorganisms (Martiny et al., 2012).

The VITEK (bioMérieux) System is a useful tool to identify different groups of bacteria by a miniaturized biochemical test with specific cards containing 64 fluorescent biochemical tests. Although the existence of few research works reporting the use of this equipment to identify bacteria from the environment, good results have been obtained in the microorganism's identification in different ecosystems (Song and Leff, 2005; Mendes et al., 2018).

These bacteria should be studied and evaluated for their use as bioremediators, as there is an increasing need for environmental protection by contaminants that are harmful to health. However an important consideration is the knowledge about the susceptibility profile to antibiotics of these environmental bacteria so that they can be disseminated in the environment.

Antibiotic-resistant bacteria already widespread in the environment as a result of the indiscriminate use of antibiotics can be detected in different environments, such as hospital sewage, domestic sewage and contaminated river water, transforming effluents into very aggressive factors for the environment (Abreu et al., 2010).

Due to its importance in the identification of environmental bacteria and determination of their susceptibility profile, the current research aimed to compare two techniques of identification: conventional and automated and to evaluate the susceptibility to antibiotics of environmental bacteria in the region of Pelotas RS Brazil.

2. Materials and Methods

2.1. Sample origin and processing

A study by Peil et al. (2016) verified the lipolytic characteristics of the isolated bacteria used in current research. These bacteria were stored in the Laboratory of Environmental Microbiology of the Biology Institute of UFPel.

Twenty-four Gram-negative bacteria were selected: five residues (isolates 1, 2, 3, 4 and 5), five commercial isolates (isolates 6, 7, 8, 9 and 10) and 14 of industrial effluent isolates).

The final identification obtained by each technique was compared individually considering that the equivalence above 90% is satisfactory (Bamford et al., 2010).

2.2. Identification and morphologic and biochemical characterization

In order to confirm the cellular morphology and classification of the bacteria, the Gram staining technique, with 100X optical microscopy visualization, should be used to determine the shape, arrangement and classification of Gram-negative bacteria. Biochemical tests and identification

cards selected to compare as two identification techniques: conventional and automated (VITEK®2).

2.3. Conventional biochemical tests

After phenotypic colony and cell identification through Gram staining by microscopic visualization were performed, the conventional biochemical tests were made to determine the isolated bacteria classification according to the Gram-negative bacterial identification protocol (Barrow and Feltham, 1993).

The conventional biochemical tests were: GS = Gram staining; OX = Oxidase; Mc = MacConkey medium; CI = Citrate; SIM = SIM medium; AR = Arabinose; CE = Cellobiose; RA = Raffinose; XY = Xylose; VP = Voges-Proskauer; LAC = Lactose; OR = Ornithine; DU = Dulcitol.

2.4. VITEK®2 System

Biochemical analyses were performed by VITEK®2 automated system. The isolated bacteria were inoculated in BHI solid culture and incubated for 24 hours at 35 °C. A homogeneous suspension with an optic density of microorganisms between 0.55 and 0.63, using a DensiCHEK™ VITEK®2 Caliper, was performed for the preparation of the samples. Samples were then analyzed by VITEK®2. Forty-eight biochemical tests were performed with Gram-negative bacterium identification cards.

The VITEK®2 biochemical tests are described in Table 1.

2.5. Antibiotic susceptibility profile

The antibiotic susceptibility profile was also determined by VITEK®2 automated system. An aliquot of 145µL of cellular suspension, with an optic density between 0.55 and 0.63, was added in 3mL saline solution at 0.85%. Samples were analyzed by VITEK®2 which indicated resistance or sensibility shown by the bacteria through several antibiotic concentrations in the anti-biogram cards. Susceptibility profiles were performed with 18 antibiotics in the specific anti-biogram AST cards, specific for Gram-negative bacteria.

Os antibióticos testados pelo Sistema VITEK®2 nas bactérias gram-negativas foram: Ampicilina, Ampicilina/Sulbactam, Ppiperacilina/Tazobactam, Cefuroxima, Cefuroxima axetil, Cefoxitina, Ceftazidima, Ceftriaxona, Cefepima, Ertapnem, Imipenem, Meropenem, Amicacina, Gentamicina, Ciprofloxacina, Tigeciclina e Colistina.

3. Results

3.1. Biochemical Conventional Identification

Biochemical tests were performed on the 24 bacterial isolates, identified as Gram-negative, bacillus and cocci, to identify the genus and the species of the Gram-negative bacteria, following Barrow and Feltham (1993).

In the case of the 24 analyzed bacteria, the biochemical conventional tests revealed six species of bacteria (Table 2), namely, *Raoutella planticola* (1 isolate – 4.17%), *K. pneumoniae* ssp. *pneumoniae* (1 isolate – 4.17%), *Serratia marcescens* (16 isolates – 66.67%), *Enterobacter cloacae* (1 isolate – 4.17%), *Raoutella* sp. (2 isolates – 8.33%) and *Klebsiella oxytoca* (3 isolates – 12.5%).

3.2. Biochemical identification by VITEK® Automated System

Gram-negative bacterial identification cards were used, specific for Gram-negative bacteria. Three out of the 24 isolated bacteria species were identified by VITEK®2, namely *K. pneumoniae* ssp. *pneumoniae* (2 isolates – 8.33%), *S. marcescens* (17 isolates – 70.83%) and *K. oxytoca* (5 isolates – 20.83%). Characterization was made by biochemical tests of the automatized system, described in Table 3.

3.3. Antibiotic susceptibility profiles

The antibiotic susceptibility profiles of all 24 isolated bacteria were determined by VITEK®2 automated system and by anti-biogram AST cards, specific for Gram-negative bacteria.

Results showed that all isolates (100%) were resistant to at least one of the 18 antibiotics tested by VITEK®2 automated system. All the identified bacteria of the same species showed the same susceptibility profile.

4. Discussion

4.1. Biochemical conventional identification and biochemical identification by VITEK®2 Automated System

Specific literature has shown that a great number of bacteria found in the effluent treatment systems are Gram-negative (Kanwar and Goswami, 2002). The most common are genus *Acinetobacter* spp. (Lima Junior, 2009), *Aeromonas* spp. (Ogino et al., 2000), *Pseudomonas* spp. (Sugimori et al., 2002), *Burkholdeira* spp. and *Serratia* spp. (Mohanan et al., 2007).

VITEK®2 Automated System showed concordance within 19 isolates (79.17%) and disagreement for five isolates (20.83%) (Table 4), when compared to results obtained in conventional biochemical tests. Current concordance results were different from those obtained by Bamford et al. (2010) when similar methodologies were compared. Clinical isolates environments.

All diagnoses when the two methodologies were used are described in table 4. The first isolate that presented a difference in the identification was Bacterium 1 that in the conventional biochemical tests identified as *K. oxytoca*, whilst the VITEK®2 Automated System identified it as *K. pneumoniae* ssp. *pneumoniae*. For the differentiation of the two species, the degradation of ornithine, dulcitol and arabinose was made by conventional biochemical tests and, in turn, ornithine and *5-ketogluconate* degradation was analyzed by VITEK®2 Automated System. These characteristics were studied by Drancourt et al. (2001) to differentiate species from *Klebsiella* and *Raoutella* genera.

K. oxytoca (isolate 13), identified belonging to the *Enterobacteriaceae* family were used in the above-mentioned study. The authors determined a higher concordance (93%) in the results obtained when compared with those in our

Table 1. Substrates present in the colorimetric identification card specific for Gram bacteria Fermenting or non fermenting (NG) negatives used in the VITEK 2 system.

Well	Teste	Mnemonic	Amount/well (mg)
2	Ala-Phe-Pro-ARYLAMIDASE	APPA	0.384
3	ADONITOL	ADO	0.1875
4	L-Pyrrolydonyl-ARILAMIDASE	PyrA	0.018
5	L-Arabitol	IARL	0.3
7	D-CELLOBIOSE	dCEL	0.3
9	BETA-GALACTOSIDASE	BGAL	0.036
10	H2S PRODUCTION	H2S	0.0024
11	BETA-N-ACETYL-GLUCOSAMINIDASE	BNAG	0.0408
12	GlutamylArylamidase pNA	AGLTp	0.0324
13	D-Glucose	dGLU	0.3
14	GAMMA-GLUTAMYL-TRANSFERASE	GGT	0.0228
15	FERMENTATION?GLUCOSE	OFF	0.45
17	BETA-GLUCOSIDASE	BGLU	0.036
18	D-MALTOSE	Dmal	0.3
19	D-MANNITOL	Dman	0.1875
20	D-MANNOSE	Dmne	0.3
21	BETA-XYLOSIDASE	Bxyl	0.0324
22	BETA-ALANINEARYLAMIDASE pNA	BaLAP	0.0174
23	L-Proline ARYLAMIDASE	ProA	0.0234
26	LIPASE	LIP	0.0192
27	PALATINOSE	PLE	0.3
29	Tyrosine ARYLAMIDASE	TyrA	0.0276
31	UREASE	URE	0.15
32	D-SORBITOL	dSOR	0.1875
33	SACCHAROSE/SUCRALOSE	SAC	0.3
34	d-TAGATOSE	Dtag	0.3
35	D-TREHALOSE	Dtre	0.3
36	CITRATE (SODIUM)	CIT	0.054
37	MALONATE	MNT	0.15
39	5-KETO-D-GLUCONATE	5KG	0.3
40	L-LACTATE alkalinisation	ILATk	0.15
41	ALPHA-GLUCOSIDASE	AGLU	0.036
42	SUCCINATE alkalinisation	SUCT	0.15
43	Beta-N-ACETYL-GALACTOSAMINIDASE	NAGA	0.0306
44	ALPHA-galactosidase	AGAL	0.036
45	PHOSPHATASE	PHOS	0.0504
46	Glycine Arylamidase	GlyA	0.012
47	ORNITHINE DECARBOXYLASE	ODC	0.3
48	LYSINE DECARBOXYLASE	LDC	0.15
52	DECARBOXYLASE bASE	ODEC	N/A
53	L-HISTIDINE assimilation	IHISa	0.087
56	COUMARATE	CMT	0.126
57	BETA-GLUCORONIDASE	BGUR	0.0378
58	O/129 RESISTANCE	O129R	0.0105
59	Glu-Gly-Arg-ARYLAMIDASE	GGAA	0.0576
61	L-MALATE assimilation	IMLTa	0.042
62	ELLMAN	ELLM	0.03
64	L-LACTATE assimilation	ILATa	0.186

Table 2. Results of biochemical tests performed to characterize genus of 24 Gram-negative bacteria isolated from residential, commercial and industrial effluents of region of Pelotas – RS.

BACTÉRIAS	PROVAS BIOQUÍMICAS													
	CG	OX	Mc	CI	SIM	AR	CE	RA	XI	VP	LAC	OR	DU	Caracterização bacteriana
1	Gram -	-	+	+	-+-	+	+	+	+	+	+	-	+	*K. oxytoca*
2	Gram -	-	+	+	--+	-	d	-	d	+	-	+	-	*S. marcescens*
3	Gram -	-	+	+	-+-	+	+	+	+	+	+	-	+	*K. oxytoca*
4	Gram -	-	+	+	--+	-	d	d	-	+	-	+	-	*S. marcescens*
5	Gram -	-	+	+	--+	-	-	-	-	+	-	+	-	*S. marcescens*
6	Gram -	-	+	+	-+-	+	+	+	+	+	+	+	-	*Raoutella* sp.
7	Gram -	-	+	+	--+	-	-	-	-	+	-	+	-	*S. marcescens*
8	Gram -	-	+	+	--+	-	-	-	-	+	-	+	-	*S. marcescens*
9	Gram -	-	+	+	--+	-	+	-	-	+	-	+	-	*S. marcescens*
10	Gram -	-	+	+	--+	-	d	-	d	+	-	+	-	*S. marcescens*
11	Gram -	-	+	+	--+	-	d	-	d	+	-	+	-	*S. marcescens*
12	Gram -	-	+	+	--+	-	-	-	d	+	-	+	-	*S. marcescens*
13	Gram -	-	+	+	-+-	+	+	+	+	+	+	-	+	*K. oxytoca*
14	Gram -	-	+	+	--+	-	-	-	d	+	-	+	-	*S. marcescens*
15	Gram -	-	+	+	--+	-	-	-	-	+	-	+	-	*S. marcescens*
16	Gram -	-	+	+	--+	-	-	d	d	+	d	+	-	*S. marcescens*
17	Gram -	-	+	+	--+	-	-	-	-	+	-	+	-	*S. marcescens*
18	Gram -	-	+	+	--+	-	-	-	-	+	-	+	d	*S. marcescens*
19	Gram -	-	+	+	--+	+	+	-	-	+	-	+	-	*E. cloacae*
20	Gram -	-	+	+	-+-	+	+	+	+	+	+	+	+	*Raoutella* sp.
21	Gram -	-	+	+	--+	-	d	-	d	+	-	+	-	*S. marcescens*
22	Gram -	-	+	+	--+	-	-	-	-	+	-	+	-	*S. marcescens*
23	Gram -	-	+	+	---	-	+	+	+	+	+	+	-	*K. pneumoniae*
24	Gram -	-	+	+	-+-	+	+	+	+	+	+	-	-	*R. planticola*

study, perhaps due to differences between isolated bacteria retrieved from different by VITEK®2, showed negative ornithine, positive dulcitol and positive arabinose results, in agreement with results from the conventional tests for isolate 13 (*K. oxytoca*). On the other hand, biochemical test results of *K. pneumoniae* ssp. *pneumoniae* demonstrated positive ornithine, negative dulcitol and negative arabinose, disagreeing with results obtained in the conventional biochemical tests, but agreeing with the results obtained by VITEK®2.

The two species from *Raoutella* genera, *Raoutella planticola* and *Raoutella* sp, identified by biochemical tests, showed discrepancy when compared with result obtained from VITEK®2 Automated System, which identified all isolates as bacteria of species *K. oxytoca*.

Based on the 16s rRNA *rpoB* gene molecular sequence analysis, Drancourt et al. (2001) proposed that *Klebsiella* had to be divided into two genera, *Klebsiella* and *Raoultella*, and that *K. oxytoca* should be left as a monophyletic species. Beyond molecular analysis, the authors determined several differences between species from these genera, based on the biochemical test.

K. oxytoca bacterium is characterized by dulcitol, L-Tartrate and 3-O-Methyl-D-glucose negative degradation. However, bacteria *Raoutella ornithinolytica*, *Raoutella planticola* and *Raoutella* sp. are dulcitol and L-Tartrate negative and 3-*O*-Metil-*D*-glucose positive. In other words, they are typical characteristics that separate *K. oxytoca* from *Raoutellas* sp. (Drancourt et al., 2001).

Current research reveals that, through the use of conventional biochemical tests, *Klebsiella* and *Raoutella* should be separated, due to dulcitol degradation. VITEK®2 failed to perform the test. Conventional biochemical tests identified them as belonging to genus *Raoutella*. Results explain the discrepancy between the two methodologies used.

In the case of the 24 isolates, the two identification methodologies tests provided different results. The conventional biochemical tests identified the isolates as *Enterobacter cloacae* and the VITEK®2 automated system identified them as *Serratia marcescens*. In the biochemical tests, *Enterobacter cloacae* and *Serratia marcescens* were differentiated by arabinose and cellobiose degradation, although VITEK®2 only evaluated the cellobiose degradation.

Results demonstrated differences between the performed tests by VITEK®2 and conventional tests with regard to cellobiose degradation. Bacterium *Enterobacter cloacae* was cellobiose positive by conventional biochemical tests, whilst bacterium *Serratia marcescens* was cellobiose

Table 3. Biochemical tests carried out to characterize 24 Gram negative bacteria isolated from effluents from food industries in the region of Pelotas - RS through VITEK®2 Automated System.

	Test	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	Ala-Phe-Pro-ARYLAMIDASE (APPA)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	ADONITOL (ADO)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	L-Pyrrolydonyl-ARYLAMIDASE (Pyra)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	L-ARABITOL (IARL)	-	+	+	+	+		+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+
5	D-CELLOBIOSE (Dcel)	+	-	+	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	+	+
6	BETA-GALACTOSIDASE (BGAL)	+	-	+	-	-	+	-	-	-	-	-	-	+	-	-	+	-	-	-	+	-	-	+	+
7	H_2S PRODUCTION (H_2S)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	BETA-N-ACETYL-GLUCOSAMINIDASE (BNAG)	-	+	-	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	-
9	Glutamyl Arylamidase pNA (AGLTp)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	D-GLUCOSE (Dglu)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
11	GAMMA-GLUTAMYL-TRASFERASE (GGT)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
12	FERMNTATION/ GLUCOSE (OFF)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
13	BETA-GLUCOSIDASE (BGLU)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
14	D-MALTOSE (dMAL)	+	+	+	+	+	+	-	-	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
15	D-MANNITOL (dMAN)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
16	D-MANNOSE (dMNE)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
17	BETA-XYLOSIDASE (BXYL)	-	-	+	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	+	+
18	BETA-Alanine arylamidase pNA (BAlap)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	L-Proline ARYLAMIDASE (ProA)	-	+	-	+	+	-	+	+	+	+	+	+	-	+	+	+	+	-	+	-	+	-	-	-
20	LIPASE (LIP)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	PALATINOSE (PLE)	+	-	+	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	+	+
22	Tyrosine ARYLAMIDASE (TyrA)	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
23	UREASE (URE)	-	-	+	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	+
24	D-SORBITOL (dSOR)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
25	SACCHAROSE/ SUCROSE (SAC)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
26	D-TAGATOSE (dTAG)	+	-	+	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	+	-
27	D-TREHALOSE (dTRE)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
28	CITRATE (SODIUM) (CIT)	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 3. Continued...

	Test	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
29	MALONATE (MNT)	+	-	+	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	+	+
30	5-KETO-D-GLUCONATE (5KG)	-	+	+	(+)	+	+	-	-	-	(-)	-	-	+	(-)	+	+	(-)	(+)	+	+	+	(-)	-	+
31	L-LACTATE alkalinisation (ILATk)	+	+	-	-	+	+	-	-	+	-	-	+	+	+	-	-	+	+	+	+	-	-	+	+
32	ALPHA-GALACTOSIDASE (AGLU)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
33	SUCCINATE alkalinisation (SUCT)	-	+	-	-	+	+	-	-	-	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+
34	Beta-N-ACETYL-GALACTOSAMINIDASE (NAGA)	-	+	-	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	-	-
35	ALPHA-GALACTOSIDASE (AGAL)	+	-	+	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	+	+
36	PHOSPHATASE (PHOS)	-	-	+	-	-	+	-	-	+	-	-	-	+	-	-	-	-	-	+	+	-	-	+	+
37	Glycine ARYLAMIDASE (GlyA)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
38	ORNITHINE DECARBOXYLASE (ODC)	-	+	-	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	-	-
39	LYSINE DECARBOSYLASE (LDC)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
40	L-HISTIDINE assimilation (IHISa)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41	COUMARATE (CMT)	+	+	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-
42	BETA-GLUCORONIDASE (BGUR)	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43	O/129 RESISTANCE (comp.vibrio) (0129R)	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	-	+	+
44	Glu-Gly-Arg-ARYLAMIDASE (GGAA)	-	+	-	+	-	-	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	-	-
45	L-MALATE assimilation (IMLTa)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
46	ELLMAN (ELLM)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
47	L-LACTATE assimilation (ILATa)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

negative. This fact corresponds to result by VITEK®2 and explains the difference in results obtained by the two methodologies.

The principal failures in VITEK®2 GNI card comprised carbohydrates, arabinose, cellobiose, sorbitol and arginine decarboxylation in all bacterial species (D'Azevedo et al., 2004).

A failure of a biochemical assimilation, due to a little inoculum or other factor can generate a different result that changes the final result.The conventional biochemical characterization performed in current study failed to reach up to the species identification of two of the 24 (8.33%) identified isolates of the genus *Raoutella* sp. after researching the schemes proposed in the literature. The schemes used by different authors were based on isolates from clinical sources and little is known about the biochemical characterization of environmental isolates (Funke et al., 1997).

A crucial difficulty, associated with the bacterial species identification based on phenotypic characteristics, relies on

Table 4. Comparison of the results of biochemical techniques of bacterial identification of bacteria isolated from effluents.

N Isolated	Conventional test	VITEK®2
1	*K. oxytoca*	*K. pneumoniae* ssp *pneumoniae*
2	*S. marcescens*	*S. marcescens*
3	*K. oxytoca*	*K. oxytoca*
4	*S. marcescens*	*S. marcescens*
5	*S. marcescens*	*S. marcescens*
6	*Raoutella* sp.	*K. oxytoca*
7	*S. marcescens*	*S. marcescens*
8	*S. marcescens*	*S. marcescens*
9	*S. marcescens*	*S. marcescens*
10	*S. marcescens*	*S. marcescens*
11	*S. marcescens*	*S. marcescens*
12	*S. marcescens*	*S. marcescens*
13	*K. oxytoca*	*K. oxytoca*
14	*S. marcescens*	*S. marcescens*
15	*S. marcescens*	*S. marcescens*
16	*S. marcescens*	*S. marcescens*
17	*S. marcescens*	*S. marcescens*
18	*S. marcescens*	*S. marcescens*
19	*E. cloacae*	*S. marcescens*
20	*Raoutella sp.*	*K. oxytoca*
21	*S. marcescens*	*S. marcescens*
22	*S. marcescens*	*S. marcescens*
23	*K. pneumoniae*	*K. pneumoniae* ssp *pneumoniae*
24	*R. planticola*	*K. oxytoca*

the occurrence of divergence or convergence. Divergence occurs in some species lineages, genetically identical, but with different phenotypic characteristics. Convergence occurs in different species lineages, genetically different, but with a similar phenotypic behavior. In both situations, the phenotypic test resulted in a wrong identification (Siqueira Junior and Roças, 2005).

Although biochemical tests revealed high efficiency in environmental bacteria identification, as in *Serratia marcescens*, a convergence possibility in the tests cannot be discarded, according to the literature (Siqueira Junior and Roças, 2005), leading to discrepancies when conventional biochemical identification methods are compared.

The isolation and identification of new microorganisms with high enzyme production potential are greatly important since they exhibit a wide applicability and are efficient in the bioremediation process of contaminated places, beyond the efficient effluent treatment with high concentrations of oil and fats (Gopinath et al., 2013). A better development of identification techniques compatible with Environmental Microbiology laboratories is required.

4.2. Antibiotic susceptibility profiles

The antibiotic resistance in current analysis was high when compared to results by Zambrano et al. (2002). When these authors determined the antibiotic susceptibility profile from bacteria isolated from residential effluents, they reported that 90% of *Enterobacteriaceae* bacteria showed resistance to at least one antibiotic.

All isolates identified as *S. marcescens* (100%) showed resistance to three kinds of antibiotics (cefuroxime, cefuroxime (axetil) and colistin), two of them belonging to the cephalosporin group (cefuroxime and cefuroxime (axetil)) in MIC>=16 and showed a sensitivity to the other antibiotics tested by VITEK®2 system.

Meanwhile, the bacterium identified as *K. pneumonia* spp. *pneumonia* showed resistance to two antibiotics (ampicillin in MIC=16 and colistin in a MIC=4) and was sensitive to the other antibiotics tested by VITEK®2 automated system. All isolates identified as *K. oxytoca* were resistant to one antibiotic (ampicillin) in MIC=16 and were sensitive to all other antibiotics tested by VITEK®2 automated system.

Results contradicted those by Pereira (2013) who evaluated the antibiotic susceptibility profile for the following bacteria species: *K. pneumonia*, *K. oxytoca* and *S. marcescens*, isolated from Municipal Water Supply System of São José do Rio Preto SP Brazil.. The species failed to show any resistance to the antibiotics under analysis.

Current study showed a prevalent resistance to cephalosporin which belongs to the β-lactam class and interacts with penicillin-binding proteins (PBP), preventing the bacteria cell wall formation. Cephalosporin is one of the most common antibiotics used in different areas, such as human and veterinary medicine. Available for a long

period on the market, the antibiotic ultimately has made selecting disseminating resistance factors or ending in selecting disseminating resistance factors. When antibiotics were discarded in the environment, they may be related to resistant microorganisms against these antibiotics (Oliveira, 2011).

In current study, it was not possible to observe a multi-resistant bacteria (Table 5) being a good microorganism characteristic being useful in the effluents treatment systems or in the bioremediation process. Several authors underscored that environmental multi-resistant bacteria populations, especially those in a contaminated environment, may increase due to the presence of resistant bacteria in industrial and hospital effluents and to the different types of genetic material exchange (Andersen, 1993), such as the horizontal gene transfer, providing an important risk to the human health.

Few studies are extant on the bacteria´s susceptibility profile in effluents (Baquero et al., 2008). Li et al. (2009) isolated bacteria from effluents of a penicillin producer industry and evaluated their susceptibility profile. The authors underscored that some species of the *Enterobacteriaceae* family did showed penicillin resistance.

Li et al. (2010) determined the presence of oxytetracycline-resistant bacteria in effluents from industries producers of this antibiotic and also from a river in which the effluent was released. Analyses revealed the presence of species of the genus *Klebsiella* spp. and other oxytetracycline-resistant enterobacteria species.

Antibiotic-resistant bacteria reach the aquatic ecosystems through the activities of animals and people. These bacteria can capture resistant genes from carrier bacteria (Alonso et al., 2001). Further, many antibiotics from the industry are present in effluents that may reach aquatic ecosystems and disturb the original microbiota (Cabello, 2006).

Bacteria, including those in a natural environment without antibiotics, may transport a great number of resistance genes (Allen et al., 2009). It is difficult to explain the role of antibiotics in natural ecosystems from an anthropocentric point of view, since they are clinically based, or rather, an antibiotic efficiency to treat a particular infection or the removal of a resistance pathogenic agent.

To conclude about the importance of studying environmental bacteria and their susceptibility to antibiotics, this this work shows that to obtain efficient bacteria for the bioremediation process, some characteristics from isolated microorganisms should be taken into account. One characteristic is its identification to verify its pathogenicity and another the probability of dispersion

Table 5. Profile of susceptibility of bacteria of environmental origin identified by the VITEK2 System against antibiotics.

Isolado	Ampicilina	Cefuroxima	Cefuroxoma Axcetil	Colistina	Piperacilina tazobactam
1	R	S	S	R	S
2	-	R	R	R	S
3	R	S	S	S	S
4	-	R	R	R	S
5	-	R	R	R	S
6	R	S	S	S	S
7	-	R	R	R	S
8	-	R	R	R	S
9	-	R	R	R	S
10	-	R	R	R	S
11	-	R	R	R	S
12	-	R	R	R	S
13	R	S	S	S	S
14	-	R	R	R	S
15	-	R	R	R	S
16	-	R	R	R	S
17	-	R	R	R	S
18	-	R	R	R	S
19	-	R	R	R	S
20	R	S	S	S	S
21	-	R	R	R	S
22	-	R	R	R	S
23	R	S	S	S	S
24	R	S	S	S	S

S= Sensitive; R=Resistant. Amicacina, Gentamicina, Cefepima, Cefoxitina, Ceftazidima, Ciprofloxacina, Tigeciclina, Ceftriaxona, Ampicilina Sulbactam, Meropenem, Ertapenem e Imipinem- All isolated were sensitive.

of multi resistant bacteria. Coliform bacteria are a high diversity heterogeneous group in terms of genera and species, all belonging to the *Enterobacteriaceae* family (Edberg et al., 2000).

The microorganism's metabolism has the capacity to eliminate residues and to produce useful substances. Therefore, biodegradation consists in an important event to improve oil pollution in the environment, minimizing the environmental impact (Gopinath et al., 2013). Consequently, several microorganisms may be efficient in the treatment of contaminated places due to their biotechnological applications.

It can be concluded that the VITEK®2 automated system showed agreement on almost 80% of the samples identified and characterized by conventional identification systems. It is a system in question to identify environmental bacteria, but not satisfactory (over 90%). The antibiotic susceptibility profile of bacteria isolated from effluents indicated resistance to at least one of the two antibiotics tested. And the absence of multiresistance and an important feature of isolated lipolytic bacteria that may possibly be used in effluent processes of residential, commercial and industrial bioremediation with high levels of lipids.

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