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The growth curve method to rapidly derive the antibacterial potential of polyoxovanadates †

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Abstract: In previous studies (Marques-da-Silva *et al.*, 2019), we measured the minimum inhibitory concentrations (MIC) of 3 polyoxovanadates, V10, MnV11, and MnV13, against *Escherichia coli*. MICs were obtained following the standard method, which requires a 16-20h culture and might neglect effects of compounds' metabolism during incubation. In this work, we studied the action of those compounds against *Enterococcus faecalis* by monitoring the bacterial growth kinetics, and we observed that the inhibition was evident right from the beginning of the exponential phase. Notably, data collected until 7h culture was enough to identify the compounds with stronger antibacterial activity according to standard MICs.

Keywords: Polyoxometalates; antibacterial agents; *Enterococcus faecalis*; manganese polyoxovanadates; drug discovery; rapid methods in Microbiology.

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1. Introduction

Polyoxometalates are a well-known group of anionic polynuclear metal oxides (containing V^{V}, Ta^{V}, Nb^{V}, W^{VI}, and Mo^{VI}, usually in their highest oxidation state) with distinct and chemically changeable cluster structures. These compounds present important therapeutic potential as anticancer, antiviral, and antibacterial agents [1].

In a previous work [2], we measured the minimum inhibitory concentrations (MIC) of 3 polyoxovanadates (POVs), namely $Na_6V_{10}O_{28}$ (abbreviated V10), $K_5Mn^{IV}V_{11}O_{32}.10\ H_2O$ (MnV11), and $K_7Mn^{IV}V_{13}O_{38}.18\ H_2O$ (MnV13) against the Gram-negative bacteria *Escherichia coli*. The MICs were obtained following the well-accepted serial two-fold dilution method. Nevertheless, this method requires a 16-20h culture and might neglect the effects of compounds' metabolism/speciation changes during the incubation.

There is a clear need for rapid methods to investigate potential antibacterial agents, antibiotic susceptibility, and novel effective combinations against different bacteria [3,4]. Sun et al. (2016) described a method based on OD600 and ATP measurements in a miniaturized set-up to identify new drugs and drug combinations against multi-drug-resistant bacteria [3]. Recently, Chakansin et al. (2022) presented a dye-based assay to rapidly screen the antibacterial activity of nanomaterials whose turbidity interferes with conventional methods [4]. Although rapid and simple, it was observed that the dye

could be degraded in the presence of nanomaterials with photocatalytic properties at high concentrations.

The application of dye-based assays to test POVs should also be regarded carefully because some of these compounds can catalyze the degradation of dyes [5]. In addition, many POVs are colored, and the color changes with the speciation.

Several (bio)chemical modifications of metallo-drugs, such as POVs, can affect their bioactivity and putative interactions with proteins [6,7]. Besides, the metabolic conversion of POVs by bacteria was reported before [2]. POVs' speciation and bioactivity can therefore change during incubation with microorganisms [6], and endpoint (single-time) assays like the conventional MIC determination method can fail to detect the influence of compounds' speciation.

Taking into account these limitations, in this work we further studied the antibacterial action of the POVs against the Gram-positive *Enterococcus faecalis* by monitoring the growth curve. The method directly following bacterial multiplication is cheap and does not requires detection reagents. Moreover, it can be automated and miniaturized for high-throughput screening of numerous compounds against different bacteria [3]. In addition, the MICs of the POVs against *E. faecalis* were determined for the first time.

2. Materials and Methods

2.1. Polyoxovanadates, bacteria and culture medium

The synthesis of the POVs and preparation of the stock solutions were performed as described in our previous work [2]. The antibacterial studies were carried out with *E. faecalis* (ATCC 29212) in LB broth.

2.2. Minimum inhibitory concentrations determination

MICs were measured by the serial two-fold dilution method using dilutions of each POV in concentrations 1024, 2048, and 4096 $\mu g \cdot ml^{-1}$. After inoculation with the bacteria and incubation for 18 hours, the occurrence or absence of bacterial growth was visually examined (turbidity or presence of a cell deposit).

2.3. Bacterial growth monitoring

Growth curves of *E. faecalis* in LB were obtained by monitoring the optical density of the cultures at 600 nm (OD600), up to 9h incubation. The POV compounds were tested at 0.5 mM concentration, based on the antibacterial data obtained before with *E. coli* [2].

3. Results and Discussion

The antibacterial action of V1, V10, MnV11, and MnV13 against *E. faecalis* was investigated by monitoring the growth of the bacteria at different incubation times and by the conventional MIC determination method.

For the growth curve method, the kinetics of bacterial growth was followed from the OD600 measured in a laboratory spectrophotometer. Figure 1 illustrates the effect of a POV with low or insignificant antibacterial action, V1, and a clearly inhibitory compound, V10. The comparison between the growth curves in the presence of the POVs and the corresponding control cultures indicated that the compounds did not affect the lag time of the culture. However, the inhibitory effects of V10, MnV11, and MnV13 on the growth of *E. faecalis* were evident right from the beginning of the exponential phase. The monomeric vanadate species (V1) showed only modest inhibition of bacterial growth.

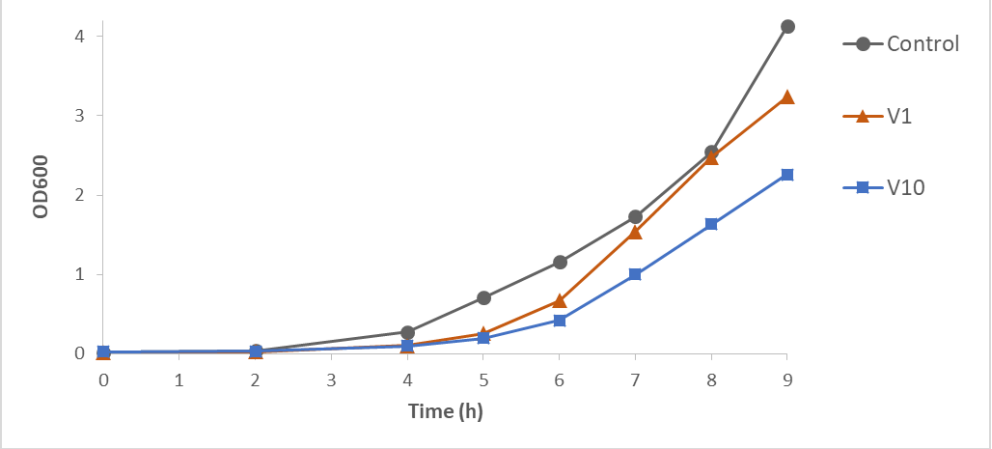

Figure 1. Time evolution of *Enterococcus faecalis* growth in the absence and in the presence of V1 or V10 compounds at 0.5 mM concentration.

In addition, the MICs of the POVs against *E. faecalis* were determined from 18h cultures in the presence of a series of concentrations up to 4096 μg·ml^{-1}. The MIC values obtained were 2048 μg·ml^{-1} for V10 and MnV11, 4096 μg·ml^{-1} for MnV13, and >4096 μg·ml^{-1} for V1. These results are in line with the efficacy of the same compounds against *E. coli* measured before [2], suggesting that the POVs have a broad antibacterial action.

There is also a good correlation between the effect of each compound in the *E. faecalis* growth curve and the corresponding MIC values (Figure 2). Notably, the inhibition data obtained until 7h culture was sufficient to identify the POVs with stronger antibacterial activity, V10, MnV11, and MnV13, in agreement with the results of MIC determination at 18h. The inhibition of bacterial growth was calculated from the increase in OD600 of cultures in the presence of the tested compound normalized to the increase observed in the control culture that started with the same bacterial inoculum but without the compounds. At 7h, V10, MnV11, and MnV13 inhibited bacterial growth by 51%, 84%, and 73%, respectively. At this incubation time, V1 only inhibited 11% of *E. faecalis* growth, an indication of its low antibacterial activity.

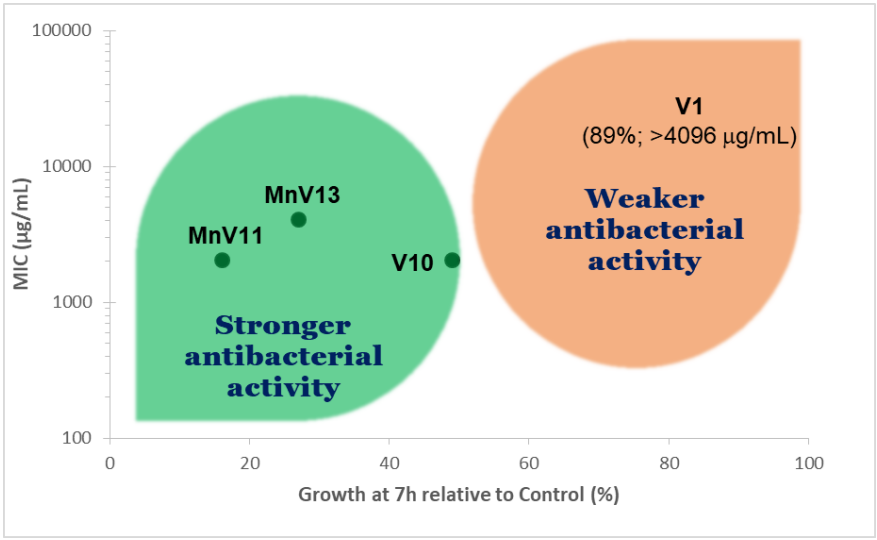

Figure 2. Relationship between the effect of the tested polyoxovanadates on *Enterococcus faecalis* growth measured at 7h incubation and the corresponding minimum inhibitory concentrations (MIC) determined by the serial two-fold dilution method at 18h.

4. Conclusion

The present study expands our understanding of the antibacterial action of vanadates and manganese polyoxovanadates. The POVs were tested against the Gram-positive bacteria *E. faecalis* by monitoring the growth of the microorganism at different incubation times and by the conventional MIC determination.

The MICs herein obtained for the POVs against *E. faecalis* and previously against *E. coli* [2] point to MnV11 as the compound with a higher potential for antibacterial applications, with activity towards both Gram-negative and –positive bacteria.

The growth curve method implemented in this work to assess the antibacterial activity of POVs returned coherent results that validate the method for fast screening of compounds. The method can provide preliminary indications of high- and low-activity compounds in a few hours.

In a future perspective, the study points out that automated monitoring of the growth curve can meet the need for rapid methods to screen antibacterial POVs against different bacteria.

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