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Study of generated genetic polymorphisms during the photocatalytic elimination of *Klebsiella pneumoniae* in water

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Abstract

Klebsiella pneumoniae is considered an emerging pathogen persistent in extreme environmental and stressed conditions. The aim of the present study is the investigation of inactivation rates of this pathogen in water by means of heterogeneous photocatalytic treatment under solar irradiation and the induced genetic variance applying RAPD-PCR as a molecular typing tool. Novel Mn and Co-doped TiO_2 catalysts were assessed in terms of their disinfection efficiency. The reference strain of *K. pneumoniae* proved to be readily inactivated, since disinfection occurred rapidly (i.e. after only 10 min of treatment) and low levels of bacterial regrowth were recorded in the dark and under natural sunlight. Binary doped titania exhibited the best photocatalytic activity verifying the synergistic effect induced by composite dopants. Applying RAPD analysis to viable cells after treatment we concluded that increasing the treatment time led to a considerable alteration of RAPD profiles and homology coefficient ranged almost between 35 and 60%. RAPD-PCR proved to be a useful typing molecular tool, that upon standardized conditions exhibits highly reproducible results. Genetic variation among isolates increased in relation to the period of treatment and prolonged irradiation in each case affected the overall alteration in band patterns. RAPD patterns were highly diverse between treated and untreated isolates when disinfection was performed with the Co-doped titania. The broad spectrum of genetic variance and generated polymorphisms has the potential to increase the already significant virulence of the species.

Keywords: Klebsiella pneumoniae; doped-TiO_2; solar photocatalysis; disinfection; RAPD

1. Introduction

Water may serve as the passive carrier of diverse bacterial groups and species, which have the potential to act as infectious agents of waterborne diseases. In recent years considerable attention has been drawn towards many opportunistic pathogens contained in water distribution systems, among which *Klebsiella pneumoniae* is prominently included.[1,2] This bacillus is gram-negative, lactose-fermenting and it belongs to the broad family of Enterobacteriaceae.[3] *K. pneumoniae* accounts for a significant proportion of hospital-acquired infections, like pneumonia, septicaemia, and soft tissue infections and is responsible for numerous nosocomial outbreaks worldwide.[4,5] The ability of this bacterial strain to spread rapidly in the hospital, as well as in the aquatic environment and its resistant nature against bactericidal factors enlist *K. pneumoniae* among the highly virulent and pathogenic microorganisms, especially for susceptible population groups and immunocompromised patients.[2,6]

The frequency of reports concerns mainly the multi-drug resistance of this specific strain, while equally significant is the resistance of *K. pneumoniae* isolates to environmental stressed conditions and deleterious agents, such as disinfectants.[6-10] The prominent polysaccharide capsule that they possess increases their virulence through protection from phagocytosis and prevents cellular destruction by bactericidal factors.[3,11]

Given the persistence of this bacterium in the environment and the fact that it may be transmitted through water consumption, it is imperative to explore effective and reliable disinfection techniques, which would eventually inactivate it, providing appropriate control measures of such pathogens. Although the beneficial effects of many treatment processes of water and wastewater have been well addressed, *K. pneumoniae* has been merely mentioned in research studies as far as its resistance against disinfection is concerned. Conventional water disinfection involves chlorination, which is widely used to remove effectively an

extended variety of microorganisms.[12-14] Yet, the diverse structural and physiological features of the latter lead to different response in the course of treatment, raising some concerns regarding the suitability of chlorination. Environmental isolates of *K. pneumoniae* display considerable resistance to chlorination due to several factors, namely extracellular polymer production, alteration of membrane lipids and increased cell aggregation.[6] Another approach towards efficient inactivation of persistent pathogens in water seems to be the application of titanium dioxide (TiO_2) photocatalysis, which has already demonstrated high reduction rates of viruses and bacteria in water/wastewater samples under ultraviolet (UV) irradiation.[15-17] Further applications are based on the exploitation of total solar light spectrum and the use of metal dopants, which have been explored for visible light-induced photocatalysis.[18] The exploitation of total solar light spectrum expands practical applications of TiO_2 photocatalysis, which has been successfully adopted as a disinfection technique.[19-22] In our previous work, *K. pneumoniae* was readily inactivated in aqueous samples under solar irradiation with the use of Mn- and Co-doped titania and disinfection occurred rapidly (i.e. after only 10 min of irradiation) with the dopant concentration affecting the overall process to a certain extent.[11]

The complexity of bacterial cells and their metabolic activity in the aquatic environment make their inactivation rather complicated under solar irradiation. Generally, sunlight damage involves the following principal mechanisms, which comprise direct DNA impairment and indirect endogenous damage caused mainly by UVB wavelengths and indirect exogenous damage involving UVB, UVA and visible wavelengths up to 550 nm.[23]

Complete bacterial inactivation, which is mostly desired, is highly dependent upon operating parameters of each water disinfection process that uses natural or simulated solar irradiation. However, even in the case of residual cells post treatment, the extent of DNA damage and the induced oxidative stress may result in loss of culturability or virulence of the

bacteria.[24] Efforts on delineating relationships among bacteria concerning alterations of their genetic material focus on molecular typing techniques, among which PCR-based methods predominate.[3,5,8,10,25,26] Among various DNA-fingerprinting techniques, Randomly Amplified Polymorphic DNA analysis (RAPD), offers a reliable, simple and cost effective tool to study the genotypic relatedness of a limited number of isolates, highlighting their generated genetic diversity through the course of a disinfection process.[25] RAPD typing has been a matter of interest mainly because of its economical and practical merits.[5] This technique is an arbitrarily primed-PCR, with which strain-specific arrays of amplified DNA fragments are generated.[25] Upon meticulous optimization, RAPD could be a sensitive and reproducible assay for the detection of DNA polymorphisms among bacterial isolates.[5,10,26,27]

In the present work the main objective was the investigation of genetic diversity applying RAPD analysis among isolates of *K. pneumoniae*, which were subject to solar photocatalysis with the use of manganese- (Mn-), cobalt- (Co-), and binary(Mn/Co-)-doped titania catalysts, prepared in previous work.[11] Also, inactivation rates and regrowth potential of the bacterium after treatment were evaluated.

2. Experimental

2.1. Metal-doped TiO_2 nanoparticles

Commercially available titanium dioxide (TiO_2 P25) was purchased from Degussa - Evonik Corp. (physicochemical characteristics are anatase:rutile 75:25, particle size of 21 nm and BET area of 50 m^2 g^{-1}) and was used as benchmark. Mn-, Co- and Mn/Co binary-doped TiO_2 catalysts were prepared by a co-precipitation method with molar ratio in different concentrations in the range of 0.02 to 0.3 wt%. Details regarding synthesis and characterization of all catalysts used in the present study can be found in previous studies.[11,28] The crystal structure, particle size, and morphology were examined with powder X-ray

diffraction (XRD), scanning electron microscopy (SEM) and transmission electron microscopy (TEM), respectfully. Powder X-ray diffraction patterns were collected on a Rigaku D/MAX-2000H rotating anode diffractometer (CuKα radiation) equipped with the secondary pyrolytic graphite monochromator operated at 40 kV and 80 mA over the 2θ collection range of 10-80°. The scan rate was 0.05° s^{-1}. The UV- Visible diffuse reflectance spectra of the final powders were measured on a Perkin Elmer LAMBDA 950 with $BaSO_4$, as reference standard. Surface morphology and elemental analysis of the samples were carried out using SEM and an energy dispersive spectrometer (EDS) on a JSM-6390LV instrument.

2.2. Disinfection experiments

The bacterial strain used in the present study was *K. pneumoniae* NCTC 5056 (Public Health England Culture Collections). The reference strain was inoculated in 10 mL of nutrient broth (HiMedia Laboratories) and grown overnight at 37 °C. The concentration of bacterial cells in the suspension was estimated measuring its optical density at 600 nm (Shimadzu UV1240 spectrophotometer) where, according to McFarland scale, an absorbance of 0.132 corresponds approximately to a cell density of 1.5×10^8 CFU mL^{-1}. Plate counts were also performed for accurate bacterial count. In each case, suspensions were properly diluted to achieve the desired initial bacterial concentration, which was used for the subsequent experiments.

Photocatalytic experiments were conducted in batch type, laboratory scale photoreactor. Solar irradiation experiments were carried out in a solar radiation simulator system (Newport, model 96000) equipped with a 150 W xenon ozone-free lamp and an Air Mass 1.5 Global Filter (Newport, model 81094), simulating solar radiation reaching the surface of the earth at a zenith angle of 48.2°. The system is described in detail in our previous work.[11] Reactions took place in an open, double-walled, cylindrical glass vessel under continuous stirring.

In a typical run, the bacteria suspension was spiked in sterile water of 200 mL which were then loaded in the reaction vessel with the appropriate amount of catalyst. The solution was left in the dark under stirring for 20 min to ensure complete equilibration of adsorption/desorption of *K. pneumoniae* bacteria onto the catalyst surface and then exposed to solar irradiation; this moment was taken as the starting point (time zero) of the disinfection experiment. Temperature was maintained at 25±2 ^{o}C with a temperature control unit. Catalyst loading was 250 mg L^{-1} in all experiments, since this concentration proved to be optimum for total elimination of *K. pneumoniae* under the current operating conditions. Disinfection rate was measured in terms of *K. pneumoniae* inactivation, according to viable counts on M-FC agar M1124 (HiMedia Laboratories) after incubation at 37 ^{o}C for 20-24 h.

Furthermore, disinfection durability experiments were carried out in the dark and at natural sunlight irradiation under continuous stirring for 48 h, in order to evaluate the regrowth potential of our strain.

2.3. DNA isolation and RAPD-PCR analysis

DNA was extracted from viable cells of *K. pneumoniae* after treatment using QIAamp DNA mini kit (Qiagen) supplemented with lysozyme lysis buffer (100 mM NaCl, 500 mM Tris [pH 8], lysozyme 10 mg mL^{-1}). The quantity and purity of all DNA samples were determined measuring their absorbance value at 260 nm and estimating the ratio of absorbance values at 260 nm and 280 nm, respectively.

For RAPD analyses different 10-nt primers of arbitrary sequence were tested and two of them (RAPD4: 5′-AAGACGCCGT-3′ and RAPD7: 5′-GTGGATGCGA-3′) were chosen for further study as they gave the most discriminating RAPD fingerprints.[5,25] PCR was performed with the use of peqlab thermal cycler (advanced primus 25) in 50μl reaction volumes containing approximately 30 ng of bacterial DNA, $MgCl_2$ 2.5 mM, 10 X PCR buffer, 20 pmol

of the primer, 1 U of AmpliTaq DNA polymerase and 250 μM of each dNTP. All data used in the present analysis were generated in a cycling program of 45 cycles of 94 ^{o}C, 1 min; 36 ^{o}C, 1 min and 72 ^{o}C, 1 min followed by a final extension at 72 ^{o}C for 9 min. PCR products were electrophoretically separated by 2% (w/v) agarose gels, which contained 1 mg mL^{-1} ethidium bromide staining. A molecular size marker (1 Kb) was used for reference in all gels, which were further visualized and photographed on a UV transilluminator. Electrophoresed agarose gels were analyzed visually and scored using a binary code. The UVIbandmap software (UVITEC Cambridge) was used to analyse the binary code, perform cluster analysis and generate dendrogram based on Jaccard coefficient and unweighted-pair group method with arithmetic mean clustering method (UPGMA).[27] The similarity index of RAPD profiles was defined as the fraction of shared bands: $S_{xy}=2n_{xy}/(n_x+n_y)$. Specifically, for individuals x and y, it is the number of common bands in their RAPD profiles (n_{xy}) divided by the average number of bands scored for both individuals. Similarity S_{xy} values range between 0 and 1. The highest value represents completely similar RAPD profiles.[29]

The reproducibility of the technique was assessed in the following ways: a) Independent RAPD assays were applied in three runs using the same DNA template and b) RAPD assays were applied to *K. pneumoniae* isolates obtained from different colonies of the reference strain, grown on the same petri dish. Band repeatability was defined as $R_b = 2b_{12} / (b_1+b_2)$, where b_{12} is the number of individuals possessing band b in both replicates and b_1 and b_2 are the numbers of individuals possessing that band in the first and the second replicate, respectively. R_b can take values between 0 and 1.[29]

3. Results & Discussion

3.1 Inactivation of *K. pneumonaie* during photocatalysis

Bacterial inactivation in water samples was recorded under solar irradiation in the presence of the prepared doped catalysts. Metal-doped catalysts showed satisfactory inactivation, as may be observed in Figure 1. Dopants improved the photocatalytic activity of pure titania, which achieved a 2-Log reduction after 30 min of treatment. Moreover, increasing the dopant concentration incorporated in titania nanoparticles, disinfection efficiency was improved as reduction of *K. pneumoniae* density was 3 orders of magnitude within almost 10 min of treatment. It is clearly shown that the 0.3 wt% Mn- and Co-doped TiO_2 catalysts showed optimum photocatalytic performance when compared to the others and disinfection took place after only 10 min, despite the fact that this specific emerging pathogen is considered persistent during various treatments and disinfection techniques.[6] These catalysts may demonstrate equal performance even in the case of higher initial concentration of *K. pneumoniae* (i.e. 10^5 CFU mL^{-1}), as it has already been shown in our previous study.[11] Moreover, it can be noticed that in certain experimental runs and when Mn-doped catalysts were employed, bacterial inactivation reached a plateau and became slow, leading in residual cells at the end of the process. Similar findings were obtained in other study referred to *K. pneumoniae* inactivation with the use of reactive plasma processed nanocrystalline TiO_2 powder.[7] Vijay et al. noticed deceleration of the decay of this bacterial strain in the course of treatment, attributing this outcome to the released metabolites from the killed bacteria, which form a screen and protect the remaining active cells. The same conclusion was drawn in the work of Yousef et al., who investigated the effect of CuO/TiO_2 nanofibers on *K. pneumoniae* elimination under visible light.[2]

Further experiments were performed with the use of Mn/Co binary-doped TiO_2, which showed enhanced disinfection efficiency, under the current experimental conditions. Complete elimination of the pathogen was recorded in really short periods of time (Figure 1). This finding verifies the general aspect that composite dopants induce a synergistic effect

such as an efficient charge separation and improvement of photostability, surpassing the disadvantages of the individual components.[30] The superiority of binary-doped catalysts has already been addressed in other cases with *E. coli* being the testing microorganism and an average 4-Log reduction of its density after 90 min of exposure to visible light.[31,32] The ratio of doping levels (0.04 and 0.01 %wt of Mn/Co) proved to be optimum for rapid killing of *K. pneumoniae* in water samples, although it should not be ignored that dopants have the potential to cause photo-induced corrosion and promote charge recombination at some metal sites.[33]

Bacterial regrowth after treatment is considered an issue of great importance and many concerns are raised for public health protection since microorganisms are capable of retaining their virulent characteristics, which may be exhibited post disinfection process. Regrowth potential of our strain was evaluated applying disinfection durability experiments. The vast majority of treatment runs did not exhibit any regrowth of the bacterium. Only in three cases was the strain reactivated during exposure under natural sunlight (Table 1). When *K. pneumoniae* was processed with 0.02% Mn:TiO_2, 0.1% Mn:TiO_2 and 0.1% Co:TiO_2, there was a photo-reactivation of the bacterium which did not exceed 0.2% of its initial population. Recovery rates in the present study are very low, indicating that damage of cell membranes during photocatalysis was extensive. According to the general observation cell death may be achieved through membrane destruction, which in turn results in leakage of cellular components. Conversely, a respective damage of the cell wall does not necessarily lead to cell death, as bacteria possess a mechanism for its repair.[34] Moreover, the change in the concentration of the cell wall components during illumination shows that, while the outer membrane serves as a barrier, the peptidoglycan layer does not have a barrier function.[2] Our results indicate that the detrimental effect of the oxidative species developed at the catalyst

surface was expanded towards the cytoplasmic membrane, increasing cell permeability which finally caused cell death.[11]

3.2 RAPD-PCR analysis

RAPD-PCR fingerprinting was employed as a typing method to highlight and discriminate possible alterations in the genomic material of *K. pneumoniae* isolates, which remained viable after treatment with the metal-doped catalysts. As a PCR-based technique, it was selected for its credit classification results relying on genomic divergence of the tested bacterial isolates. Generally, in RAPD analysis arbitrary oligonucleotides are used to promote DNA amplification at low annealing temperatures determining genomic diversity. This generates strain-specific arrays of amplified DNA fragments. The formation of these arrays does not depend on prior knowledge of the nucleotide sequence, nor is it affected by DNA modifications that complicate typing by restriction endonuclease digestion of genomic DNA. This technique has been preferred in many studies because of its speed, simplicity and highly discriminatory results.[3-5,8,10,25-27]

3.2.1 Reproducibility of RAPD analysis

The reproducibility of the method was evaluated with preliminary trials using the same DNA template and genetic material from different *K. pneumoniae* colonies. Despite the advantages of RAPD there are some considerable concerns and criticism regarding the lack of repeatability, due to low stringency conditions employed and variable factors that may affect RAPD profiles.[5] The reproducibility of the technique was assessed as mentioned before and the average R_b (band repeatability) was estimated in profiles generated with the two selected primers RAPD7 and RAPD4. When the same DNA template was used R_b ranged between 0.96 and 0.99 with both primers used. The corresponding values when DNA was isolated

from different colonies of the reference strain were in the range of 0.93-0.97. Generally, the results were highly reproducible with both primers and under the specific experimental conditions.

It is of great importance that reaction conditions, including DNA template concentration, annealing temperature and the choice of the primers should be standardized to avoid variations in RAPD patterns.[27] Upon optimization of conditions, RAPD offers the possibility of studying, at least within a single run, the genotypic relatedness of a limited number of isolates.[25] Ashayeri-panah et al., who worked with *Klebsiella* isolates and RAPD analysis, concluded that the reproducibility of the technique may be achieved even when different thermal cyclers are used under strictly defined conditions.[5] In this sense, this method may provide a stable and highly reproducible discrimination of bacterial isolates, as it has already been documented in the literature.[3,4,8]

3.2.2 RAPD profiles of viable cells after treatment

Several primers were tested for RAPD fingerprinting, among which RAPD7 and RAPD4 were the most discriminatory in relation to the number and intensity of generated bands, size range and smear formation. Both primers were selected for detailed testing of sequence divergence among isolates. Viable isolates after photocatalysis with the metal-doped titania were subject to RAPD-PCR and all of them proved to be typeable with both primers, developing a variety of amplification bands. RAPD profiles and the corresponding cluster analysis of treated *K. pneumoniae* with Mn-, Co- and binary-doped catalysts are shown in Figures 2, 3 and 4, respectively. The protocol performed in this study allowed the amplification of 6-23 bands ranging in size from 200 to 2700 bp with primer RAPD7 and 7-29 bands ranging from 200 to 4000 bp with primer RAPD4.

Reviewing the patterns obtained from isolates treated with Mn-doped TiO_2 in comparison to that of intact cells, different combinations of amplification bands were obvious with both primers resulting in their discrete grouping applying cluster analysis (Figure 2). Isolates were divided into different groups, according to their homology with the intact *K. pneumoniae*. In the course of treatment and increasing the period of irradiation a considerable alteration of RAPD profiles occurred in viable bacterial cells. Homology of RAPD patterns ranged between 27-88% and 38-90% with primers RAPD7 and RAPD4, respectively. Significant discrimination among isolates was recorded after long treatment periods. For example when bacteria were treated with 0.02% Mn:TiO_2, the homology of RAPD patterns between untreated and viable cells after 30 min of irradiation was only 27%. The concentration of the dopant did not affect significantly the profiles. Conversely, the period of irradiation in each case affected the overall alteration in band patterns. Interestingly, although the band patterns were different for each primer similar dendrograms and clusters were obtained but with different similarity index S_{XY} (Table 2). Primer RAPD4 yielded patterns with lower values of similarity index, compared with those recorded with primer RAPD7. The average S_{XY} of profiles were 0.46 and 0.60 with RAPD4 and RAPD7, respectively, when Mn:TiO_2 was applied. This finding indicates that the former primer exhibits higher discriminatory power, in terms of highlighting the induced changes in genetic material of *K. pneumoniae* isolates under solar irradiation with the use of the prepared metal-doped catalysts.

Screening results obtained when Co:TiO_2 was used it is evident that they follow the same trend (Figure 3). Band patterns obtained with primer RAPD4 showed significant heterogeneity among *K. pneumoniae* isolates, with the untreated one being grouped separately from all others and the homology coefficient reaching a value of almost 35%. A period of 15 min of irradiation with the 0.3% Co:TiO_2 was sufficient to cause such alteration in the genetic material of viable bacteria. The similarity index among profiles obtained with primer RAPD4

in the presence of Co-doped titania had even lower average value than that recorded with Mn-doped titania (i.e. 0.23), as may be seen in table 2. The differences in the discriminatory power of the tested primers became apparent after analysis of all viable isolates after disinfection with the three sets of metal-doped catalysts (i.e. Mn-, Co- and Mn/Co bimary-doped). The use of two different primers to generate RAPD profiles gave an extended flexibility and sensitivity to the typing method. The use of more than one primer seems to be advantageous, since it adds to the consistency of the established groups and in many studies applying RAPD it is preferred to employ a set of oligonucleotides for accurate and reliable bacterial clustering.[5,25]

In the case of binary-doped catalysts, lower homology coefficient among isolates was achieved with primer RAPD4, reaching a value of almost 42%, whereas better discrimination and grouping of patterns was generated with primer RAPD7 (Figure 4) but with higher average value of similarity index (i.e. 0.69; Table 2). The demonstrated dendrogram showed all treated isolates into one major group from a common lineage with further separation into smaller sets, whose similarity level with the untreated *K. pneumoniae* was approximately 60%. It should be noted though that when treatment process was performed with binary-doped titania, the recorded genetic variation was referred to isolates that were exposed to solar irradiation for only 10 min, as complete removal of the bacteria occurred within this period of time.

RAPD fingerprinting method could be used to rapidly identify major clones of this species, including multi-drug resistant and particularly virulent clones. According to de Souza et al., pathogenic *K. pneumoniae* populations are highly heterogeneous.[27] The numerous serotypes in this species could explain the relevant degree of genetic diversity highlighted by RAPD analysis, as it was exhibited in our study. Polymorphisms detected throughout the entire genome may include point mutation and genetic rearrangement, which have the potential to

increase the already significant virulence of the species. The occurrence of broad spectrum of *K. pneumoniae* variants in stressed environments portrays the ability of this species to survive abundantly under extreme conditions.[10]

4. Conclusions

The present study focused on the inactivation of *K. pneumoniae* in aqueous matrix under solar irradiation with the use of metal-doped titania and the induced genetic variation among viable cells after treatment. In this perspective RAPD-PCR was performed as a molecular typing tool, that has the potential to highlight genetic diversity among bacterial isolates from various sources. The main conclusions derived from this work are as follows:

- Inactivation rates of *K. pneumoniae* in water samples under simulated solar irradiation were highly improved in the presence of metal-doped catalysts. Of all the catalysts used, Mn/Co binary-doped titania exhibited the best photocatalytic activity and complete bacterial removal was recorded in less than 10 min of treatment. This finding verifies the synergistic effect induced by composite dopants.
- All catalysts were effective for considerable inactivation of *K. pneumoniae* with very low levels of bacterial regrowth in the dark and under natural sunlight.
- RAPD-PCR proved to be a useful typing molecular tool, that upon standardized conditions exhibits highly reproducible results.
- Genetic variation among isolates increased in relation to the period of treatment. The period of irradiation in each case affected the overall alteration in band patterns.
- RAPD patterns were highly diverse between treated and untreated isolates when disinfection was performed with the Co-doped titania.
- Generated polymorphisms may add to the virulence of the species.

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424 **Table 1**

425 *K. pneumoniae* survival after the end of disinfection durability experiments either in the dark
426 or under natural sunlight irradation.

Operating conditions			*K. pneumoniae* regrowth (CFU mL^{-1})			
Run	**Catalyst**	**Initial bacterial concentration**	**Dark conditions** (24 h of exposure)	**Natural sunlight** (24 h of exposure)	**Dark conditions** (48 h of exposure)	**Natural sunlight** (48 h of exposure)
1	0.02% Mn:TiO_2	10^3 CFU mL^{-1}	0	0	0	1
2	0.1% Mn:TiO_2	10^3 CFU mL^{-1}	0	1	0	2
3	0.1% Co:TiO_2	10^3 CFU mL^{-1}	0	0	0	1

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Table 2

Average similarity index (S_{XY}) of generated RAPD profiles of viable isolates after treatment with the metal-doped catalysts.

Catalysts used during photocatalysis	**Average similarity index (S_{XY}) of RAPD profiles**	
	Primer RAPD 7	***Primer RAPD 4***
Mn-doped TiO_2	0.60 ± 0.14	0.46 ± 0.18
Co-doped TiO_2	0.68 ± 0.08	0.23 ± 0.07
Mn/Co binary-doped TiO_2	0.69 ± 0.05	0.39 ± 0.17

Figure captions

Figure 1

K. pneumoniae inactivation in the presence of different a) Mn- doped TiO_2 and the commercially available TiO_2 (P25, Evonik) and b) Co- and Mn/Co binary-doped TiO_2. Catalyst concentration is 250 mg L^{-1}.

Figure 2

RAPD patterns generated with a) primer RAPD7 and b) primer RAPD4 and corresponding cluster analysis of *K. pneumoniae* isolates treated with Mn-doped TiO_2. Prefix NT indicates the not treated isolate. Lane numbers correspond to *K. pneumoniae* treated isolates as follows, 1, 2: 0.02% Mn:TiO_2 after 6 and 30 min, respectively; 3, 4: 0.04% Mn:TiO_2 after 6 and 30 min, respectively; 5, 6: 0.1% Mn:TiO_2 after 3 and 10 min, respectively; 7, 8: 0.3% Mn:TiO_2 after 6 and 15 min, respectively. Dendrogram generated with Jaccard's coefficient and the UPGMA clustering method.

Figure 3

RAPD patterns generated with a) primer RAPD7 and b) primer RAPD4 and corresponding cluster analysis of *K. pneumoniae* isolates treated with Co-doped TiO_2. Prefix NT indicates the not treated isolate. Lane numbers correspond to *K. pneumoniae* treated isolates as follows, 1, 2: 0.02% Co:TiO_2 after 6 and 15 min, respectively; 3, 4: 0.04% Co:TiO_2 after 6 and 10 min, respectively; 5, 6: 0.1% Co:TiO_2 after 6 and 10 min, respectively; 7, 8: 0.3% Co:TiO_2 after 3 and 15 min, respectively. Dendrogram generated with Jaccard's coefficient and the UPGMA clustering method.

Figure 4

RAPD patterns generated with a) primer RAPD7 and b) primer RAPD4 and corresponding cluster analysis of *K. pneumoniae* isolates treated with Mn/Co binary-doped TiO_2. Prefix NT indicates the not treated isolate. Lane numbers correspond to *K. pneumoniae* treated isolates

458 as follows, 1, 2: 0.04% Mn/Co:TiO_2 after 6 and 10 min, respectively; 3, 4: 0.1% Mn/Co:TiO_2
459 after 6 and 10 min, respectively. Dendrogram generated with Jaccard's coefficient and the
460 UPGMA clustering method.
461

462 **Figure 1**

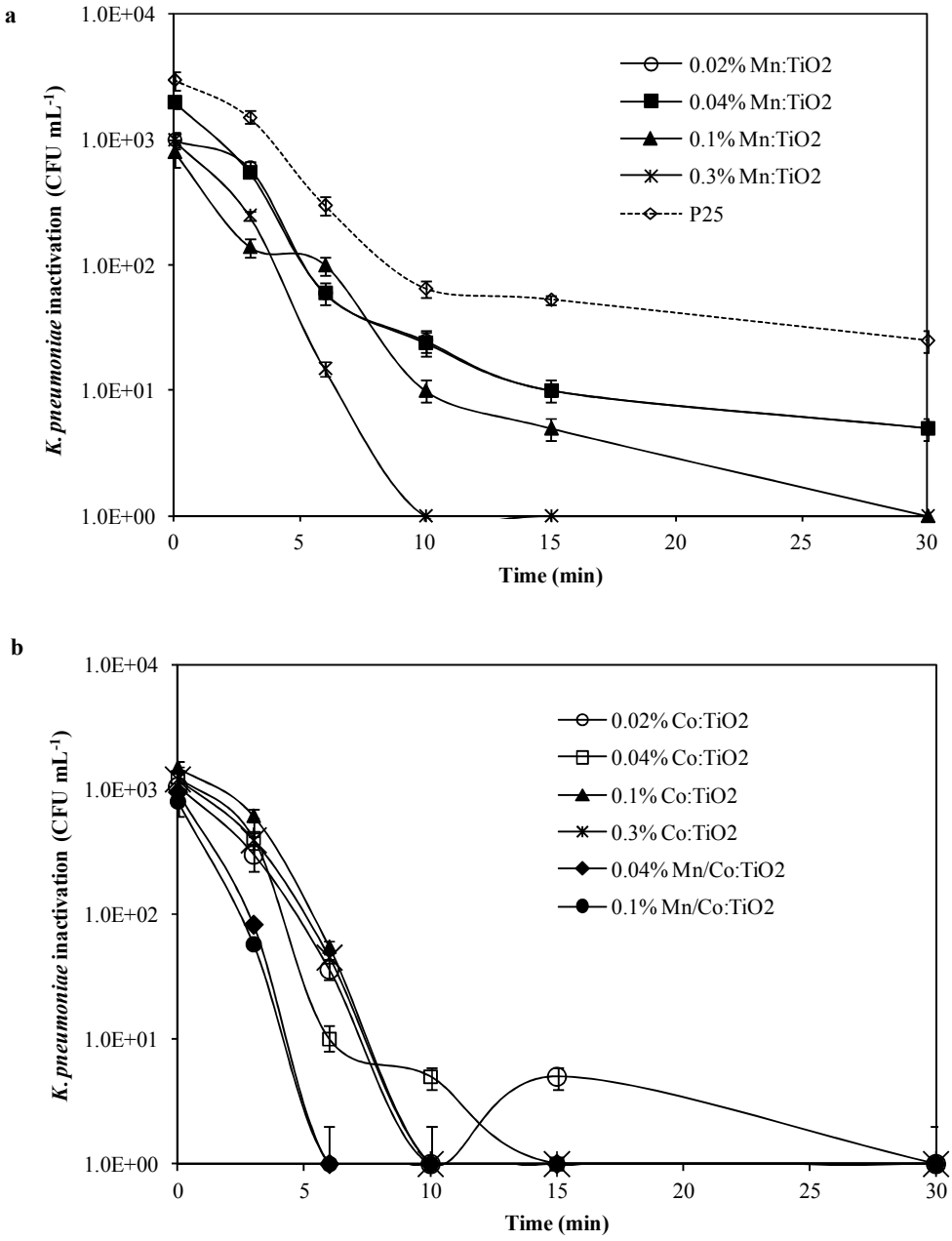

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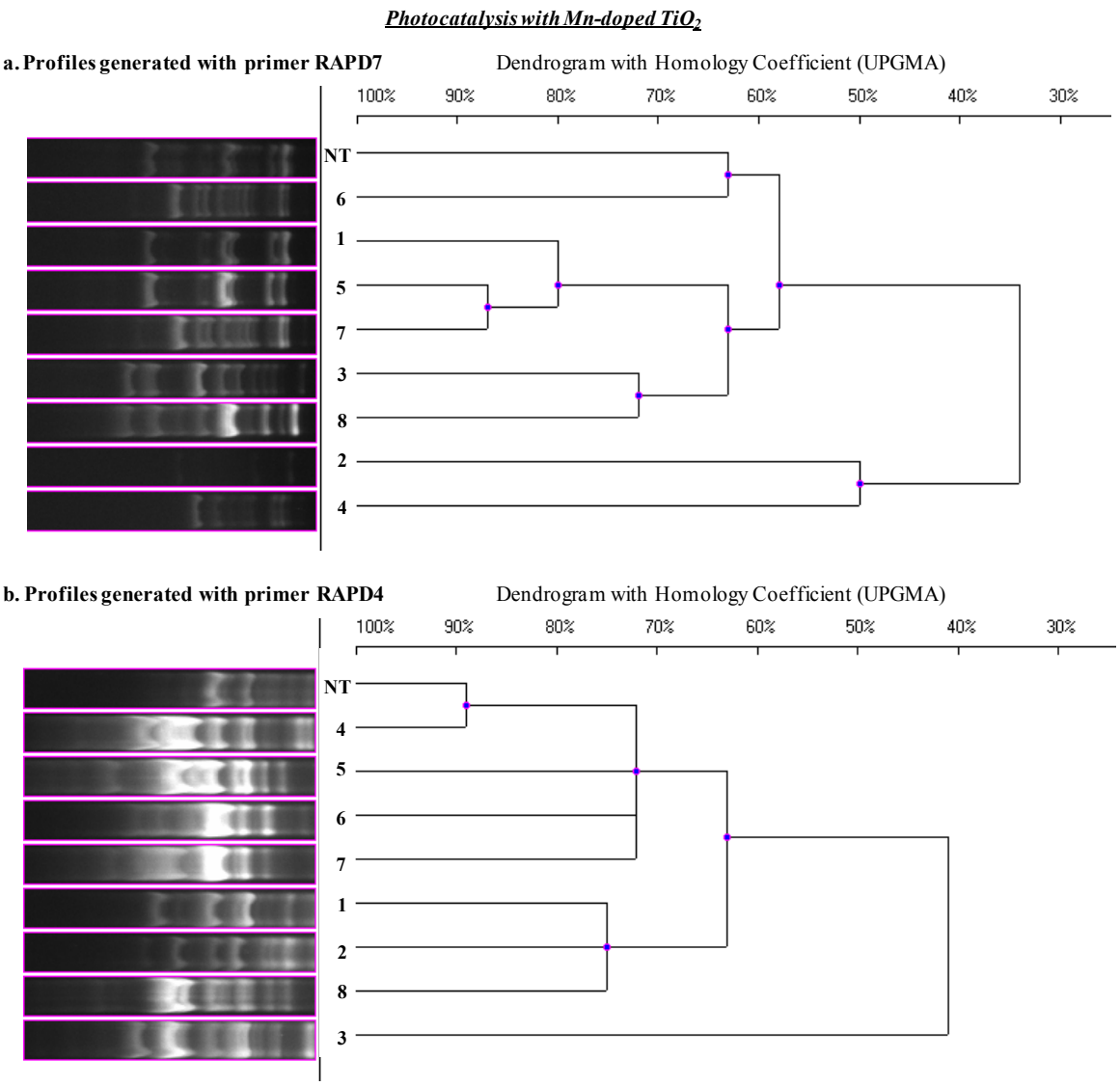
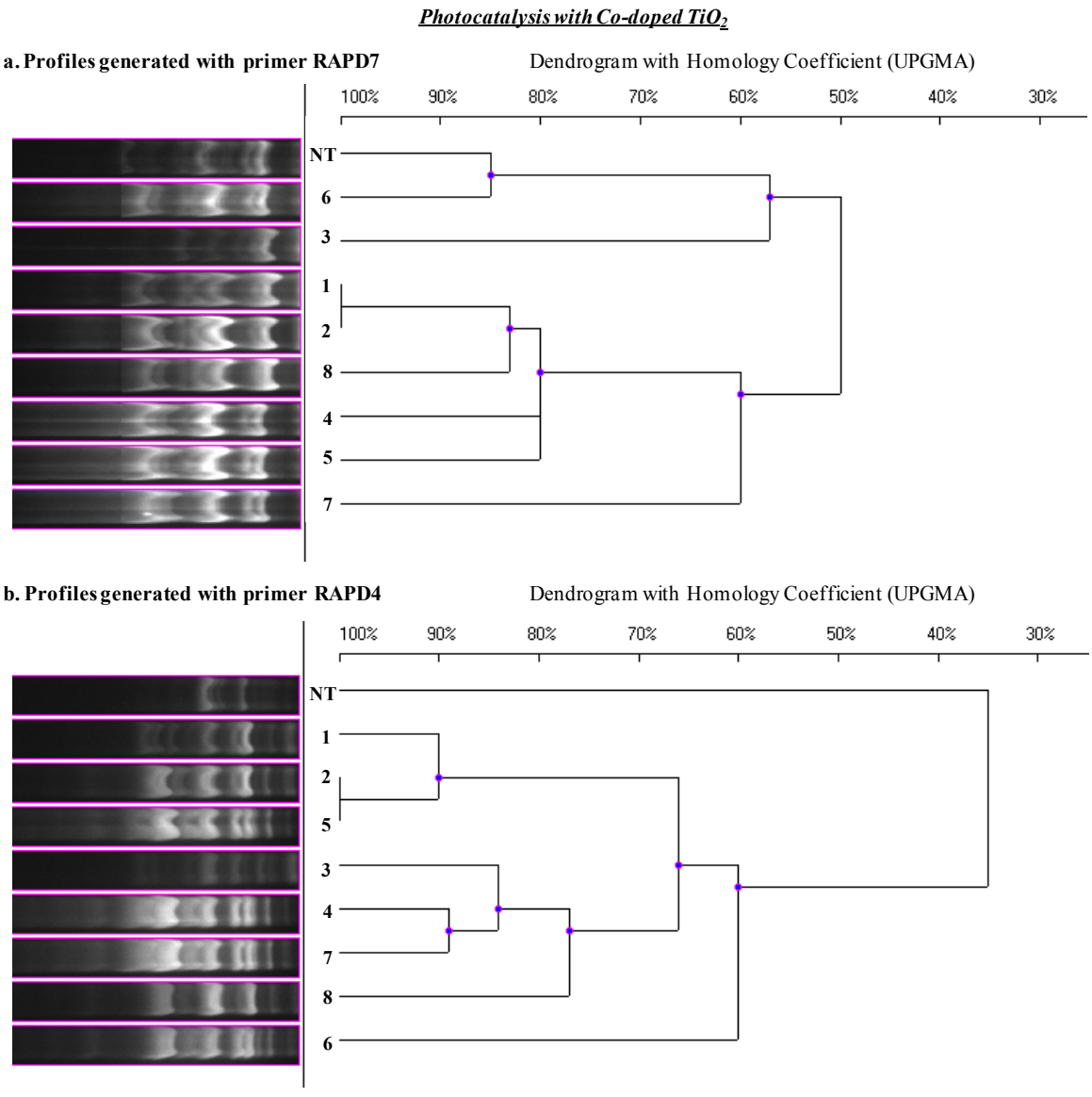
Figure 2



468 **Figure 3**



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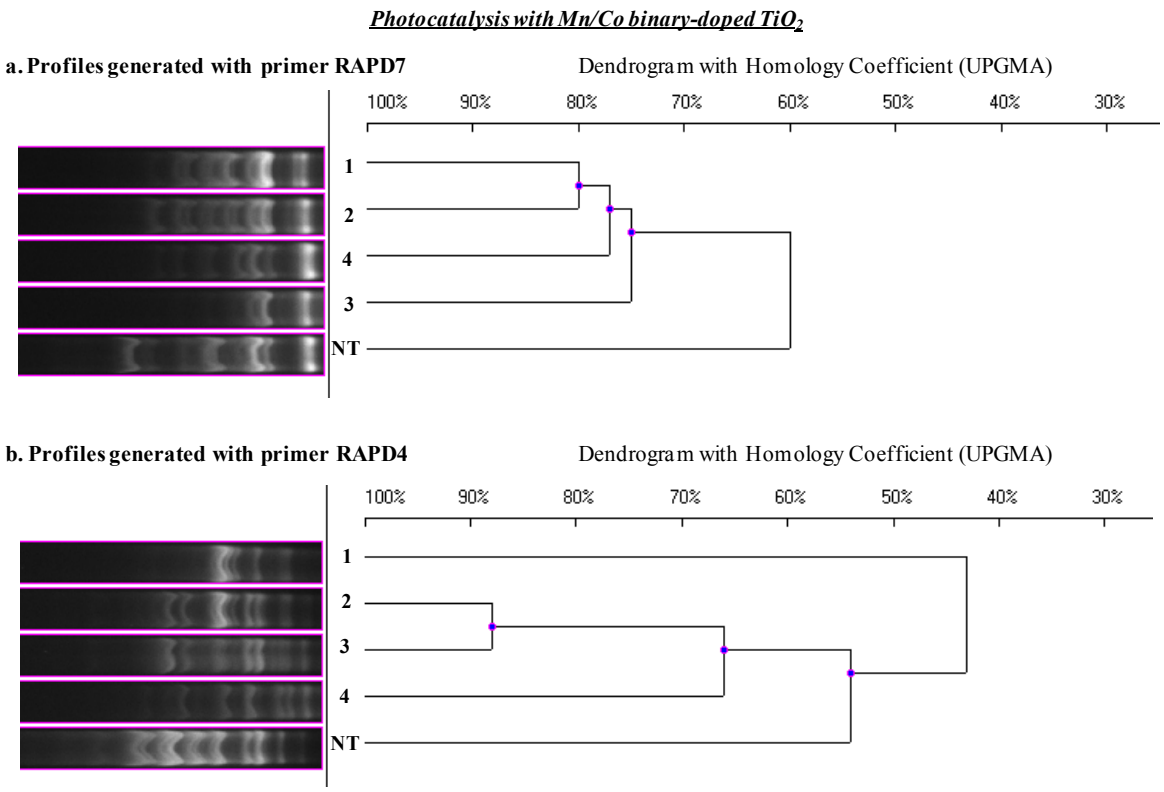
471 **Figure 4**



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