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Rhizospheric iron and arsenic bacteria affected by water regime: Implications for metalloid uptake by rice

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ABSTRACT

Rice is characterized by high levels of arsenic accumulation, even if cultivated in non-contaminated soils. Given the limits for arsenic concentration in rice grain recently established by the European Community, it is essential to understand the mechanisms and find solutions to this issue. Arsenic bioavailability is strictly related to water management of the rice paddy as well as to iron- and arsenic-cycling bacterial populations inhabiting the rice rhizosphere.

To evaluate the effect of different agronomic conditions on the root-soil microbiota involved in arsenic mobilization, rice plants were grown in macrocosms containing non contaminated field soil under either continuous flooding, aerobic rice regime or continuous flooding with a 14 day-period of drainage before flowering. Specific groups of iron- and arsenic-cycling bacteria were assessed by real time quantitative PCR and fluorescence *in situ* hybridization.

Continuous flooding led to the release of arsenate and iron in soil solution and produced rice grains with arsenite and organic arsenic above the recently established limits, contrary to the other agronomic conditions.

Iron-reducing bacteria affiliated to the family *Geobacteraceae* significantly increased under continuous flooding in rhizosphere soil, in concomitance to arsenate dissolution from iron minerals. The 14 day-period of drainage before flowering allowed the recycling of iron, with the increase of *Gallionella*-like iron-oxidizing bacteria. This phenomenon likely influenced the decrease of arsenic translocation in rice grains.

Regardless of the water regime, genes for arsenite oxidation (*aioA*) were the most abundant arsenic-processing genes, explaining the presence of arsenate in soil solution. The presence of arsenite and organic arsenic in rice grains produced under continuous flooding might be related to the retrieval of genes for arsenate reduction (*arsC*) and for arsenite methylation (*arsM*) in the proximity of the roots.

These outcomes indicate a potential active role of rhizospheric iron- and arsenic-cycling bacteria in determining arsenic accumulation in rice grains from plants cultivated under continuous flooding, even in soil with a low arsenic content.

1. Introduction

Rice is one of the crops with the highest levels of arsenic (As) and one of the most important contributors to human exposure (EFSA, 2014; Hojsak et al., 2015; Singh et al., 2015). Noteworthy, even if rice is cultivated in soil with a low As concentration (i.e. tot As < 20 mg kgdw^{-1}), rice grains may accumulate inorganic As (iAs)

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exceeding the recently introduced limits for food quality of 100 and 200 μg kg^{-1}, respectively for baby food production and for adult consumption (Commission regulation (EU) 2015/1006). The reason for the high content of As in rice grains is that these plants are mainly cultivated under continuous flooding for the whole cropping cycle. In oxygenated soils, the most abundant form of As is arsenate [As(V)], firmly bound to iron minerals. Continuously flooded conditions of the rice paddy lead to the rapid depletion of O_2 with the consequent decrease of the reduction potential. As a consequence, the reduction of ferric iron [Fe(III)] releases As from Fe-As minerals into the porewater. Furthermore, mineral-bound As(V) is reduced by microorganisms to arsenite [As(III)], which is more mobile and toxic than As(V) (Takahashi et al., 2004; Yamaguchi et al., 2014). These reactions are carried out by microorganisms that either use As(V) as an electron acceptor for respiration [dissimilatory As(V) reductase, *arrA* gene] or reduce it for detoxification purposes [As(V) reductase, *arsC* gene, Zhu et al., 2014]. Therefore, under continuous flooding, As bioavailability and consequent rice plant uptake increases. Several studies have documented the reduction of rice grain As content by cultivating the plants under intermittent flooding or with sprinkler irrigation (Das et al., 2016; Li et al., 2009; Ma et al., 2014; Somenahally et al., 2011b; Spanu et al., 2012). The amount of As accumulated in rice grains varies among different rice varieties, with the lowest accumulation rate being 95 μg kg^{-1}, very close to the limit for baby food production (Spanu et al., 2012).

In addition to the physical and chemical factors, microorganisms affect the As cycle with a variety of direct and indirect processes. A wide range of genes are present in bacteria and archaea that encode for As-processing enzymes and transporters. With these enzymes, microorganisms can reduce As(V) to As(III), oxidize As(III) to As(V), methylate As(III) and extrude As(III) from the cell (processes reviewed by Cavalca et al., 2013; Slyemi and Bonnefoy, 2012; Yamamura and Amachi, 2014). Given that Fe minerals have a higher affinity for As(V) with respect to As(III) (Liu et al., 2005; Martin et al., 2014; Yamaguchi et al., 2014), the activity of Fe(III)-reducing bacteria (FeRB) as well as As(V)-reducing bacteria could promote the dissolution of As from soil Fe (hydr)oxides into the porewater, increasing its bioavailability (processes reviewed by Zhu et al., 2014). On the other hand, in the proximity of rice roots, where oxygen is released by root aerenchyma, the activity of As(III)- and Fe(II)-oxidizing bacteria (AOB and FeOB) can both contribute to the formation and co-precipitation of As with Fe minerals, decreasing its bioavailability (Das et al., 2016; Jia et al., 2014).

Microorganisms also influence As speciation in rice grains. Arsenic in rice grains is mainly present as iAs and dimethylarsinic acid (DMA), with great variation between different countries of origin (Meharg et al., 2009). Recent studies indicate that methylated As found in rice grains is not produced by the plant, but derives from the activity of rhizospheric microorganisms (Arao et al., 2011; Jia et al., 2012; Lomax et al., 2012; Zhao et al., 2013). Although several studies reported higher toxicity of iAs if compared to organic As, dimethylarsenite [DMA (III)] and monomethylarsenite [MMA (III)] have been demonstrated to be more genotoxic than iAs (Stýblo et al., 2002; Thomas et al., 2001). Therefore, understanding which microorganisms are involved in As methylation within the rice plant rhizosphere and what conditions favor their growth is of great importance.

Arsenic mobilization into rice is becoming a world-wide health issue for millions of people, yet little is known about the factors influencing microbial As solubilization in low arsenic soils of European countries. In the context of better understanding microbial As mobilization, a comprehensive study on connections between iron and arsenic-cycling bacteria in different oxic/anoxic conditions of soil in the different root compartments was carried out. The aim of the present study was to set up a detailed experiment in which iron and arsenic cycles could be deciphered by physico-chemical and biological parameters, in order to define their role in As contamination of rice grains established in different agronomic conditions.

2. Material and methods

2.1. Experimental setup and water regimes

The experiment was carried out at the Rice Research Centre (Ente Nazionale Risi) in Castello d'Agogna (Pavia, Italy) in 2013, in macrocosms set up in 0.83 m^2 plastic tanks filled with 30 cm of gravel and 25 cm of soil from a paddy field (As concentration and other selected physic and chemical characteristics are reported in Table 1). The macrocosms were located in an open air area in front of the Rice Research Center. Water was supplied with a garden hose and capped holes at the bottom of the containers allowed water control and maintenance of aerobic conditions when required. Rice plants (*Oryza sativa* subsp. *japonica*, variety Loto) were grown under three different water regimes: continuous flooding (CF); rotational irrigations over the cropping season (aerobic rice, AR) and continuous flooding with a 14 day-period of drainage before flowering (2nd internode elongation drainage, 2IED). Three replicates randomly located were set up for each water management. Dry seeding was performed on 10th June in the soil fertilized with 22 g m^{-2} of urea and 40 g m^{-2} of P-K fertilizer (14–18 units respectively). The plants germinated within 10 days, and on 2nd July the plants under CF and 2IED regimes were flooded and AR plants were watered. Watering of AR plants was performed only when the soil water content was below field capacity (approximately every 10 days). After nearly one month from flooding (6th August), when the plants were at the 2nd internode elongation stage, the 2IED macrocosms were drained for 14 days and then re-flooded until 30th September and harvested on 9th October. At harvesting, rice grain was separated from rice straw and then polished and ground for As extraction and speciation.

In the macrocosms soil pH and temperature were measured with SenTix® 41-3 pH electrodes directly placed in the soil. Porewater was sampled according to Cattani et al. (2006) through Rhizon soil moisture samplers (Rhizosphere®, Rhizosphere Research Products, Wageningen, NL) at three growing stages: tillering (28th June), flowering (20th August), and senescence (30th September). An aliquot was immediately mixed with orthophenantroline for the measurement of Fe(II), another aliquot was acidified with 2% nitric acid (HNO_3) for As determination, while the rest was transferred into 10 mL polyethylene tubes without headspace, refrigerated and immediately transferred to the laboratory for the analysis of DOC and major dissolved anions.

Table 1
Selected soil physic and chemical characteristics. Values represent the means of samples from all macrocosms ± standard deviation. Fe_R and As_R are *aqua regia* extractable Fe and As respectively.

Parameter	Value	Measure unit
Sand (2.00–0.05 mm)	54.4 ± 1.98	%
Silt (0.05–0.002 mm)	39.0 ± 1.62	%
Clay (<0.002 mm)	6.6 ± 0.71	%
pH	5.9 ± 0.05	–
Organic C	15.3 ± 0.45	g kg^{-1}
Total N	1.2 ± 0.05	g kg^{-1}
Olsen P	36.9 ± 1.33	mg kg^{-1}
Fe_R	33.1 ± 1.04	g kg^{-1}
As_R	11.4 ± 0.74	mg kg^{-1}