Global Advanced Research Journal of Agricultural Science (ISSN: 2315-5094) Vol. 8(6) pp. 185-193, July, 2019 Issue.
Available online http://garj.org/garjas/home

Full Length Research Paper

Characterization of an anaerobic bacterial consortium isolated from chicken manure capable to degrade organic arsenical compound into inorganic arsenic and methane

Javiera Ravanal[1], Ruben Moraga[2], Carla G. Leon[1], Italo A. Fernandez[1], Rodrigo Riquelme[1], Victor Hernández[3], Jorge Yañez[4]., Carlos T. Smith[1], Maria A. Mondaca[1], Victor L. Campos[1]

[1]Environmental Microbiology Laboratory, Department of Microbiology, Faculty of Biological Sciences, University of Concepción, Concepción, Chile.
[2]Microbiology Laboratory, Faculty of Renewable Natural Resources, Arturo Prat University, Iquique, Chile
[3]Natural Products Chemistry Laboratory, Department of Botany, University of Concepción, Chile.
[4]Department of Analytical and Inorganic Chemistry, Faculty of Chemical Sciences, University of Concepción, Chile.

Accepted 19 May, 2019

Roxarsone (ROX) (3-nitro-4-hydroxybenzene arsenic acid), an arsenic (As) containing compound is widely used as a food additive in the production of broiler chickens to control coccidial intestinal parasites and to favour rapid growth. Broiler chickens receiving ROX in their diet (between 23 and 45 g ton^{-1} food) excrete it untransformed in manure. This manure is commonly used as fertilizer, polluting farming fields. However, several soil bacteria can degrade ROX, releasing inorganic As. The results of this work demonstrated that a bacterial consortium, isolated from chicken manure and cultured under anaerobic conditions, was mainly composed by bacilli. DGGE analysis of the 16s rDNA sequences demonstrated that Firmicutes, which has been reported as main components in soils, sediments and animal faeces under anaerobic conditions, was the predominant tax a present in the studied consortium. The growth kinetics of the consortium was higher in the presence of ROX than in its absence, suggesting that ROX could be used as carbon source by the consortium. ROX was degraded by the consortium producing inorganic As, mainly arsenite (As(III)). Concomitantly with ROX biotransformation, the consortium produced As free methane. These results provide the first evidence that an anaerobic bacteria consortium isolated from chicken manure can rapidly biotras form ROX to inorganic arsenic and produce arsenic free biogas.

Keywords: biotransformation, roxarsone, chicken manure, bacterial-community, arsenic, methane.

INTRODUCTION

Roxarsone (ROX) (3-nitro-4-hydroxybenzene arsenic acid), is widely used as a food additive in broiler chickens production. About 70% of broiler chickens receive ROX in their diet (between 23 and 45 g ROX·ton^{-1} food) to control coccidial intestinal parasites and to sustain rapid growth (Chapman and Johnson, 2002). Most of the ROX fed to chickens is excreted untransformed in manure. Fresh chicken manure typically contains between 14 and 48 mg As·kg^{-1} (Garbarino et al., 2003). Farmers commonly spread poultry litter on the soil, as a valuable source of nitrogen, potassium and phosphorus (Liu et al., 2014), incorporating considerable amounts of As into farming fields. This practice may lead to polluting side effects. In fact, Wershaw et al. (1999) concluded that approximately 1x10^{6} kg·year^{-1} of ROX and its degradation products are added to the environment when fowl manure is used as fertilizer. On the other hand, Hancock et al. (2002) detected total As concentrations of 27 mg·kg^{-1} in fresh fowl manure, being most of it organic As; nevertheless, in soil samples of agricultural fields where chicken manure was used as fertilizer, mainly inorganic As was detected. Between the years 2013 and 2015, the Federal Drug Administration of the USA (FDA, 2015) decided to withdraw the approval for nitarsone, ROX and other arsenicals in the United States.

However, there is no indication that the marketing and use of arsenicals will be discontinued internationally as an additive for animal food (Nigra et al., 2017; FDA, 2015; Yao et al., 2013).

ROX is highly soluble in water, facilitating its rapid migration through the soil and contaminating both superficial and groundwater water. During manure storage and composting, ROX is transformed to even more toxic As species, including arsenate (As(V)), arsenite (As(III)), dimethylarsinic acid (DMA) and 4-hydroxy-3-amino-phenylarsonic acid (HAPA), depending on litter storage period and redox conditions (Nachman et al., 2005; Zhang et al., 2014). In addition, Mangalgiri et al. (2015), using chicken litter slurries or sewage sludge, suggested that biological processes are responsible for the transformation of ROX. However, neither the microorganisms nor the biological processes involved were identified.

There are few reports about the isolation and identification of microorganisms involved in ROX transformation. A study by Stolz et al. (2007) demonstrated that *Clostridium* species present in fowl manure rapidly transform ROX into inorganic arsenate under anaerobic conditions. On the other hand, Garbarino et al. (2003) performed experiments that demonstrated the biotransformation of ROX into inorganic As and other organo-arsenical metabolites, but they did neither isolated nor identified the microorganisms involved in this process or the impact of ROX on the bacterial community of the soil.

Therefore, the aim of this work was to characterize an indigenous bacterial consortium isolated from chicken manure capable to degrade ROX under anaerobic conditions, to evaluate its potential implications in the mobilization of As inorganic compounds and its possible capability to produce biogas.

MATERIALS AND METHODS

Sampling

A poultry litter sample was collected at a poultry industry using ROX as chicken food additive. Sampling took place in the vicinity of Florida, Bio Bio Region, Chile (36º47'31.02" South, 72º44'13.57" West).

Samples were obtained and stored in anaerobic vials at 4°C until further analysis. All further analyses were done at the Laboratory of Environmental Microbiology, Department of Microbiology, Faculty of Biological Sciences, University of Concepción, Concepción, Chile.

Enrichment of the bacterial consortium

A bacterial consortium was enriched inoculating 5 g of a sample of chicken faeces in a flask containing 100mL of the basal medium (Stolz et al., 2007) and incubated at room temperature (nearly 25°C) with agitation (100 rpm) under anaerobic condition (mixture of sterilized 80% N_2 and 20% CO_2) during four weeks in darkness. Starting at day seven, 100 ml of fresh culture media were added once a week for three weeks. After the four weeks period, the bacterial consortium obtained was inoculated into two flasks, one containing basal medium plus 0.5 mM ROX and the second one with basal medium alone (negative control). Both bacterial consortiums were cultured for 72 h under the same conditions described above for further studies.

Scanning Electronic Microscopy

Consortiums were studied by scanning electron microscopy (SEM). Aliquots of each consortium were obtained and they were harvested and processed as described by Campos et al. (2011). Samples were studied using a JEOL JSM 6380LV (JEOL USA, Inc., Peabody, MA, USA) scanning electron microscope.

*Corresponding Author's Email: vcampos@udec.cl

DNA Extraction, 16s rDNA amplification and denaturing gradient gel electrophoresis (DGGE) analysis

The bacterial consortiums were characterized by PCR-DGGE. The total DNA of each bacterial consortium, anaerobically grown in basal medium in the presence or absence of ROX, was extracted using the Ultra Clean soil DNA extraction kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA) following the instructions provided by the manufacturer. Both total DNAs were amplified using rDNA 16s universal primers EUB 9-27 and EUB 1542 (Leon et al. 2012). Nested PCR of each consortium was performed using the primer pair 341f and 534r-GC clamp attached to the forward primer (Leon et al., 2012). The PCR conditions were those described by Campos et al. (2011). DGGE was performed using a DGGE 1001 system (C.B.S. Scientific Company Inc., Thermo Fisher Scientific, USA). Fifteen µl of PCR products of the V3 region of rDNA 16S were applied directly onto 6% (w/v) polyacrylamide gels in 1X TAE (40 mM Tris-acetate, 1 mM EDTA) with a denaturant gradient from 20 to 60% (100% denaturant contained urea 7 M and 40% formamide). Electrophoresis was performed at a constant voltage of 200 V at 60°C for 6 h. After electrophoresis, gels were stained for 20 min with SYBR Gold nucleic acid gel stain (Invitrogen, Thermo Fisher Scientific, USA), as specified by the manufacturer, and they were visualized on a transilluminator (UVP Inc.,Germany) (Campos et al. 2011). The most representative bands were removed, reamplified and purified to be sequenced by Macrogen Inc. (South Korea). The sequences reported were edited and analysed using the ARB bioinformatics tool (Campos et al., 2010)

Growth kinetics of bacterial consortiums

The growth kinetics of bacterial consortiums (cultured with or without ROX) was determined, in triplicate, under anaerobic condition, up to 48 h of incubation, by optical density measurements (600 nm) in 96-well plates using a microplate spectrophotometer (Epoch, BIOTEK, Wisconsin, USA) (Guzman-Fierro et al., 2015). The curves obtained were analyzed using mathematical modelling approaches: Gompertz, logistic, exponential Malthusian, exponential plateau and Weibull (Zwietering et al., 1990). The best model was selected by the Fisher test and comparing the coefficients of determination (R^2). Graphs, statistics and models were made using Graph Pad Prism version 5.0 (GraphPad software, San Diego, CA, USA).

Degradation of ROX by the bacterial consortium

Bacterial degradation of ROX was measured by means of a spectrophotometric technique. The bacterial consortium was grown in basal medium plus 0.5 mM ROX during 48 h under anaerobic conditions. Sterile basal medium plus 0.5 mM ROX was used as negative control. Aliquots (1 mL) were obtained every 6h and filtered (sterile 0.22 □m Millipore). Then, 200 □L of each aliquot were transferred, in triplicate, to 96-wells plates. ROX degradation was quantified using a microplate spectrophotometer (Epoch, BIOTEK), by means of spectrograms integration (310-500 nm) by the trapezoid method using the Gen5 software (BIOTEK) (Guzman-Fierro et al., 2015). For detecting ROX transformation into inorganic As, a 1 mL sample of the supernatant was aseptically obtained every 6 h up to 48 h from each consortium and filtered through a sterile Millipore 0.22 µm pore size filter. Inorganic As species were detected by means of high performance liquid chromatography (HPLC) coupled to atomic absorption (AAS) according to Yañez et al. (2015).

Assessment of biogas production by gas chromatography with mass detector

The qualitative determination of biogas was performed by means of gas chromatography with mass detector (GC-MS). The bacterial consortium was grown in basal medium with 0.5 mM ROX or without it during 48h under anaerobic conditions. The biogas production was detected using a gas chromatograph Agilent 7890A coupled to a mass detector Agilent 5975C. Carrier gas was helium electronic degree at a flow rate of 9.3mL·min^{-1} and a split flow of 32.4 mL·min^{-1}. The column used was an Agilent J&W type HP-5MS, 30 m length, 0.25 mm internal diameter (id) and 0.25 mm thickness film of fused silica. The temperature of the column, the injector port and the detector was 140, 180, and 285 ºC, respectively. Samples (100 □L) from the headspace were collected using a pressure-lock gas syringe (Manzano et al., 2013). The results were obtained comparing the chromatographic peaks obtained with those available at the NIST05 database.

Statistical analysis

Growth rates (*k*) of the models that best conformed to the kinetics of bacterial consortiums growth and bacterial kinetics ROX degradation were analysed by means of Student's t-tests using the MINITAB version-15 software (Minitab Inc, Pennsylvania, USA). P values <0.05 were considered as statistically significant.

RESULTS AND DISCUSSION

A microbial consortium was isolated after 28 days of enrichment, under anaerobic conditions, of a faeces sample obtained from poultry of chicken feed with ROX supplemented food. In order to assess the effect of ROX on the consortium, it was cultured it in the presence of 0.5 mM ROX or its absence. Both cultures were observed under SEM, the consortiums obtained were characterized

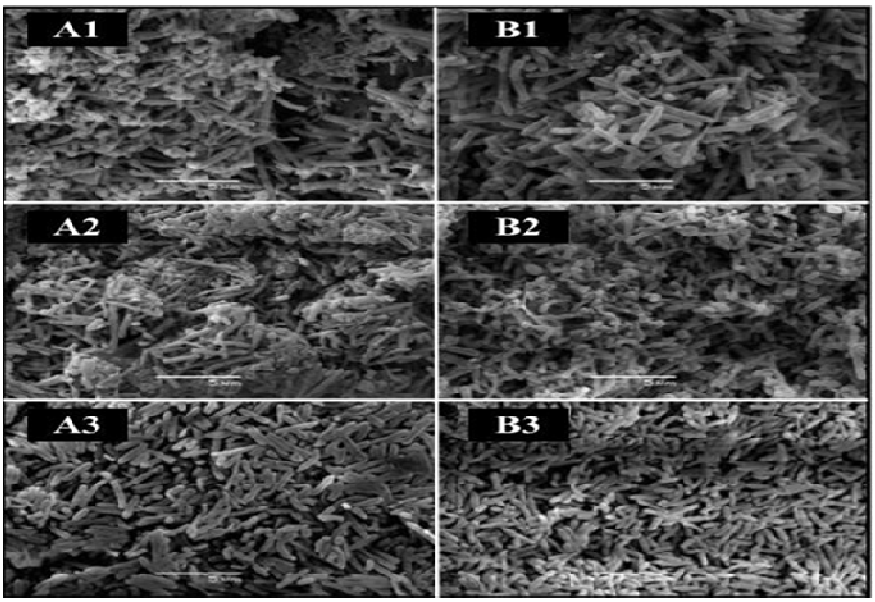

Figure 1. Scanning electron micrographs of bacterial consortiums after 72 h of incubation under anaerobic conditions. A1-A3: bacterial consortium cultured in the absence of roxarsone. B1-B3: bacterial consortium cultured in the presence of 0.5 mM roxarsone. Bar indicates 5 □m.

Table 1. Microorganisms identified (by analysis of 16S rDNA sequences obtained by DGGE) in the consortiums grown in the presence of 0.5 mM roxarsone or its absence

Band	Closest relative sequenced	Growth ROX Pr[1]	Growth ROX Ab[2]	Phylum	Gen Bank Access
1	*Bacillus ginsengisoli*	+	-	Firmicutes	NR109068.1
2	*Alcaligenes faecalis*	+	-	Proteo bacteria	NR025357.1
3	*Bacillus thermocopriae*	-	+	Firmicutes	NR109664.1
4	*Bacillus horikoshii*	-	+	Firmicutes	KF625181.1
5	*Lysinibacillus fusiformis*	+	+	Firmicutes	KF261599.1
6	*Bacillus cereus*	+	+	Firmicutes	GU384235.1
7	*Lysinibacillus* sp.	+	+	Firmicutes	KJ188109.1
8	*Lysinibacillus odysseyi*	-	+	Firmicutes	KC149512.1
9	*Bacillus niacini*	-	+	Firmicutes	AY509228.1
10	Uncultured *Clostridium* sp.	-	+	Firmicutes	JX826397.1
11	*Bacteroides fragilis*	-	+	Bacteroidetes	NC003228.3
12	*Clostridium aceticum*	+	-	Firmicutes	AB910752.1
13	*Clostridium ljungdahlii*	+	-	Firmicutes	L34419.1

[1]Pr: microorganism present in the consortium grown in the presence of 0.5 mM roxarsone. [2]Ab: microorganism present in the consortium grown in the absence of roxarsone.

with respect to taxa present, growth kinetics, ROX biotransformation and their capacity to produce biogas.

SEM analyses showed that after 72 h of incubation both bacterial consortiums (with and without ROX) were heterogeneous communities. No visual differences were observed between both consortiums, being the bacillar morphology predominant (Fig. 1). Guzman-Fierro et al. (2015) also reported the predominance of bacillary species in a consortium obtained from soil fertilized with ROX containing chicken manure after 168h of culture under aerobic conditions.

In order to assess the composition of the bacterial communities of the consortiums, a nested PCR followed by a DGGE under a denaturing gradient of 16s rDNA was performed for each consortium (Fig. 2). The closest GenBank matches for 16s rDNA sequences revealed that the highest percentage of taxa belonged to phylum Firmicutes (84.6%) (Table 1). Wiegel (2009) also reported

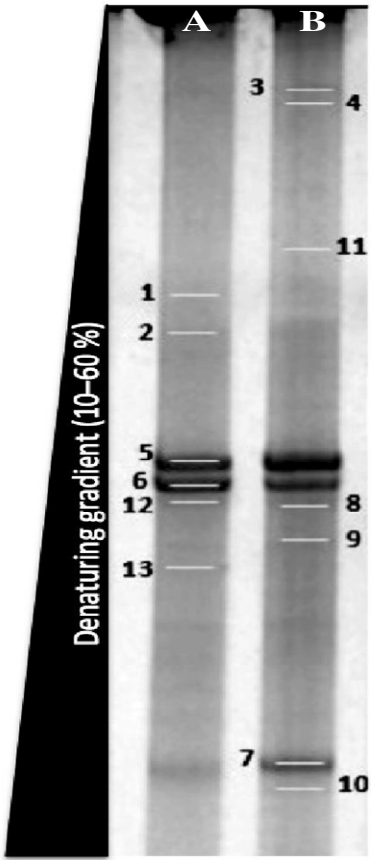

Figure 2. Ten to sixty per cent denaturant gradient gel electrophoresis (DGGE) of amplified fragments of the variable region V3 of 16S rDNA of each consortium. Consortiums were grown in the presence of 0.5 mM roxarsone (A) or in the absence of roxarsone (B). Numbers of the bands correspond to the sequences reported in Table 1.

the predominance of Firmicutes in soils, sediments and animal faeces under anaerobic conditions. In this work, the microorganisms found only in the consortium cultured in the presence of ROX were *Bacillus ginsengisoli*, *Alcaligenes faecalis*, *Clostridium aceticum* and *Clostridium ljungdahlii*, suggesting that these are the microorganisms able to metabolize this compound. Stolz et al. (2007) reported that *Clostridium* spp. isolated from chicken faeces biotransformed ROX into inorganic arsenical compounds under anaerobic condition. Being present in the cultures with and without ROX, *Lysinibacillus fusiformis*, *Bacillus cereus* and *Lysinibacillus* sp. should be able to at least tolerate ROX. In fact, Mafla et al. (2015) had already reported the presence of *Lysinibacillus* sp. in a microcosm, obtained from underground water, able to degrade ROX. On the other hand, *Bacillus thermocopriae*, *Bacillus horikoshii*, *Lysinibacillus odysseyi*, *Bacillus niacini*, uncultured *Clostridium* sp. and *Bacteroides fragillis* were only present in the consortium cultured in the absence of ROX; therefore they should not be able to tolerate ROX or its degradation products. *Bacillus thermocopriae* is a Gram positive anaerobic facultative bacterium described as a new species isolated from a compost sample of sludge sewage and crop straw by Han et al. (2013).

The effect of the presence or absence of ROX on the growth kinetics of each consortium was studied and the Weibull algorithm applied (Zwietering et al., 1990). This model demonstrated that after 48 h of incubation, the bacterial consortium cultured in the presence of ROX showed a higher growth (Fig. 3). Growth rates (k) were 0.01790 $OD \cdot h^{-1}$ and 0.01291 $OD \cdot h^{-1}$ in the presence or absence of ROX, respectively. Student's t-test (with 95% confidence) demonstrated significant differences between both conditions (P=0.031), showing a 1.3 fold k increase when ROX was present. Thus, it is possible that the bacterial consortium having ROX available used it as an additional carbon source. Guzman-Fierro et al. (2015) reported similar results (k increase of 1.4 fold) in a ROX degrading aerobic bacterial consortium isolated from an agricultural soil fertilized with ROX fed chicken manure.

ROX biotransformation assessed by means of spectrophotometric analysis revealed that 90.5% of the compound was transformed after 48 h of anaerobic incubation in the presence of the consortium (Fig. 4).

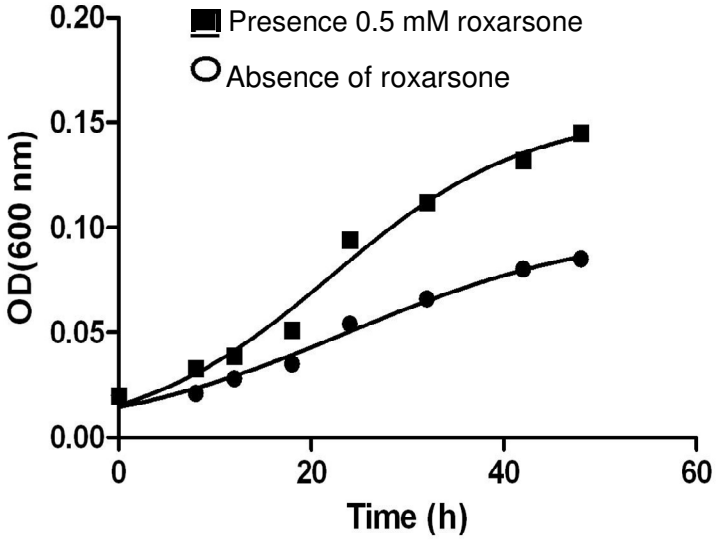

Figure 3. Growth kinetics, determined by spectrophotometry at 600 nm, of the bacterial consortium cultured in the presence of 0.5 mM roxarsone or in the absence of this compound

Under the same conditions but absence of the consortium there was no ROX degradation, confirming that it was, in fact, a biotransformation. The percentage of ROX degradation achieved by the consortium obtained in this work is in agreement with the percentage degradation reported by Garbarino et al. (2003) in poultry litter.

Stolz et al. (2007) using *Clostridium* species cultured under anaerobiosis reported ROX biotransformation rates close to 100%, but after 9 days of culture. Therefore, using a bacterial consortium including different bacterial groups, such as this one isolated from broiler chicken manure, acting in a collaborative fashion, may increase microbial activity and consume more rapidly this organoarsenical compound.

Studies have reported that lactate increased ROX biodegradation under anaerobic conditions (Cortinas et al., 2006; Stolz et al., 2007; Sierra-Álvarez et al., 2010), favouring the growth of microorganisms. But, as showed in this study, in the absence of lactate, the presence of ROX in the medium increased the growth rate of the consortium and it would stimulate the growth of certain microorganisms, as observed in the results obtained by DGGE. Thus, it is possible that ROX, or its degradation intermediate compounds, may provide the electrons required by the electron transport chain. In addition, Jiang et al. (2013), studying soil bacterial communities, showed significant effects of ROX in their diversity and metabolism.

In accordance with Wershaw et al. (1999), during ROX metabolizing as a carbon source, firstly occurs the reduction of the nitro group, then the oxidative fission of the aromatic ring (most important stage) and finally the breaking the C–As bond. Guzman-Fierro et al. (2015), studying an aerobic consortium isolated from an agriculture soil fertilized with ROX feed chicken manure, proposed that the oxidation of aromatic and non-aromatic compounds did not change despite ROX presence. These authors suggested that the reduction of the nitro group, producing a functional amine, should occur first, facilitating a decrease in the stability of the aromatic ring resonance energy.

As well as monitoring ROX degradation, its biotransformation into inorganic As species was measured by HPLC-AAS. Results showed that under the cultured conditions assayed, 95.6% of ROX was biotrans formed by the consortium into inorganic As after 48 h of incubation (Fig. 4). As(V) was the initially most abundant species but it was rapidly reduced making As(III) the predominant inorganic As species. This reduction could be the consequence of the changes in the microbiological composition of the consortium after 48 h of incubation, favouring species of microorganisms that provide electrons to As(V) and transform it into As(III).

Stolz et al. (2007) and Liang et al. (2014) reported similar ROX degradation values after 168 h of incubation under anaerobic conditions. Huang et al. (2019) reported that during ROX biotransformation by *Enterobacter* sp strain CZ-1, five metabolites including arsenate (As[V]), arsenite (As[III]), N-acetyl-4-hydroxy-m-arsanilic acid (N-AHPAA), 3-amino-4-hydroxyphenylarsonic acid (3-AHPAA) and a novel sulfur-containing As species ($AsC_9H_{13}N_2O_6S$) were detected. D'Angelo et al. (2012) reported the production of considerable amounts of arsenate and several other ROX transformation intermediates while litter is accumulated in broiler houses.

Yin et al. (2018) evaluated, in a lab-scale pilot employing an anoxic-oxic (A-O) process, the effect of adding ROX to the treatment process of livestock wastewater, reporting

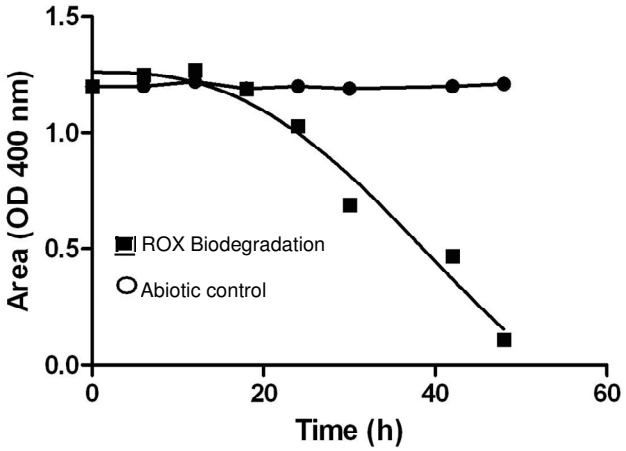

Figure 4. Roxarsone degradation determined by spectrophotometric analysis associated to spectrograms integration (310-500 nm) using the trapezoid method. Squares correspond to roxarsone degradation by the microbial consortium in the presence of 0.5 mM roxarsone while circles correspond to the control with 0.5 mM roxarsone but without the microbial consortium.

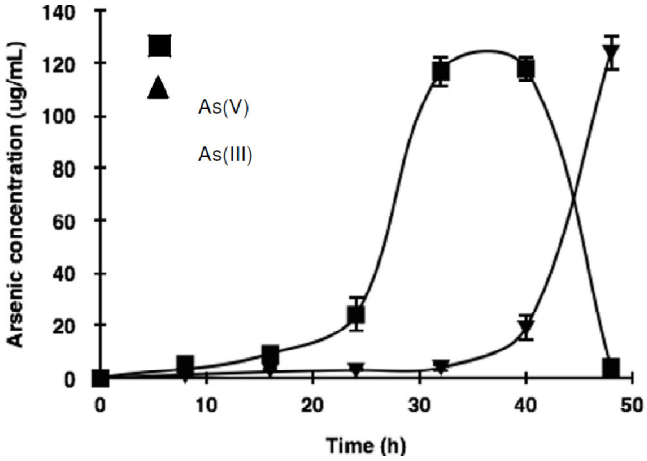

Figure 5. Quantification, by high performance liquid chromatography coupled to atomic absorption spectrophotometry (HPLC-AAS), of the inorganic arsenic species resulting from roxarsone biotransformation.

that after 85 days of operation of this process, As(V) and residual ROX were the main As forms present. Furthermore, the mass balance of As at steady state revealed that around 0.08%, 3.91% and 96.01% of total As was transformed into gas (biogas), solid (excess sludge) and liquid (effluent), respectively. In this study, the assay for detecting biogas demonstrated the generation of methane gas (CH_4), both in the presence and absence of ROX, and no arsine was detected in the consortium incubated in the presence of ROX. On the other hand, the results showed that the methanogenesis was not inhibited by the presence of ROX (Fig. 6). This result is in opposition to previous studies reporting that methanogenic microorganisms are inhibited by the presence of ROX or its degradation metabolites and by inorganic As species (Sierra-Alvarez et al., 2004; Sierra-Álvarez et al., 2010).

In general, it is known that inorganic As(V), the dominating As species in soils, is subject to microbial reduction and methylation leading to volatilization as arsines. However, the reduction and methylation rates of As, necessary prerequisites to arsine production, vary greatly depending on soil properties, such as soil moisture and temperature, abundance of different species of As and microbial populations in soil (Turpeinen et al., 2002). Furthermore, the search for volatile arsines by gas chromatography gave negative results, suggesting that volatile As species would not be liberated into the environment if the gas is used as fuel.

In conclusion, these results provide the first evidence of the feasibility of profiting from the metabolic activity of a microbial community isolated from chicken manure to simultaneously degrade ROX and to produce an As free

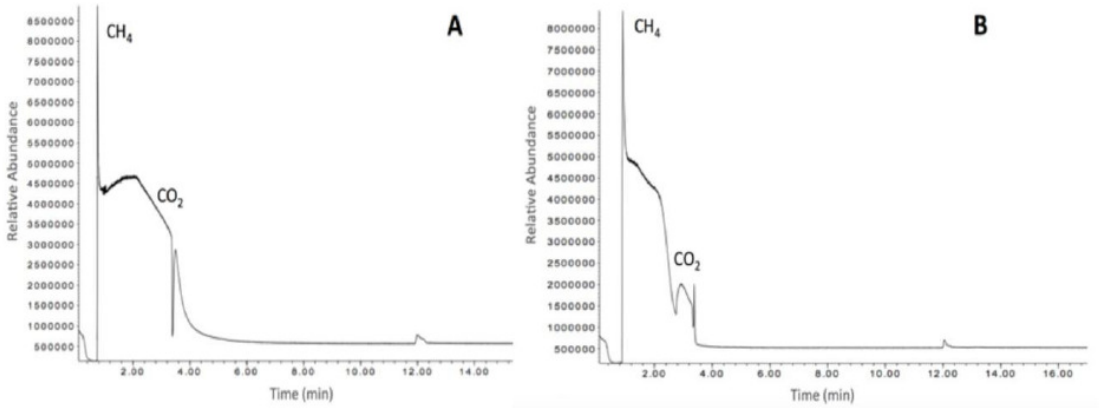

Figure 6. Biogas detection by gas chromatography. A) Consortium grown without roxarsone; B) Consortium grown in the presence of 0.5 mM roxarsone. Retention times: methane (CH_4) 1 min; carbon dioxide (CO_2) 3 min.

biogas. In addition, the methane yield was not affected by the presence of the various arsenical compounds derived from the degradation of ROX at a concentration of up to 0.5 mM.

ACKNOWLEDGEMENTS

This work was supported by Grant FONDECYT (Chile) Nº1110876, Grant FONDECYT (Chile) Nº 11130383 and Grant VRID (University of Concepción, Chile) Nº 216.036.043.1.0.

REFERENCES

Campos VL, León C, Mondaca MA, Yañez J, Zaror C (2011). Arsenic Mobilization by Epilithic Bacterial Communities Associated with Volcanic Rocks from Camarones River, Atacama Desert, Northern Chile. Arch. Environ. Contam. Toxicol. 61:185–192.

Chapman HD, Johnson ZB (2002). Use of antibiotics and roxarsone in broiler chickens in the USA: analysis for the years 1995 to 2000. Poult. Sci. 81:356–364.

Cortinas I, Field J, Kopplin M, Garbarino J, Gandolfi A, Sierra-Alvarez R (2006). Anaerobic biotransformation of roxarsone and related N-substituted phenylarsonic acids. Environ. Sci. Technol. 40:2951–2957.

D'Angelo EM, Zeigler G, Beck EG, Grove J, Sikora F (2012). Arsenic species in broiler (*Gallus gallus domesticus*) litter, soils, maize (*Zea mays L.*), and groundwater from litter-amended fields. Sci. Total Environ. 438:286–292.

FDA. 2015. FDA Announces Pending Withdrawal of Approval of Nitarsone.http://www.fda.gov/AnimalVeterinary/NewsEvents/CVMUpdates/ucm440668.htm

Garbarino JR, Bednar AJ, Rutherford DW, Beyer RS, Wershaw RL (2003). Environmental fate of roxarsone in poultry litter. I. Degradation of roxarsone during composting. Environ. Sci. Technol. 37:1509–1514.

Guzmán-Fierro VG, Moraga R, León CG, Campos VL, Smith C, Mondaca MA (2015). Isolation and characterization of an aerobic bacterial consortium able to degrade roxarsone. Int. J. Environ. Sci. Technol. 12(4):1353–1362.

Han L, Yang G, Zhou X, Yang D, Hu P, Lu Q, Zhou S (2013). *Bacillus thermocopriae* sp. nov., isolated from a compost. Int. J. Syst. Evol. Microbiol. 63:3024–3029.

Hancock T, Denver J, Riedel G, Miller C (2002) Source transport and fate of arsenic in the Pocomoke River

Basin a poultry dominated Chesapeake Bay Watershed. In: Proceedings of Arsenic in the Environment Workshop February 21–22 2001 US Geological Survey Denver Colorado.

Huang K, Peng H, Gao F, Liu Q, Lu X, Shen Q, Le XC, Zhao FJ (2019). Biotransformation of arsenic-containing roxarsone by an aerobic soil bacterium Enterobacter sp. CZ-1. Environ. Pollut. 247: 482-487.

Jiang Z, Li P, Wang YH, Li B, Wang YX (2013) Effects of roxarsone on the functional diversity of soil microbial community. Int. Biodeterior. Biodegrad. 76:32–35.

Leon C, Campos V, Urrutia R, Mondaca M (2012). Metabolic and molecular characterization of bacterial community associated to Patagonian Chilean oligotrophic-lakes of quaternary glacial origin. World J. Microbiol. Biotechnol. 28:1511–1521

Liang T, Ke Z, Chen Q, Liu L, Chen G (2014). Degradation of Roxarsone in a Silt Loam Soil and Its Toxicity Assessment. Chemosphere. 112:128-133.

Liu H, Wang G, Ge J, Liu L, Chen G (2014). Fate of roxarsone during biological nitrogen removal process in wastewater treatment systems. Chem. Eng. J. 255:500–505.

Mafla S, Moraga S, León CG, Guzmán-Fierro V, Yañez J, Smith CT, Mondaca MA, Campos VL (2015).

Biodegradation of Roxarsone by a Bacterial Community of Underground Water and Its Toxic Impact. World J. Microbiol. Biotechnol. 31:1267-1277.

Manzano P, Miranda m, Abreu j, Silva M, Hernández V, Peralta E (2013). Gas Chromatography-Mass

Spectrometry Study from the Leaves Fractions Obtained of Vernonanthura patens (Kunth) H. Rob. International Journal of Organic Chemistry 3:105-109.

Mangalgiri KP, Adak A, Blaney L (2015). Organoarsenicals in poultry litter: Detection, fate, and toxicity. Environ. Int. 75:68–80.

Nachman KE, Graham JP, Price LB, Silbergeld EK (2005). Arsenic: a roadblock to potential animal waste management solutions. Environ. Health Perspect. 113:1123.

Nigra AE, Nachman KE, Love DC, Grau-Perez M, Navas-Acien A (2017). Poultry consumption and arsenic exposure in the U.S. population. Environ. Health Perspect. 125:370–377; http://dx.doi.org/10.1289/EHP351

Sierra-Alvarez R, Cortinas I, Yenal U, Field J (2004). Methanogenic Inhibition by Arsenic Compounds. Appl. Environ. Microb. 40:5688-5691.

Sierra-Alvarez R, Cortinas I, Field J (2010). Methanogenic inhibition by roxarsone (4-hydroxy-3-nitrophenylarsonic acid) and related aromatic arsenic compounds. J. Hazard Mater. 175:352-358.

Stolz JF, Perera E, Kilonzo B, Kail B, Crable B, Fisher E, Ranganathan M, Wormer L, Basu P (2007). Biotransformation of 3-nitro-4-hydroxybenzene arsonic acid (roxarsone) and release of inorganic arsenic by Clostridium species. Environ. Sci. Technol. 41:818–823.

Turpeinen R, Kallio MP, Kairesalo T (2002). Role of microbes in controlling the speciation of arsenic and production of arsines in contaminated soils. Sci. Total Environ. 285:133-145.

Wershaw RL, Garbarino JR, Burkhardt MR (1999). Roxarsone in natural water systems. In: Effects of animal feeding operations on water resources and the environment. US Geological Survey. Open-File Report 00-204, p 95.

Wiegel J (2009). Family I. Clostridiaceae Pribram 1933, 90AL. In: Whitman WB (eds) Bergey's manual of systematic bacteriology, Volume Three: The Firmicutes, 2nd edn. Springer, New York, 737.

Yañez J, Mansilla H, Santander P, Fierro V, Cornejo L, Barnes M, Amarasiriwardena D (2015) Urinary arsenic speciation profile in ethnic group of the Atacama desert (Chile) exposed to variable arsenic levels in drinking water. J. Environ. Sci. Heal. A 50:1–8.

Yao L, Huang L, He Z, Zhou C, Li G (2013). Occurrence of arsenic impurities in organoarsenics and animal feeds. J. Agric. Food Chem. 61:320–324.

Yin Y, Wan J, Li S, Li H, Dagot C, Wang Y (2018). Transformation of roxarsone in the anoxic–oxic process when treatingthe livestock wastewater. Sci. Total Environ. 616:1224–1234

Zhang FF, Wang W, Yuan SJ, Hu ZH (2014) Biodegradation and speciation of roxarsone in an anaerobic granularsludge system and its impacts. J. Hazard Mater. 279:562–568.

Zwietering MH, Jongenburger I, Rombouts FM, Van't Riet K (1990) Modeling of the bacterial growth curve. Appl. Environ. Microbiol. 56:1875-1881.