BACTERIAL INVASION POTENTIAL IN WATER IS DETERMINED BY NUTRIENT AVAILABILITY AND THE INDIGENOUS COMMUNITY

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AIMS

In drinking water and the distribution systems, bacterial growth and biofilm formation has to be controlled for drinking water safety, limiting taste or odour development and preventing clogging or bio-corrosion problems. After a contamination with undesired bacteria, factors like temperature, nutrient availability and predation will influence the survival of these invaders (Hellweger, *et al.*, 2009). For controlling microbial growth in drinking water, two main pathways are used; either a disinfection process is used, or a biologically stable water is created by extensive nutrient limitation, preventing bacterial regrowth. This is mainly monitored by focussing on organic carbon as main nutrient, measured by total organic carbon (TOC) or, in best case, AOC (assimilable organic carbon) (Van Der Kooij, 2000). However, specific for pathogens, Vital *et al*. showed that growth did not correlate with dissolved organic carbon and only weakly with AOC concentrations (Vital, *et al.*, 2010). The growth of invading bacteria in drinking water has always been a big concern and the influencing parameters are poorly understood. In this study, *Pseudomonas putida* – a model invader – was spiked in different drinking water samples in order to examine the effect of different nutrient concentrations and the indigenous microbial community on the growth and survival of *P. putida.*

METHODS

The *gfp*-labeled *Pseudomonas putida* SM1699 (Sternberg, *et al.*, 1999) was grown overnight at 27°C in Tryptic Soy Broth, harvested by 4 min centrifugation at 3000g and washed twice in 0.22 µm filtered drinking water to remove traces of medium. For assessing intact and damaged bacterial cell concentrations, the combination of SYBR Green I and propidium iodide was used as a live/dead staining and measured with a Cyan ADP LX flow cytometer. For the flow cytometric counting of *gfp*-labeled *P. putida* cells, no stain was added to the samples.

10 mL samples of drinking or surface water (Table 1) were spiked with a concentrated *P. putida* solution, until a final concentration of half of the initial concentration of the present indigenous community of that water sample. When C, N or P was dosed, it was in the form of Na-acetate, NH_4Cl and Na_2HPO_4. When the bacterial communities of drinking and surface water were switched, this was done by filtration on a 0.22 µm filter membrane. All tests were performed in at least triplicate and all samples were incubated in the dark on a shaker at 27°C.

Table 1: Chemical and biological properties of the samples.

	Drinking water	Surface water
pH	6,88	7,72
Intact cells.mL^{-1}	4,4 x 10^4	1,4 x 10^7
Damaged cells.mL^{-1}	4,4 x 10^4	3,7 x 10^6
AOC (µg C.L^{-1})	57 ± 33	616 ± 77
Conductivity(mS.cm^{-1})	0,417	0,666
COD (mg.L^{-1})	< 15	25
NH_4^+ (mg.L^{-1})	< 0,1	< 0,1
Cl^- (mg.L^{-1})	8,95	78,2
NO_2^- (mg.L^{-1})	< 0,05	0,713
NO_3^- (mg.L^{-1})	4,24	21,0
PO_4^{3-} (mg.L^{-1})	< 0,05	< 0,05
SO_4^{2-} (mg.L^{-1})	13,5	9,49

RESULTS

In this study, *P. putida* has been spiked in several waters with different nutrient concentrations and indigenous bacterial communities. When spiking in plain drinking water, the *P. putida* concentration tripled within one day, but this concentration lowered soon again (Fig. 1). When adding different carbon concentrations up to 37 mg C.L^{-1}, *P. putida* reached 5.87 x 10^5 cells.mL^{-1}, which was 5 times the maximum concentration of the previous experiment. However, after two days, this concentration lowered and no clear differences could be detected anymore compared to plain drinking water (Fig. 1). Carbon possibly promoted initial growth of *P. putida*, however, the survival time in drinking water was not elongated. Apparently, carbon availability is not fully explaining biological stability and regrowth. Addition of only phosphate and/or nitrogen addition indicated the importance of phosphate for growth. After two days, the *P. putida* concentrations had risen very similar if either only phosphate or only carbon was dosed (Fig. 1). When only N was added, the initial *P. putida* concentrations only decreased (data not shown). When adding combined carbon, phosphate and nitrate, *P. putida* grew for 5 days until a maximum concentration 3 orders of magnitude higher than the initially spiked concentration. This was followed by a initial slight die-off and survival for a longer period of time (Fig. 1). The combination of C, N and P enabled extended growth and survival of the invader. Surprisingly, when a similar set of experiments was conducted in surface water, there was some initial growth but in each case, after five days, no *P. putida* could be detected anymore in surface water (Fig. 2). While nutrients were brought to the same level drinking water and surface water, the indigenous bacterial community stayed different. To examine this effect, indigenous bacterial communities of both samples were switched, resulting in drinking water harbouring surface water bacteria and vice versa. Carbon, nitrogen and phosphorus were added in the same concentrations like before. After spiking *P. putida* in surface water with drinking water bacteria, a similar extended growth and survival was seen like before in the nutrient-enriched drinking water (Fig. 1).

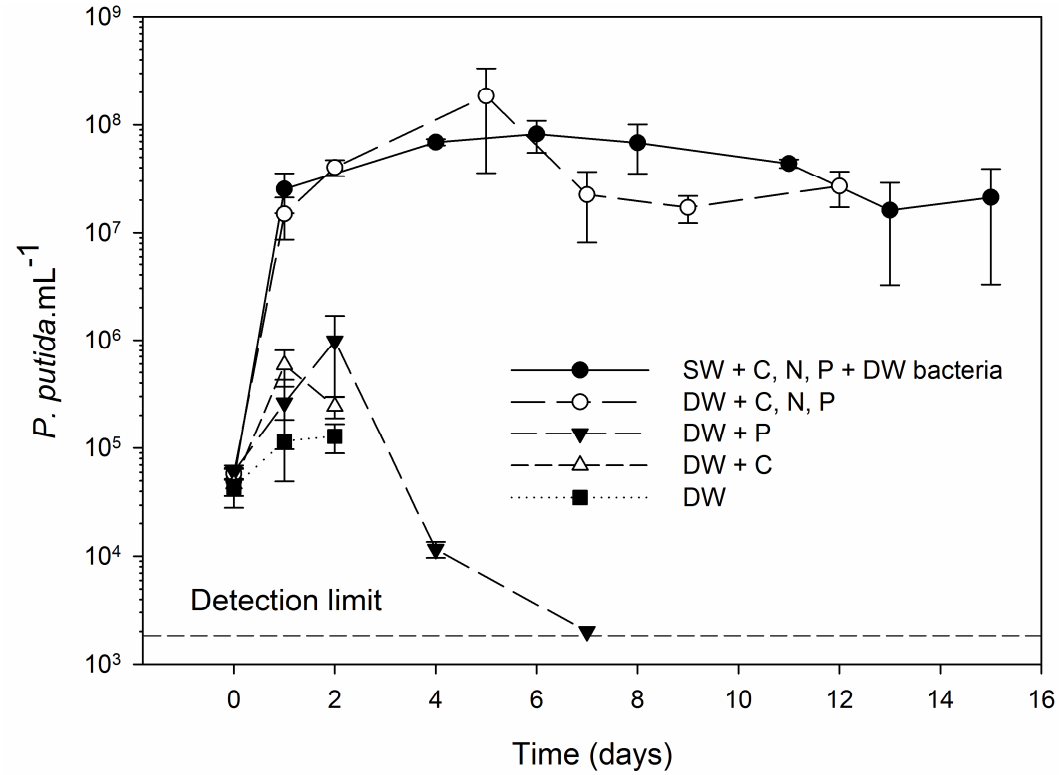

Fig. 1: The growth and survival of *Pseudomonas putida,* spiked in different types of water. DW: drinking water. SW: surface water. Only in water samples with C, N and P added and drinking water bacteria as indigenous community, a survival was possible for an extended time (n=3).

In the drinking water with the surface water community on the other hand, growth was more limited this time, although the survival time was also extensive (data not shown). Previous authors already reported either antagonistic or protagonistic effects of indigenous bacteria on the survival of invaders (Ducluzeau, *et al.*, 1984, Kerr, *et al.*, 1999). However, none of the theories could fully explain our results and a microbial community analysis with DGGE was performed. The higher richness in surface water could possibly lead to the higher invasion resistance, as stated by the diversity-invasibility hypothesis (Kennedy, *et al.*, 2002), however, the difference in richness was limited. Alternatively, a higher initial community evenness can also result a higher invasion resistance (De Roy, *et al.*, 2013). This was also in accordance with our results, where the invasion-resistant surface water was shown to have a higher evenness compared to drinking water.

CONCLUSIONS

In conclusion, the antagonistic or protagonistic effect of indigenous water communities on growth of invaders is hard to predict. This depends on the bacterial community structure and each water source has its own unique bacterial community, influencing invasion. It will remain difficult to draw general conclusions about these complex effects without testing for the specific bacteria in the specific water for each situation. However, these experiments could confirm the combined importance of carbon and phosphate; extensive limitation of one of both can prevent the long-term growth or survival of an invader. A combined extensive

carbon and phosphate removal is a possible pathway for providing safe, biostable drinking water, not susceptible for invasion.

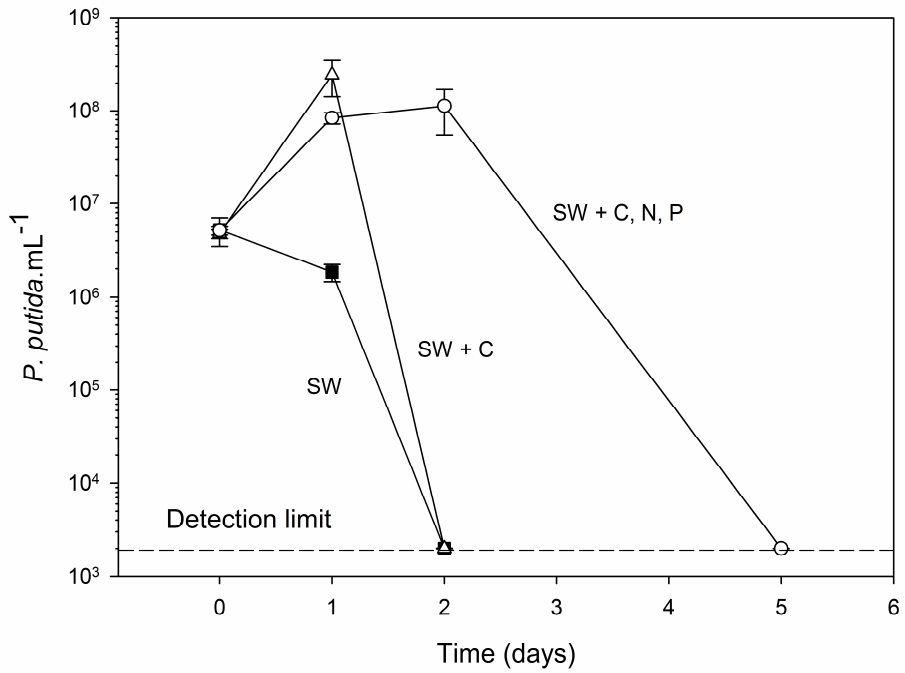

Fig 2: The growth and survival of *Pseudomonas putida,* spiked in surface water. In none of the surface water samples, *P. putida* (n=3) could be detected after 5 days (n=3).

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