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Research Article

RESEARCH ARTICLE

# The long-term effect of uranium and pH on the community composition of an artificial consortium

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**One sentence summary:** The composition of an artificial consortium differs depending on the conditions (pH7, pH4.5 and presence of uranium).

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## ABSTRACT

In the environment, microorganisms are living in diverse communities, which are impacted by the prevailing environmental conditions. Here, we present a study investigating the effect of low pH and elevated uranium concentration on the dynamics of an artificial microbial consortium. The members (*Caulobacter* sp. OR37, *Asinibacterium* sp. OR53, *Ralstonia* sp. OR214 and *Rhodanobacter* sp. OR444) were isolated from a uranium contaminated and acidic subsurface sediment. In pure culture, *Ralstonia* sp. OR214 had the highest growth rate at neutral and low pH and only *Caulobacter* sp. OR37 and *Asinibacterium* sp. OR53 grew in the presence uranium. The four strains were mixed in equal ratios, incubated at neutral and low pH and in the presence uranium and transferred to fresh medium once per week for 30 weeks. After 30 weeks, *Ralstonia* sp. OR214 was dominant at low and neutral pH and *Caulobacter* sp. OR37 and *Asinibacterium* sp. OR53 were dominant in the presence of uranium. After 12 weeks, the cultures were also transferred to new conditions to access the response of the consortia to changing conditions. The transfers showed an irreversible effect of uranium, but not of low pH on the consortia. Overall, the strains initially tolerant to the respective conditions persisted over time in high abundances in the consortia.

**Keywords:** interaction; competition; artificial consortium; community composition; uranium; pH

## INTRODUCTION

In the environment, microorganisms live in communities, which are important in many ecosystem functions such as nutrient cycling, oxygen production, catabolism of complex compounds and many others. Bacteria in these communities can form positive associations that improve the function of the community as well as negative interactions that limit the performance of the community (Keller and Surette 2006; Williams *et al.* 2007; Little, Robinson and Peterson 2008; Hibbing *et al.* 2010; Mitri and Foster 2013).

Molecular methods have been heavily used in recent years to describe natural microbial communities (Gill *et al.* 2006; Akob, Mills and Kostka 2007; Biddle *et al.* 2008; Szczepanowski *et al.* 2009; Math *et al.* 2010; Liang *et al.* 2012). While natural communities offer a realistic reflection of the community's capabilities, it is often difficult to discern the contribution of single microbial populations in the community using molecular methods. Conversely, studying microbial communities in the laboratory is often limited by our inability to mimic the natural conditions as well as to cultivate many strains that are part of the natural community (Lawrence *et al.* 2012; De Roy *et al.* 2014). Artificially assembled microbial communities, which can be manipulated under laboratory conditions, offer an alternative to natural communities to understand how communities respond to different environmental conditions.

Co-culture experiments in the laboratory have been used to investigate the interactions in and development of microbial communities. Studies using communities containing only two organisms have demonstrated that bacteria can form mutualistic or commensalistic interactions in a short period of time

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(Christensen *et al.* 2002; Harcombe 2010; Hosoda *et al.* 2011; Xu *et al.* 2013). These interactions typically form as a result of cross-feeding related mechanisms and are unstable associations that can be disrupted by changing growth conditions (Harcombe 2010; Mitri, Xavier and Foster 2011). Artificial multispecies communities containing more than two species have been used to study the degradation of organic pollutants and cellulose and nitrate removal (Kato *et al.* 2005, 2008; Khehra *et al.* 2005; Hudcova *et al.* 2011; Miller *et al.* 2010; Spain and Krumholz 2012). Short-term studies over one growth cycle showed that communities with higher diversity of microorganisms tend to remove a larger array of substrates or remove a single substrate more quickly than individual populations (Hudcova *et al.* 2011; Xu *et al.* 2013). However, one study reported a decrease in the degradation of the antibiotic sulfamethoxazole in co-cultures relative to an individual population indicating that factors other than microbial diversity are important in forming a stable community (Larcher and Yargeau 2011). Therefore, short-term studies can give insights how microbial populations interact under a given condition, but the resulting community might not represent the functionally stable community that exists in the environment (Lawrence *et al.* 2012; De Roy *et al.* 2014). Long-term growth experiments involving 10 or more successive transfers of the mixed cultures to fresh medium offer an alternative to short-term experiments because they allow interactions between artificially assembled bacterial populations to stabilize and adjust to the growth conditions as a community.

Only a few long-term growth experiments using artificial communities have been conducted, but some of these studies showed that microbial communities are more productive at the end of the long-term growth experiment than the starting cultures (Hillesland and Stahl 2010; Summers *et al.* 2010; Lawrence *et al.* 2012; Fiegna *et al.* 2015). For example, the synthetic mutualism between *Desulfovibrio vulgaris* and *Methanococcus maripaludis* that allowed the community to use lactate when neither species could metabolize it on their own required 300 generations to emerge (Hillesland and Stahl 2010).

The subsurface sediment at the Integrated Field Research Challenge (IFRC) at Oak Ridge (TN) is acidic, highly contaminated with heavy metals, radio nucleotides, organic compounds and nitrate (http://www.esd.ornl.gov/orifrc). The groundwater in that area is contaminated with uranium up to 250 $\mu$M (60 mg $l^{-1}$) and had a pH of 3–3.5 (http://www.esd.ornl.gov/orifrc). The diffusion chamber has been used to isolate bacteria from sediment samples obtained from the IFRC subsurface sediment (Bollmann *et al.* 2010). A total of 71 different bacteria have been isolated of which 26 were detected also by molecular methods on the IFRC area in earlier studies (Fields *et al.* 2005; Abulencia *et al.* 2006; Akob, Mills and Kostka 2007; Bollmann *et al.* 2010; Green *et al.* 2010; Hemme *et al.* 2010).

Here, we present a study investigating the impact of different stress factors on the community composition and biomass of an artificial consortium of four bacterial isolates from the IFRC. The consortium consisted of *Caulobacter* sp. OR37, *Asinibacterium* sp. OR53, *Ralstonia* sp. OR214 and *Rhodanobacter* sp. OR444. It was exposed to low pH and high uranium (two stress factors prevalent at the IFRC) and incubated for 30 weeks (or ~300 generations) to determine the ability of the different strains to persist as members of the consortium in the presence of these stress factors over an extended period of time. Quantitative PCR was used to measure the relative abundance of each strain in the consortium, and optical density was used to assess the amount of biomass produced by the consortium in all treatments. The results indicated that strains initially tolerant to high concentrations of uranium or low pH exhibited the ability to persist over time in high abundance under the respective conditions.

## MATERIALS AND METHODS

### Strains

Four bacterial strains that were previously isolated from the contaminated subsurface sediment at the IFRC in Oak Ridge (TN, USA) were used for the experiment (Bollmann *et al.* 2010). Three strains belong to the phylum *Proteobacteria* (*Caulobacter* sp. OR37, *Ralstonia* sp. OR214 and *Rhodanobacter* sp. OR444) and one to the phylum *Bacteroidetes* (*Asinibacterium* sp. OR53) (Bollmann *et al.* 2010).

### Media

The bacterial isolates were grown in mineral salts (MS) medium containing 5 mM $NH_4Cl$, 1 mM NaCl, 0.25 mM $MgSO_4 \bullet 7H_2O$, 5 $\mu$M $CaCl_2 \bullet 2H_2O$, 5 $\mu$M $KH_2PO_4$ and 1 mL $L^{-1}$ trace elements (Bollmann, French and Laanbroek 2011). The medium was buffered with 10 mM HEPES (pH 5–8) or 10 mM HOMO-PIPES (pH 3.5-4.5). After autoclaving, sterile solutions of glucose, tryptone and yeast extract were added to obtain a final concentration of 0.05% (v/v) for each carbon source. For agar plates, the medium was solidified with 15 g $L^{-1}$ Bacto agar. The MS medium was supplemented with uranium (VI) (as sterile filtered uranyl acetate solution) to obtain final concentrations between 25 and 200 $\mu$M uranium directly before the start of the experiment.

### Growth dynamics of the individual strains

All strains were inoculated from MS agar plates into liquid MS medium and incubated for 2 days at 27°C. MS medium (150 $\mu$l) at different pH values (3.5–8) and with different uranium concentrations (0–200 $\mu$M) was distributed into 96-well plates. The plates were inoculated with 10% (v/v) of the 2-day cultures and incubated for 2 days in a plate reader (Synergy HT BioTek Instruments, Winooski, VT, USA) at 27°C. The optical density ($OD_{600}$) was measured for every 10 min after shaking the plate for 10 s. The growth rates were determined by calculating the slope of the natural log-transformed $OD_{600}$ data plotted against time.

### Long-term growth experiment

All strains were inoculated from MS agar plates into liquid MS medium and incubated for 2 days at 27°C. The cultures were diluted with MS medium to the same cell density based on $OD_{600}$ and inoculated in equal parts into 12-well plates with different media. Three different media were used: MS medium at pH 7 (pH7), MS medium at pH 4.5 (pH4.5) and MS medium at pH 7 with 200 $\mu$M uranium (pH7U) (Table 1). We chose this uranium concentration and pH because the values reflect the conditions in the groundwater in the subsurface sediment where the bacteria were isolated (http://www.esd.ornl.gov/orifrc). Each medium was inoculated in six replicates. The plates were incubated without shaking for 1 week at 27°C. After 1 week, the cultures were resuspended by pipetting up and down several times, the $OD_{600}$ was measured, the cultures (1% (v/v)) were inoculated into fresh medium and samples for molecular analysis were taken. This procedure was repeated for an overall incubation time of 30 weeks.

New culture conditions were added after 12 weeks to investigate how the cultures would react to a change in the growth environment (Table 1). Therefore, the pH7 cultures were

**Table 1.** Experimental conditions in the long-term incubation experiments.

| Condition | pH | U [$\mu$M] | Comment |
|---|---|---|---|
| Original culture incubated for 30 weeks | | | |
| pH7 | 7 | 0 | Original culture at pH 7 |
| pH4.5 | 4.5 | 0 | Original culture at pH 4.5 |
| pH7U | 7 | 200 | Original culture at pH 7 with 200 $\mu$M uranium |
| Transfer cultures after 12 weeks | | | |
| pH4.5 → pH7 | 7 | 0 | Transferred from pH 4.5 to pH 7 |
| pH7 → pH4.5 | 4.5 | 0 | Transferred from pH 7 to pH 4.5 |
| pH7U → pH7 | 7 | 0 | Transferred from pH 7 with 200 $\mu$M uranium to pH 7 |
| pH7 → pH7U | 7 | 200 | Transferred from pH 7 to pH 7 with 200 $\mu$M uranium |

transferred to pH4.5 (pH7 → pH4.5) and pH7U (pH7 → pH7U), pH7U was transferred to pH7 (pH7U → pH7), and pH4.5 was transferred to pH7 (pH4.5 → pH7). These culture conditions were treated in the same way as the other cultures and incubated for 18 more weeks until the end of an overall incubation time of 30 weeks.

### Relative abundance of each strain at the beginning of the experiment

To determine the relative abundance of each strain directly at inoculation, we grew all four cultures for 2 days under the same conditions as the starting cultures for the experiment, adjusted the $OD_{600}$ to 0.3, mixed 250 $\mu$l of each culture and used molecular analysis to determine the relative abundance of each strain.

### Molecular analysis

Bacterial cultures (1 ml) were centrifuged (24 328 × g, 10 min) at the end of each week, the supernatant was discarded and the cell pellets were stored at −80°C until further analysis.

### DNA isolation

DNA was isolated using the DNeasy blood and tissue kit (Qiagen, Valencia, CA, USA) according to the manufacturers recommendations. DNA concentration and quality was measured with a NanoDropTM 3300 Fluorospectrometer (Thermo Fisher Scientific, Willmington, DE, USA). The DNA was diluted to 5 ng $\mu L^{-1}$ stored in aliquots at −20°C until further analysis.

### Primer design and optimization for quantitative PCR (qPCR)

Primers to quantify the four different strains were used as previously published or designed using the NCBI blast primer tool (Table S1, Supporting Information) (Muyzer, de Waal and Uitterlinden 1993; Fierer *et al.* 2005; Bacchetti De Gregoris *et al.* 2011). The optimal annealing temperatures of the primers for each strain were determined on a PCR machine with a temperature gradient (Biorad, Hercules, CA, USA) using GoTaq Green master Mix (Promega, Madison, WI, USA) and the following protocol: initial denaturation 5 min at 95°C, 30 cycles of denaturation for 30 s at 95°C, annealing for 30 s at a temperature gradient of 50–65°C, extension for 45 s at 72°C and final extension for 5 min at 72°C. The size of the PCR products was determined using agarose electrophoresis. The specificity of the primers was determined by amplification of the DNA of each strain individually and in different mixtures with all four different primer sets.

**Table 2.** Maximum uranium concentration and minimum pH value in the MS medium that allowed growth of the individual bacterial strains as pure cultures. The strains were incubated over a range of uranium concentrations (0–200 $\mu$M) and pH values (3.5–8) to determine the growth at the different conditions.

| | Minimum pH value | Uranium [$\mu$M] |
|---|---|---|
| *Caulobacter* sp. OR37 | 3.5 | 200 |
| *Asinibacterium* sp. OR53 | 4.5 | 200 |
| *Ralstonia* sp. OR214 | 3.5 | 25 |
| *Rhodanobacter* sp. OR444 | 4.5 | 100 |

### Quantitative PCR

The qPCR reactions were performed using SensiFAST Sybr No-ROX PCR mix (Bioline, London, UK) on an Illumina Eco Real-Time PCR system (Illumina, San Diego, CA, USA) with primers and under conditions presented in Tables S1 and S2 (Supporting Information). Standard curves were constructed using plasmids containing the 16S rRNA gene sequence of the strain of interest. The efficiency of the reactions ranged from 91% to 108% and $R^2$ was in all experiments 0.99 (Table S2, Supporting Information). All samples were run as technical duplicates. The relative abundance was calculated by dividing the absolute copy number of the 16S rRNA genes of each strain in the mixture by the summation of the copy number of all strains.

## RESULTS

### Growth of the individual bacterial strains at different uranium concentrations and pH values

The growth of all four strains as individual cultures was determined over a range of uranium concentrations (0–200 $\mu$M) and pH values (3.5–8). The results were used to determine the minimum pH and maximum uranium concentration that sustained growth of each of the individual strains after two days (Table 2). *Ralstonia* sp. OR214 and *Caulobacter* sp. OR37 grew at pH 3.5, while pH 4.5 was the lowest pH at which *Rhodanobacter* sp. OR444 and *Asinibacterium* sp. OR53 grew (Table 2). *Ralstonia* sp. OR214 and *Rhodanobacter* sp. OR444 did not grow at concentrations above 25 or 100 $\mu$M uranium, respectively. *Caulobacter* sp. OR37 and *Asinibacterium* sp. OR53 grew in 200 $\mu$M uranium (Table 2). In addition, the growth rates of each strain were determined under the same conditions that were used for the long-term growth experiment: pH 7 (pH7), pH 4.5 (pH4.5) and pH 7 in the presence of 200 $\mu$M uranium (pH7U) (Table 3). *Ralstonia* sp. OR214 exhibited the highest growth rates at pH 7 and pH 4.5, while *Caulobacter* sp. OR37

**Table 3.** Specific growth rates of the bacterial strains as pure cultures under the three experimental conditions at pH 7, pH 4.5 and pH 7 with uranium (pH7, pH4.5, pH7U) (mean ± SD, $n = 3$). The corresponding growth curves ($OD_{600}$ over time) were presented in Fig. S1 (Supporting Information).

| | pH7 | pH4.5 | pH7U |
|---|---|---|---|
| *Caulobacter* sp. OR37 | 8.52 ± 0.10 | 3.09 ± 0.39 | 7.14 ± 0.09 |
| *Asinibacterium* sp. OR53 | 4.80 ± 0.02 | 2.43 ± 0.06 | 3.57 ± 0.02 |
| *Ralstonia* sp. OR214 | 10.43 ± 0.03 | 7.84 ± 0.24 | 0 |
| *Rhodanobacter* sp. OR444 | 4.89 ± 0.01 | 3.95 ± 0.03 | 0 |

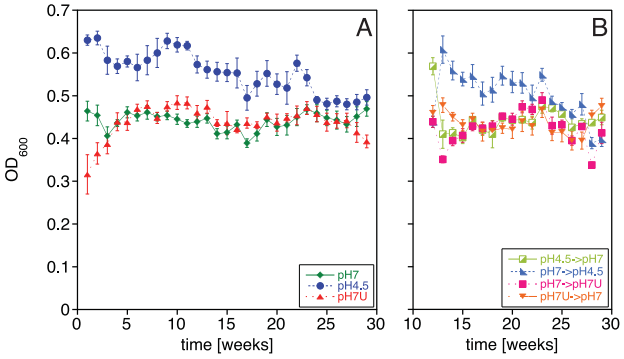


**Figure 1.** Biomass concentration ($OD_{600}$) at the end of each incubation week before transfer to fresh medium. See Table 1 for the description of the individual treatments. Arrows indicate the transfers at week 12 described in Table 1. **A**: pH and **B**: uranium (mean ± SD, $n = 6$).

exhibited the highest growth rate at pH 7 in the presence of 200 $\mu$M uranium. *Rhodanobacter* sp. OR444 and *Ralstonia* sp. OR214 did not grow at pH 7 in the presence of 200 $\mu$M uranium.

## Biomass concentration ($OD_{600}$) during the long-term growth experiment

The four bacterial strains were grown together for 30 weeks at pH 7, pH 4.5 and pH 7 with 200 $\mu$M uranium (pH7, pH4.5 and pH7U; Table 1). The biomass measured as $OD_{600}$ in the mixed cultures grown at pH7 was stable at an $OD_{600}$ of 0.4 to 0.5 throughout the whole experiment (Fig. 1A). The $OD_{600}$ of pH7U increased over the first 6 weeks and stayed stable for the rest of the incubation period around 0.4–0.5. The highest $OD_{600}$ was measured at pH4.5 at the beginning of the 30 weeks incubation period and decreased over time to the same level as the $OD_{600}$ of the cultures incubated at pH7 and pH7U (Fig. 1B). After 12 weeks, the original cultures were transferred to new conditions (Table 1). The $OD_{600}$ of the cultures transferred from pH 7 to pH 7 with uranium (pH7 → pH7U) initially decreased but leveled off at the same value as the original pH7 and pH7U cultures (Fig. 1A). No changes in the $OD_{600}$ were detected when the cultures were transferred from pH 7 with uranium to medium without uranium (pH7U → pH7) (Fig. 1A). When the cultures were transferred from pH 7 to pH 4.5 (pH7 → pH4.5) and vice versa (pH4.5 → pH7), the $OD_{600}$ of the cultures increased or decreased, respectively.

## Relative abundance in different conditions during 30-week incubations

The relative abundance of each of the four strains in the consortium was determined using qPCR targeting their respective 16S rRNA gene. At the beginning of the experiment, the relative abundance of all four strains under all conditions was between 12% and 48% (Figs 2 and 3).

## Relative abundance at pH 7 (pH7)

At pH7, all strains were present during the 30 weeks (Fig. 2B). Throughout the course of the experiment, *Ralstonia* sp. OR214 was the most abundant strain and represented around 60% of the detected 16S rRNA genes. The relative abundance of the other three strains ranged between 5% (*Rhodanobacter* sp. OR444) and 20% (*Caulobacter* sp. OR37 and *Asinibacterium* sp. OR53) (Fig. 2B). The community composition at pH 7 was stable during the 30-week incubation period (Figs 2B and 3).

## Relative abundance at pH 4.5 (pH4.5)

At pH4.5, the community composition was also stable over the course of the experiment. *Ralstonia* sp. OR214 was the dominant strain (80%) and *Caulobacter* sp. OR37 and *Asinibacterium* sp. OR53 each made up around 10% of the community (Fig. 2C). The relative abundance of *Rhodanobacter* sp. OR444 was very low (<0.05%) (Fig. 2C).

## Relative abundance at pH 7 with 200 $\mu$M uranium (pH7U)

*Caulobacter* sp. OR37 and *Asinibacterium* sp. OR53 were the dominant members in the consortium at pH7U, while *Ralstonia* sp. OR214 had a relative abundance of 0.2% (Fig. 2A). *Rhodanobacter* sp. OR444 was not detected in the artificial consortium. By week 5, *Caulobacter* sp. OR37 was dominant with a relative abundance of more than 90% in the consortium whereas *Asinibacterium* sp. OR53 had a relative abundance of less than 10% (Fig. 2A). Over time the relative abundance of *Caulobacter* sp. OR37 decreased to 60% and the relative abundance of *Asinibacterium* sp. OR53 increased up to 40% (Figs 2A and 3).

## Relative abundance in the cultures after transfer to different conditions in week 12 (Table 1)

The relative abundance of the strains when transferred from pH 7 to pH 4.5 (pH7 → pH4.5) and vice versa (pH4.5 → pH7) was similar to the relative abundance observed in the original consortia under each respective condition (Figs 2B, C, F, G and 3).

When the cultures were transferred from medium with uranium to medium without uranium (pH7U → pH7), the relative abundance of *Caulobacter* sp. OR37 decreased to around 40% and the relative abundance of *Asinibacterium* sp. OR53 increased to 60% (Fig. 2D). However, the two strains were still the dominant strains in the consortium with a combined relative abundance higher than 99.5% (Figs 2D and 3). The transfer from medium without uranium to medium with 200 $\mu$M uranium (pH7 → pH7U) resulted in a decrease of the relative abundance of *Ralstonia* sp. OR214 and *Rhodanobacter* sp. OR444 to values lower than 0.1% (Fig. 2E). *Asinibacterium* sp. OR53 and *Caulobacter* sp. OR37 became the dominant members of the consortium (Figs 2E and 3).

In summary, pH4.5 and pH7 had a similar effect on the community composition of the artificial consortium (Fig. 3). *Ralstonia* sp. OR214 was the dominant strain at pH7 and pH4.5, and the other three strains were present in different abundances. Uranium (pH7U, pH7 → pH7U and pH7U → pH7) had a very different effect on the composition of the consortium. *Caulobacter*

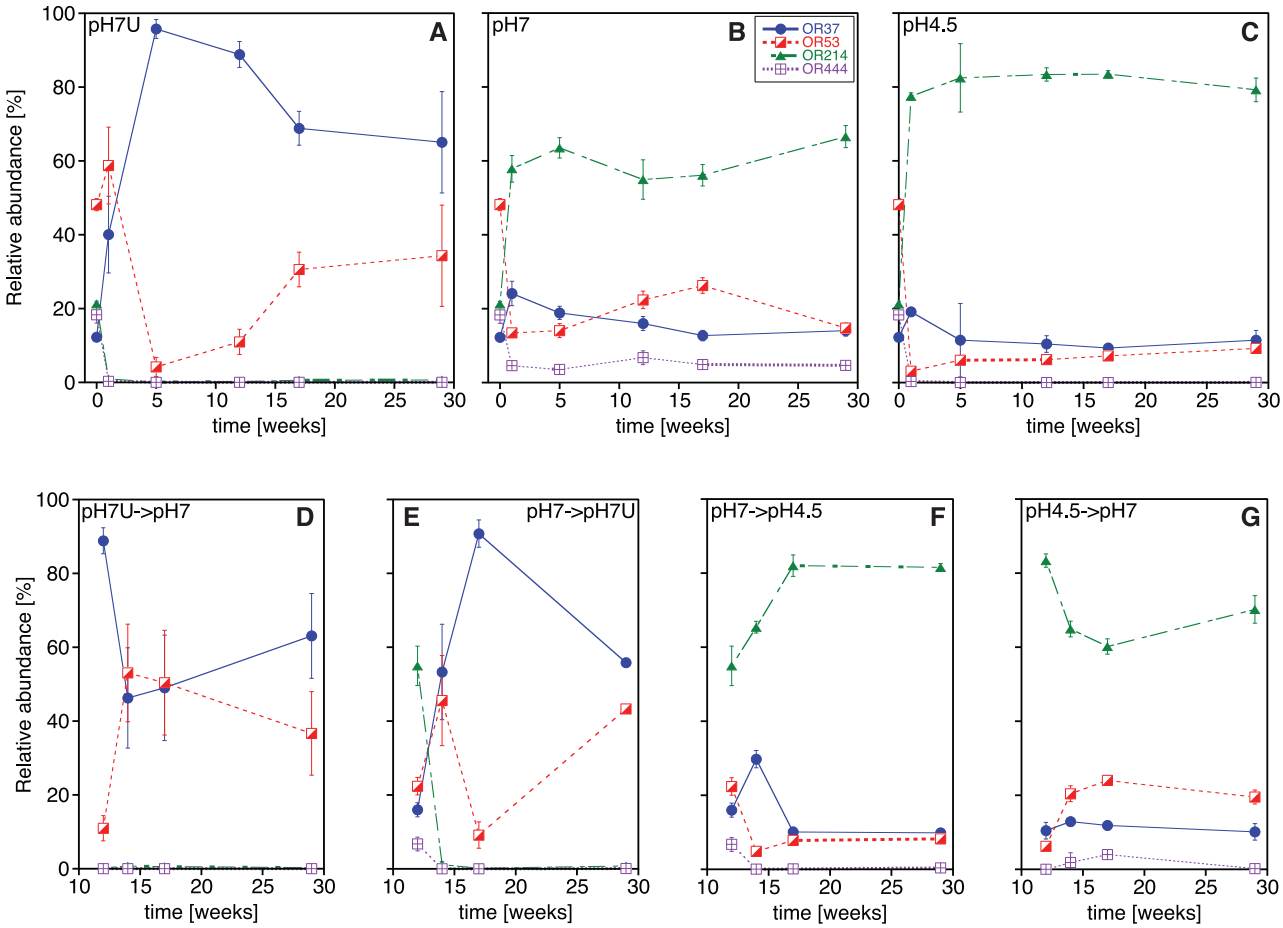


**Figure 2.** Relative abundance [%] of the 16S rRNA genes of *Caulobacter* sp. OR37, *Asinibacterium* sp. OR53, *Ralstonia* sp. OR214 and *Rhodanobacter* sp. OR444 in an artificial consortium at **A**: pH 7 with 200 $\mu$M uranium (pH7U), **B**: pH 7 (pH7), **C**: pH 4.5 (pH4.5), **D**: after transfer from pH 7 with 200 $\mu$M uranium to pH 7 (pH7U → pH7), **E**: after transfer from pH 7 to pH 7 with 200 $\mu$M uranium (pH7 → pH7U), **F**: after transfer from pH 7 to pH 4.5 (pH7 → pH4.5) and **G**: after transfer from pH 4.5 to pH 7 (pH4.5 → pH7) (mean ± SD, $n = 3$).

sp. OR37 and *Asinibacterium* sp. OR53 were dominant in the presence of uranium (pH7U and pH7 → pH7U) and stayed dominant if the consortium was transferred back to conditions without uranium (pH7U → pH7).

## DISCUSSION

The overall goal of this study was to determine the development of the community structure of an artificial consortium of four bacterial strains under three different conditions (Table 1). We chose these four isolates out of a larger number of isolates, because they showed different growth patterns under each of the tested conditions (Tables 2 and 3; Fig. S1, Supporting Information).

### Growth as individual strains and composition of the artificial consortium

The strains with the highest growth rates under each condition as pure cultures were also the dominant strains in the artificial consortium under that particular condition. *Ralstonia* sp. OR214 was dominant at pH7 and pH4.5 and *Caulobacter* sp. OR37 dominated in pH7U (Fig. 3, Table 3). The dominant strains are considered to be the best competitors under the prevailing conditions (Tilman 1977). Therefore, it can be assumed that the faster growing strains were the strains with the best competitive abilities compared to the other strains under each of the applied conditions. The biomass produced by the consortium did not increase over the 30-week incubation period under any of the tested conditions (Fig. 1). A significant increase in biomass would be an indication of fewer negative interactions or even positive interactions between the strains in the artificial consortium (Foster and Bell 2012; Fiegna *et al.* 2015). Based on these observations, it is very likely that the negative interaction competition was the prevalent interaction between the four strains in the artificial consortium.

### Impact of pH7 on the composition of the artificial consortium

At pH7, *Ralstonia* sp. OR214 was consistently the most abundant strain and the strain with the highest growth rate (Figs 2B and 3; Table 3). Compared to the whole sequenced genomes of *Caulobacter* sp. OR37, *Asinibacterium* sp. OR53 and *Rhodanobacter* sp. OR444, the genome of *Ralstonia* sp. OR214 had a high number of ABC transporters for the uptake of amino acids and sugars

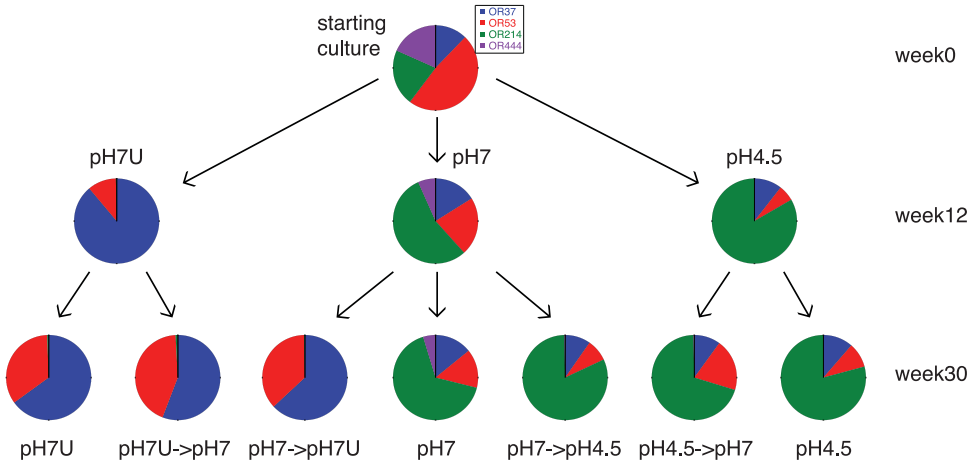


**Figure 3.** Relative abundance [%] of the 16S rRNA genes of *Caulobacter* sp. OR37, *Asinibacterium* sp. OR53, *Ralstonia* sp. OR214 and *Rhodanobacter* sp. OR444 in the artificial consortium under all experimental conditions at the beginning of the experiment (week 0), at week 12 and at the end of the experiment (week 30) ($n = 3$). Data summarized from Fig. 2.

suggesting that this strain was able to take up a broader range of substrates than the rest of the artificial consortium (Utturkar *et al.* 2013a,b; Brzoska and Bollmann, unpublished). The medium used in the long-term growth experiment contained a wide variety of carbon sources such as glucose, casamino acids and yeast extract suggesting that *Ralstonia* sp. OR214 had a competitive advantage due to its increased number of transporters for carbon uptake. The remaining community was made up by the other three strains (Figs 2B and 3) indicating that all strains were able to grow in the presence of a dominant strain with the competitive advantage for resources.

## Impact of pH 4.5 on the composition of the artificial consortium

At pH 4.5, *Ralstonia* sp. OR214 dominated the artificial community (Figs 2C and 3). Low pH in the environment results in an influx of protons, which acidify the cytoplasm of neutrophilic bacteria (Krulwich, Sachs and Padan 2011). F-type ATPases and kup-type potassium transporters are involved in pH regulation and were found in the genomes of all four strains (Utturkar *et al.* 2013a,b; Brzoska and Bollmann, unpublished). In addition, the genome of *Ralstonia* sp. OR214 contained an arginine/lysine/ornithine decarboxylase (Utturkar *et al.* 2013b), which is involved in arginine-dependent acid resistance in *Escherichia coli* (Foster 2004). Therefore, the high abundance of *Ralstonia* sp. OR214 in the artificial consortium at low pH values compared to the other isolates could be due to a combination of the presence of the arginine/lysine/ornithine decarboxylase allowing the strain to better withstand the effects of high proton concentrations and the increased number of transporters for carbon sources in the genome for the uptake of a wide variety of carbon sources.

## Impact of the presence of 200 $\mu$M uranium on the composition of the artificial consortium

*Caulobacter* sp. OR37 and *Asinibacterium* sp. OR53 were dominant in the pH7U condition (Figs 2A and 3). Uranium as a heavy metal is toxic to microorganisms in small quantities due its ability to cause DNA damage and to reduce enzyme activities (Nies 2003). *Caulobacter* sp. OR37 and *Asinibacterium* sp. OR53 have mechanisms to withstand the effects of uranium. *Caulobacter crescentus*, a close relative of *Caulobacter* sp. OR37, precipitates uranyl phosphate complexes outside of the cells and withstands uranium concentrations up to 1 mM in short-term experiments (Hu *et al.* 2005). Precipitation of uranium and uranyl phosphate has also been observed for *Caulobacter* sp. OR37 (Brzoska and Bollmann, unpublished). Unpublished results show that *Asinibacterium* sp. OR53 very likely utilized biosorption to tolerate uranium (Brzoska and Bollmann, unpublished). During biosorption, phosphate groups in the lipopolysaccharides passively bind uranium (Newsome, Morris and Lloyd 2014).

In addition to *Caulobacter* sp. OR37 and *Asinibacterium* sp. OR53, *Ralstonia* sp. OR214 was detected in pH7U with a relative abundance of 0.5%, while *Ralstonia* sp. OR214 as pure culture was not able to grow in the presence of uranium (Fig. 2A; Table 3). *Caulobacter* sp. OR37 and *Asinibacterium* sp. OR53 very likely remove uranium from the medium and thereby reduce the stress for *Ralstonia* sp. OR214 allowing the strain to survive as part of the consortium.

## Transfer of the cultures to different conditions at week 12

When transferred to new conditions at week 12, the community composition at pH4.5 → pH7 and pH7 → pH4.5 shifted slightly. At the end of the experiment (week 30), the relative abundances of the different community members at pH7 → pH4.5 resembled those at pH4.5 and vice versa (Figs 2B, C, F, G and 3) indicating that changes in pH did not alter the community composition on a large scale. Uranium had a different effect on the community composition. The relative abundances of the strains in the consortium in pH7 → pH7U shifted in the same manner as in pH7U (Fig. 2A and E). In pH7U → pH7, the relative abundances of the different strains did not shift back to the same community composition that was found at pH7 (Fig. 2D). In summary, these observations indicate that the effects of pH on the consortium were minimal while uranium altered the consortium irreversibly. *Ralstonia* sp. OR214 was able to survive the presence of uranium as part of the consortium with *Caulobacter* sp. OR37 and *Asinibacterium* sp. OR53 but did not increase in abundance when

transferred from pH7U to pH7. Uranium very likely had a negative effect on *Ralstonia* sp. OR214 that changed the competitive abilities of the strain in comparison to *Caulobacter* sp. OR37 and *Asinibacterium* sp. OR53 and resulted in the irreversible change in the overall community structure.

*Rhodanobacter* sp. OR444 was below the detection limit under both stress conditions, but behaved differently when transferred back to pH7. In pH4.5 → pH7 the strain grew back to almost the same abundance as in pH7, but in pH7U → pH7 it could not be detected anymore indicating that the strain reacted differently to pH and uranium as stress factors. Uranium had a detrimental effect on *Rhodanobacter* sp. OR444 while pH 4.5 allowed survival of the strain in very low abundance.

### *Rhodanobacter* sp. OR444 and *Ralstonia* sp. OR214 at pH7U and under field conditions

*Ralstonia* sp. OR214 was detected in abundances around 0.5% at pH7U, while *Rhodanobacter* sp. OR444 was below detection limit. Both strains did not grow as individual cultures at pH7U (Table 3) indicating that both strains did not have efficient mechanisms to withstand the toxic effects of uranium. However, close relatives of *Ralstonia* sp. OR214 and *Rhodanobacter* sp. OR444 were detected at the IFRC often even in high abundances (Akob, Mills and Kostka 2007; Hemme *et al*. 2010; Spain and Krumholz 2011) suggesting that these strains should have the ability to survive in the presence of heavy metal contaminants. Besides the low pH and high uranium concentration, high nitrate and low oxygen concentrations are prevalent conditions in the subsurface sediment at Oak Ridge indicating that the conditions might be suited well for denitrification (http://www.esd.ornl.gov/orifrc/). The genomes of both strains contain the full array of enzymes necessary for denitrification (Utturkar *et al*. 2013b; Brzoska and Bollmann, unpublished), and denitrification has been demonstrated in relatives of *Rhodanobacter* sp. OR444 as well as in the environment from which these strains were isolated (Green *et al*. 2010, 2012; Spain and Krumholz 2011). The ability to denitrify might change the susceptibility of *Ralstonia* sp. OR214 and *Rhodanobacter* sp. OR444 to the toxic effects of uranium and thereby explain the ability of the strains to survive and thrive in the original environment in the presence of uranium but not under the applied experimental conditions.

## CONCLUSIONS

This experiment adds to the small number of long-term experiments that investigated the changes in biomass and community composition of artificial consortia (Hillesland and Stahl 2010; Lawrence *et al*. 2012; Fiegna *et al*. 2015). Some of the studies found that positive interactions evolved over time in the consortia and resulted in the ability of the consortium to use substrates more efficiently (Hillesland and Stahl 2010; Fiegna *et al*. 2015). The biomass of the artificial consortium in this study did not change and the community composition under the different conditions was very stable (Figs 1–3). Therefore, it is very likely that no positive interactions evolved and that competition was the main interaction in the artificial consortium.

## SUPPLEMENTARY DATA

Supplementary data are available at FEMSEC online.

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