Ochrobactrum anthropi, contaminants of *in vitro* culture of sugarcane cells and tissues

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ABSTRACT

The purpose of this paper was to report, for first time, the presence of *Ochrobactrum anthropi* as contaminant of *in vitro* cell and tissue culture of sugarcane. Bacteria contaminant of *in vitro* plants were isolated directly from visibly contaminated plant culture on Tryptone soy agar medium and some drops of sugarcane cell suspension contaminated were inoculated on tubes with 3ml of Tryptone Soy Broth (TSB). Isolated bacteria were examined microscopically (shape, motility) and some biochemical test (Gram´s stain (Hucker´s modification), oxidase, catalase, O/F (oxidation/fermentation), were performed. Additional tests, according to Bergey´s Manual of Determinative Bacteriology, were performed. Identification was complemented by fatty acid analysis. Different bacteria can be contaminating plant cell and tissue culture. However, references about the presence of *Ochrobactrum anthropi* as contaminant of *in vitro* culture of plant cell and tissue have not been reached. For controlling microbial contaminants, is necessary to identify it.

Key words: bacterial contamination,cell suspension, identification, *in vitro* plants, *Saccharum*

RESUMEN

El propósito de este trabajo fue informar, por primera vez, la presencia de *Ochrobactrum anthropi* como contaminante del cultivo *in vitro* de células y tejidos de caña de azúcar. La bacteria contaminante de las plantas *in vitro* se aisló directamente en el medio de cultivo Agar Triptona soya a partir de plantas visiblemente contaminadas y algunas gotas de suspensiones celulares contaminadas se inocularon en tubos de ensayo con 3ml de Caldo Triptona soya. Las bacterias aisladas se examinaron microscópicamente (forma, motilidad) y mediante prueba bioquímicas (tinción de Gram; modificación de Hucker), oxidasa, catalasa, O/F (oxidación/fermentación). Otras pruebas adicionales fueron realizadas de acuerdo con lo descrito en el Manual de Bergey. La identificación se complementó por análisis de ácidos grasos. Diferentes bacterias pueden aparecer contaminando el cultivo *in vitro* de células y tejidos vegetales, sin embargo, no se han encontrado referencias sobre la presencia de *Ochrobactrum anthropi* como contaminante del cultivo *in vitro* de caña de azúcar. Para controlar los contaminantes bacterianos es necesario primero identificarlos.

Palabras clave: contaminación bacteriana, identificación, plantas *in vitro*, *Saccharum*, suspensiones celulares

INTRODUCTION

Different bacteria can be contaminating plant cell and tissue culture. Species of the genera *Pseudomonas, Bacillus, Enterobacter, Klebsiella, Staphylococcus, Lactobacillus*, etc. (Leifert and Cassells, 2001) have been identified frequently.

Ochrobactrum, formerly known as CDC group Vd, is an oxidase-positive, nonfermenting, gram-negative bacillus that was first described by Holmes *et al.* (1988) with a single species, *Ochrobactrum anthropi*. Hospital environments (Bizet and Bizet, 1995), activated sludge (Danilo *et al.,* 1996), seawater samples (Croci *et al.*, 2001), plant rhizosphere (Tripathi *et al*., 2002) or root nodules (Ngom *et al.,* 2004) have been frequently places to be found. However, references about the presence of this bacterial specie as a contaminant of *in vitro* culture of plant cell and tissue have not been reported.

Development of *in vitro* techniques in sugarcane has resulted in more efficient and effective means for international exchange of germoplasm (Taylor and Dukic, 1993;). According to Dookun (1998) and Lakshmanan *et al.* (2005) the application of biotechnology to sugarcane has a major role to play in increasing the productivity of this crop. Bacteria constitute the most common and troublesome kind of contaminating microorganism in plant tissue culture (George, 1993), however, only few authors (Taylor and Dukic; 1993; Moutia and Dookun, 1999) described the problems of bacterial contamination on sugarcane tissue culture. For controlling microbial contaminants, is necessary to identify it.

The purpose of this study was to report for first time the presence of *Ochrobactrum anthropi* as contaminant of sugarcane cell and tissue culture.

MATERIAL AND METHODS

Plant material

In vitro sugarcane (*Saccharum* spp. hybrid) plants, variety C 87-51 in multiplication stage (on culture medium containing Murashige and Skoog salts (Murashige and Skoog, 1962) supplemented with 0.3mg.l^{-1}6-benzylaminopurine, 1.0mg.l^{-1} tiamine, 30g.l^{-1} sucrose and solidified with 7g. l^{-1} agar) visibly contaminated by bacteria were used. Besides, sugarcane cell suspension, cultured as indicated by Freire *et al*. (2002) and previously detected as contaminated by microscopically observation, were employed.

Isolation and identification

Bacteria contaminants of *in vitro* plants were detected as whitish exudates around the base of *in vitro* plants. They were isolated directly from visibly contaminated plant culture by streaking of bacterial growth on Tryptone soy agar (TSA) medium. Plates were incubated at 30°C for 24-72h. Final purification was obtained by restreaking single colonies on similar medium and were maintained by transferring them to TSA slants for short-term storage and by preparing the inocula for further identification test.

Some drops of contaminated sugarcane cell suspension were inoculated on tubes with 3ml of Tryptone Soy Broth (TSB). Tubes were incubated in static conditions at 30°C for 24-72h during a week. Purification was developed by the same protocol those bacterial isolates from *in vitro* plants.

Cultural characteristics of bacterial growth in bacteriological media (colony shape, colour, borders, consistency, optical and surface characteristics) were recorded.

Isolated bacteria were examined microscopically (shape, motility) and some biochemical test (Gram´s stain (Hucker´s modification), oxidase, catalase, O/F (oxidation/fermentation), were performed according to Lelliot and Stead (1987) and Klement *et al.* (1990).

Additional test were performed according to Bergey´s Manual of Determinative Bacteriology (Krieg and Holt, 1994), and identification was complemented by fatty acid analysis, following the protocol described by manufacturer (MIDI, Sherlock, Microbial Identification System, version 3.0, 1999). Fatty acid profiles were compared using MIDI library software.

RESULTS AND DISCUSSION

Two typical contaminants were isolated from each type of plant material (strain CCIBP-M29 from *in vitro* plant and strain CCIBP-Sp108 from cell suspensions).

Both strains produced colonies on TSA whitish, circular, convex, smooth and translucent with entire edges.

Microscopic observation showed Gram negative rods, singly and motile, forming in the cell characteristics described in Bergey´s Manual (Krieg and Holt, 1994). Results of other performed test are listed in table 1. Two strains were different for their growth at 42ºC, and fatty acid composition. Identification by the analysis of cultural and biochemical characteristics was not possible.

Fatty acid analysis, however, was conclusive and the strains CCIBP-M29 and CCIBP-Sp108 were identified as most closely related to *Ochrobactrum anthropi* (similarity index: 0.658 and 0.896, respectively). Table 2 shows fatty acid profiles from both.

The specie *Ochrobactrum anthropi* found in sugarcane cell suspensions and *in vitro* plants were not known to be contaminant in plant cell and tissue culture. In this crop, Moutia and Dookun (1999) identified *Bacillus cereus, B. megaterium, B. pumilus, Xanthomonas maltophilia, Burkholderia gladioli* and some *members* of *Enterobacteriacea* family as contaminants of buds culture using fatty acid analysis of isolates.

According to Leifert and Woodward (1998), very few bacterial species produced characteristic symptoms or growth in plant tissue cultures. For this reason, different bacterial genera can not be separated based on their colonies morphology. Methods that give as much taxonomic information as possible are required (Stead *et al*., 1998).

Holmes *et al.* (1988) created genus *Ochrobactrum*. It included the species *O. anthropi* (Holmes *et al.* 1988), *O. intermedium* (Velasco *et al.* 1998), *O. tritici* and *O. grignonense* (Lebuhn *et al.,* 2000) and *O. gallinifaecis* (Kämpfer *et al.*, 2003).

Bacteria found as contaminants of plant tissue culture are diverse and belong to a range of ecological groups. They include plant pathogens, epiphytes, endophytes and accidental contaminants (Stead *et al.,* 1998). Identification of *O. anthropi* as contaminant of sugarcane *in vitro* culture indicated the capacity of this bacterium for growing together with plants and cells in controlled condition.

O.anthropi has been isolated, besides, from internal tissue of sweet corn (*Zea mays*) (Mclnroy and Kloepper, 1995), rhizosphere of different plants (Tripathi *et al*., 2002) and soil (Lebuhn *et al*., 2000).Since the presence of this specie on sugarcane whole plants, not been reported, the source of it could be related with initial explants because appear.

Table 1. Principal characteristics of two bacterial strains of *Ochrobactrum anthropi* isolated from sugarcane cell suspension (CCIBP-Sp108) and *in vitro* plants culture (CCIBP-M29).

Characteristics	CCIBP-Sp 108	CCIBP-M29
Fluorescent pigments in King B medium	-	-
Catalase	+	+
O/F test oxidative	+	+
Oxidase	+	+
Indole production	-	-
Urease	+	+
Pigment	-	-
Growth on MacConkey Agar	+	+
Simmons citrate utilization	+	+
H_2S production	-	-
Arginine dihydrolase	+	+
Lysine decarboxylase	-	-
Ornithine decarboxylase	-	-
*Acid from Glucose	-	-
*Gas from Glucose	-	-
Growth at 5°C	-	-
Growth at 42°C	-	+
Starch hydrolysis	-	-
Caseine hydrolysis	-	-
Gelatin hydrolysis	-	-
Growth on lactose 10.0%	-	-
Growth on NaCl 4.0%	-	-
Methyl red	-	-
Voges Proskauer test	-	-
NO^-_3 reduction	-	-

*Peptone water medium

Table 2. Fatty acids profiles of two strains of *Ochrobactrum anthropi* contaminants of sugarcane cell suspension (CCIBP-Sp108) and *in vitro* plants culture (CCIBP-M29).

	Area (%)	
Name	CCIBP-Sp108	CCIBP-M29
16:0	4.56	5.23
17:1 w8c	0.08	-
17:1 w6c	0.13	-
17:0	0.57	-
16:0 3OH	0.13	-
18:1 w7c	81.50	78.96
18:0	4.58	5.19
19:0 CYCLO w8c	2.61	1.00
18:1 2OH	2.52	4.70
18:0 3OH	0.51	1.11
20:1 w7c	0.27	-

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