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The influence of pH and external K^+ concentration on caesium toxicity and accumulation in *Escherichia coli* and *Bacillus subtilis*

Joy Perkins and Geoffrey M. Gadd

Department of Biological Sciences, University of Dundee, Dundee DD1 4HN, Scotland, UK

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SUMMARY

Toxicity screening of *Escherichia coli* NCIB 9484 and *Bacillus subtilis* 007, NCIB 168 and NCIB 1650 has shown Cs^+ to be the most toxic Group 1 metal cation. However, toxicity and accumulation of Cs^+ by the bacteria was affected by two main external factors; pH and the presence of other monovalent cations, particularly K^+. Over the pH range 6–9 both *E. coli* and *B. subtilis* showed increasing sensitivity towards caesium as the pH was raised. The presence of K^+ and Na^+ in the laboratory media used lowered caesium toxicity and lowered accumulation of the metal. In order to assess accurately Cs^+ toxicity towards the bacterial strains it was therefore necessary to define the K^+:Cs^+ ratio in the external medium. The minimum inhibitory K^+:Cs^+ concentration ratio for the *Bacillus* strains tested was in the range 1:2–1:3 while *E. coli* had a minimum inhibitory K^+:Cs^+ concentration ratio of 1:6.

INTRODUCTION

Renewed interest in caesium has arisen as a result of the increasing exposure of living systems to radiocaesium, including that from the Chernobyl accident. Several studies have concentrated on uptake by different organisms and passage through the food chain of different radioactive forms of Cs [12,15]. The ability of caesium to transfer along food chains is enhanced because its physical and chemical properties resemble those of the essential element potassium [16]. Microbes have a high requirement for K^+ and in bacteria, cytoplasmic K^+ concentrations can vary between 0.2 and 1.0 M [4]. Functions of potassium in prokaryotes include osmoregulation by maintenance of cell wall turgor [39] and involvement in cellular pH homeostasis [9]. It can also act as an activator of enzymes by maintaining the specific protein conformation necessary for optimum catalytic efficiency [11]. Recent research has also implicated the stabilizing nature of potassium with the phosphate groups of nucleic acids in *E. coli* [29].

The role of monovalent cations, particularly K^+, has been studied extensively in bacteria, primarily *E. coli* [5,36], with a main area of investigation being the energetics/kinetics of K^+ transport. In *E. coli*, K^+ influx depends on two distinct transport systems: TrK and Kdp. The Kdp system is a high affinity K^+-scavenging system ($K_m = 2\ \mu M\ K^+$) which is driven by ATP and is repressed by high K^+ concentrations [17]. TrKA is however the main constitutive K^+ uptake system in *E. coli* (K_m = 1.5 mM). TrKD (Kup) is an accessory system to TrKA (K_m = 0.5 mM) and has a lower affinity and transport rate for K^+ [34]. A third system is also thought to exist, TrKF, which does not follow Michaelis–Menten kinetics and may therefore be passive [6]. Despite a significant amount of work on other aspects of physiology [23], little research on K^+ transport systems in *Bacillus* spp. has been performed. However, it has been postulated that *B. subtilis* has a turgor-sensitive K^+-uptake system similar in function to the system characterized in *E. coli* [41].

Accumulation of other monovalent cations via the described K^+ transport systems has also been observed. ^{204}Tl and ^{86}Rb have been used to probe potassium transport by the TrKA system in *E. coli* [3,14]. In contrast, the TrKD system shows relatively little discrimination in the transport of K^+, Rb^+ and Cs^+ and is the main route of Cs^+ transport in *E. coli* [10]. Bacterial monovalent cation transport in all the described cases depends on two main factors: the electrical component ($\Delta\psi$) of the transmembrane proton gradient ($\Delta\tilde{\mu}_{H+}$) and the requirement for an energy source such as glucose [18].

Most information on Cs^+ and bacteria to date has arisen indirectly from studies on K^+ transport, with little or no investigations of Cs^+ toxicity or how the presence of other monovalent metal cations, particularly K^+, may affect toxicity. This is surprising in view of the previously mentioned competition between K^+ and Cs^+ for K^+ transport and possible correlations between accumulation and toxicity. There is also a lack of information on external pH which is a prime determinant of metal–microbe responses, including metal association with cell walls and transport. The aims of this study were therefore to characterize and compare the toxicity of Cs^+ to other Group 1 monovalent metal cations (K^+, Na^+, Li^+ and Rb^+), which have similar ionic radii and may be accumulated by K^+ transport systems in bacteria, and to examine the influence of external pH and monovalent cation concentration, particularly K^+, on Cs^+ toxicity and accumulation. For this, we have used strains of two different neutrophilic bacteria (Gram-negative

Correspondence to: G.M. Gadd, Department of Biological Sciences, University of Dundee, Dundee DD1 4HN, Scotland, UK.

Escherichia coli NCIB 9484 and Gram-positive *Bacillus subtilis*), transport assays with ^{137}Cs, and well diffusion assays in solid medium. The latter provides a simple toxicity assessment system which, as well as clarifying the interactions described above, can establish differences between organisms and strains with potential applications for isolation and screening [19,20].

MATERIALS AND METHODS

Organisms, media and cultural conditions

The organisms used were *Escherichia coli* NCIB 9484, *Bacillus subtilis* 007, NCIB 1650 and NCIB 168. All organisms were maintained on nutrient agar (Lab M). For growth in liquid medium, Oxoid no. 2 nutrient broth was used. Starter cultures of the bacteria were prepared by loop-inoculating a single colony into 5 ml of nutrient broth, which was incubated for approximately 17 h at 37 °C on an orbital shaker (200 cycles min^{-1}). Optical densities at 550 nm were measured using a Pye Unicam (Unicam Ltd, Cambridge, UK) SP600 series 2 spectrophotometer.

Diffusion assays

Well diffusion assays, modified from Gadd [19], were carried out using nutrient agar (10 cm^3 per 9-cm diameter Petri dish) which incorporated the test organism at 0.1 ml per 10 ml agar to an $OD_{550\,nm}$ of 0.2. Plates were set on a levelling table and three wells made per Petri dish using a sterile 10-mm diameter sterile cork borer. Fifty-microlitre aliquots of the desired caesium solutions were added to wells. Plates were incubated overnight (~17 h) at 37 °C. The maximum and minimum zone width [24] were measured using back illumination.

Caesium gradient analysis

Fifty microlitres of metal solution were added to 10-mm wells cut in plates (10 cm^3 agar per plate) and left overnight (~17 h) at 37 °C. A sterile cork borer was used to remove four agar discs (4-mm diameter) from specific points on the plates. These discs were digested in 0.5 ml 6 M nitric acid at 90 °C for 30 min after which time 4.5 ml of distilled deionized water was added. Metal concentrations in these solutions were determined using a Pye Unicam SP9 atomic absorption spectrophotometer with reference to appropriate standard solutions.

Short term Cs^+ uptake experiments

Overnight cultures were harvested by centrifugation at 4 °C (10 000 × *g*, 10 min), using a Sorvall (Dupont (UK) Ltd, Stevenage, UK) RC-5B refrigerated Superspeed centrifuge and washed twice in 5 mM PIPES buffer, pH 6.5. Cell density was adjusted to an $OD_{550\,nm}$ ~5.0 in 10 ml of the same buffer and cells were starved at 37 °C with shaking for 1 h. The suspension was then equilibrated for 30 min at 37 °C with 50 mM glucose before adding CsCl to the required concentration with ^{137}Cs (Amersham International, Amersham, UK) added as a tracer to a final activity of between 0.67–0.80 MBq ml^{-1}. Where required, either KCl or NaCl at the desired concentration was added to the cell suspension together with the CsCl and ^{137}Cs. At intervals, 200-μl samples were removed and harvested by centrifugation (8000 × *g*, 30 s) through a layer (200 μl) comprising 60% (by volume) Dow Corning (Merck Ltd, Lutterworth, UK) 550 silicone oil and 40% bis 3,3,5-trimethylhexylphthalate (Fluka Chemicals Ltd, Gillingham, UK). Reversed (v/v%) ratios of oils were used in the presence of NaCl to aid separation using 500-μl Beckman (Beckman Instruments (UK) Ltd, High Wycombe, UK) PRO22 centrifuge tubes and an Eppendorf 5412 microcentrifuge (Merck Ltd, Lutterworth, UK). After centrifugation, the bottom of each tube was cut off and placed in 2 ml Ecoscint A scintillation fluid (National Diagnostics, Maville, NJ, USA) for 24 h before measuring radioactivity using a Wallac (EG and G Ltd, Crownhill, Milton Keynes, UK) 1409 liquid scintillation counter.

Other analytical methods

When required, the pH of nutrient agar was adjusted with either 1 m KOH or 1 M HCl. After autoclaving the medium, the pH was determined using a Kent (Kent Industrial Measurements, Stonehouse, UK) 1070-2 surface electrode attached to a Kent EIL 7055 pH meter. In some control experiments, 50 μl of 0.1% (w/v) pararosaniline was added to a 10-mm well, 3 per 9-cm Petri dish (10 cm^3 agar), and left overnight at 37 °C; zones formed by the dye were measured as above. Dry weights of cells were determined after harvesting them by centrifugation (1200 × *g*, 10 min) and washing twice with distilled deionized water. Washed pellets were resuspended in 1 ml water and dried at 160 °C for 24 h using tared aluminium foil cups dried to a constant weight.

RESULTS

Toxicity of caesium and other monovalent cations towards Bacillus subtilis *and* Escherichia coli

Toxicity experiments, where aliquot volumes of metal solutions were added to wells in plates of nutrient agar (containing 0.13 M NaCl and 8.3 mM KCl) revealed marked differences between the four organisms studied. Inhibition of growth of *Escherichia coli* NCIB 9484 and *Bacillus subtilis* strains 007, NCIB 168 and NCIB 1650 occurred in the presence of the monovalent cations Cs^+ and Li^+. Other Group 1 monovalent cations tested (K^+, Na^+ and Rb^+) had no discernible toxic effect on the above organisms. Caesium was the most toxic metal of Group 1 (Fig. 1A,B) especially towards *B. subtilis* 007. Use of 2 M CsCl in well diffusion assays showed that *B. subtilis* 007 was the most Cs^+-sensitive organism; *B. subtilis* NCIB 168 and NCIB 1650 were of similar sensitivity while *E. coli* was the least Cs^+-sensitive organism (Table 1).

The influence of other anions (carbonate and sulphate) was also assayed for Cs^+ inhibition of growth on the above bacteria (Fig. 2). Caesium nitrate was also examined but the low solubility of this salt prevented further use (solubilities, in cold water, are $CsNO_3$, 9.16 g per 100 ml; Cs_2SO_4, 167.0 g per 100 ml; Cs_2CO_3, 260.5 g per 100 ml; CsCl, 162.2 g per 100 ml). Equimolar Cs^+ concentrations, using Cs_2SO_4 and CsCl, inhibited growth to a similar extent. The pH of the agar (initial pH 7.15) was also unaffected at 1-, 2- and 3-cm inter-

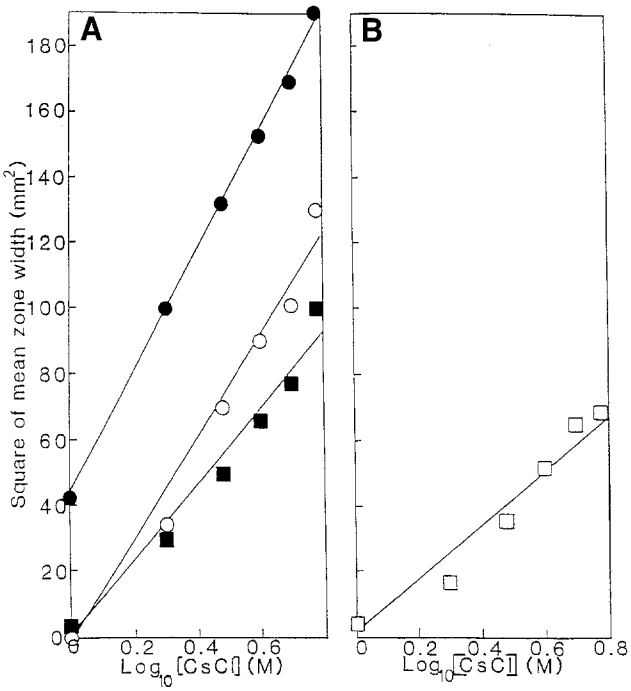

Fig. 1. The inhibitory effect of CsCl on the growth of four bacterial strains; (A) *Bacillus subtilis* 007 (●), *B. subtilis* NCIB 1650 (○), *B. subtilis* NCIB 168 (■) and (B) *Escherichia coli* NCIB 9484 (□), assessed using agar well diffusion assays. Aliquot volumes (50 μl) of CsCl solutions were added to 10-mm diameter wells in the plate. Each point is a mean of six replicates; bars indicating standard error of the mean (SEM) were smaller than the dimensions of the symbols.

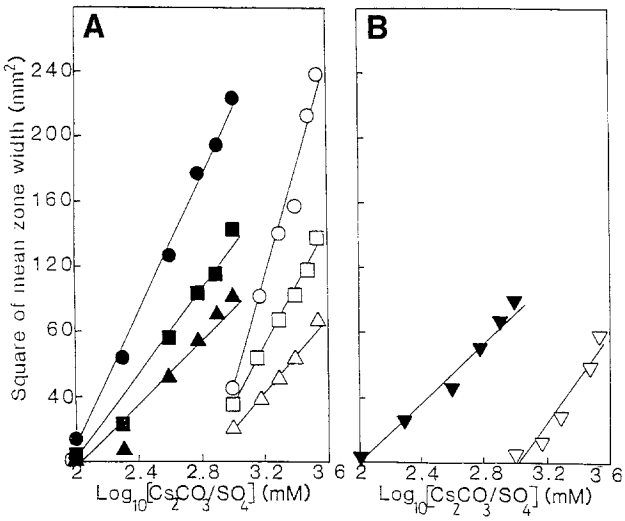

Fig. 2. Inhibitory effect of Cs_2CO_3 and Cs_2SO_4 (closed and open symbols respectively) towards four bacterial strains; (A) *Bacillus subtilis* 007 (●, ○), *B. subtilis* NCIB 1650 (■, □), *B. subtilis* NCIB 168 (▲, △) and (B) *Escherichia coli* NCIB 9484 (▼, ▽) assessed using agar well diffusion assays. Aliquot volumes (50 μl) of metal solutions were added to 10-mm diameter wells in the plate. Each point is a mean of six replicates; bars indicating SEM were smaller than the dimensions of the symbols.

vals from the well edge after diffusion of the CsCl and Cs_2SO_4 into the agar after overnight incubation. In comparison, the diffusion of Cs_2CO_3 into the agar created a pH gradient, pH values of 9.2, 8.4 and 7.8 being recorded at 1-cm intervals from the well edge respectively. When the toxicity of Cs_2CO_3, Cs_2SO_4 and CsCl was compared, inhibition of growth at equimolar Cs^+ concentrations was significantly higher in all the bacterial assays for the carbonate.

The kinetics of Cs^+ gradient formation when CsCl solution was added to wells in the plate centre is shown in Fig. 3. There was a decrease in Cs^+ concentration over a distance of 3.5 cm from the well edge after a 17-h incubation period. Using these data, the apparent minimum inhibitory concentrations (MIC) of CsCl at the periphery of the zone of inhibition after 17 h were 22 mM for *B. subtilis* 007, 25 mM for *B. subtilis* NCIB 168, 24 mM for *B. subtilis* NCIB 1650 and 26 mM for *E. coli* NCIB 9484. These similar values do not adequately distinguish the observed sensitivities of the bacterial strains, probably because of progressive equalization ('flattening') of the Cs^+ gradient after 17 h incubation.

TABLE 1

Influence of the other Group 1 monovalent metal cations on Cs^+ toxicity assessed using agar well diffusion assays

Organism	Square of mean zone width (mm^2)				
	Cs^+ only	Cs^+/K^+	Cs^+/Rb^+	Cs^+/Na^+	Cs^+/Li^+
B. subtilis 007	100.0 ± 0	NT	90.3 ± 0	51.1 ± 0.3	56.3 ± 0
B. subtilis NCIB 1650	34.8 ± 0.2	NT	45.5 ± 0.2	11.6 ± 0.4	25.8 ± 0.3
B. subtilis NCIB 168	29.3 ± 0.3	NT	36.0 ± 0	9.0 ± 0	20.3 ± 0
E. coli NCIB 9484	16.7 ± 0.2	NT	NT	NT	8.5 ± 0.2

Aliquot volumes (50 μl) of equimolar (2 M) metal chloride solutions were added to 10-mm diameter wells in the plate. Each value is a mean of six replicates ± SEM (NT indicates no detectable toxicity).

pH-dependence of caesium toxicity in Bacillus subtilis *and* Escherichia coli

Cs^+ toxicity was dependent on pH and was inversely proportional to the size of the zone of inhibition (Fig. 4). *E. coli* NCIB 9484 and *B. subtilis* NCIB 168 both showed increasing

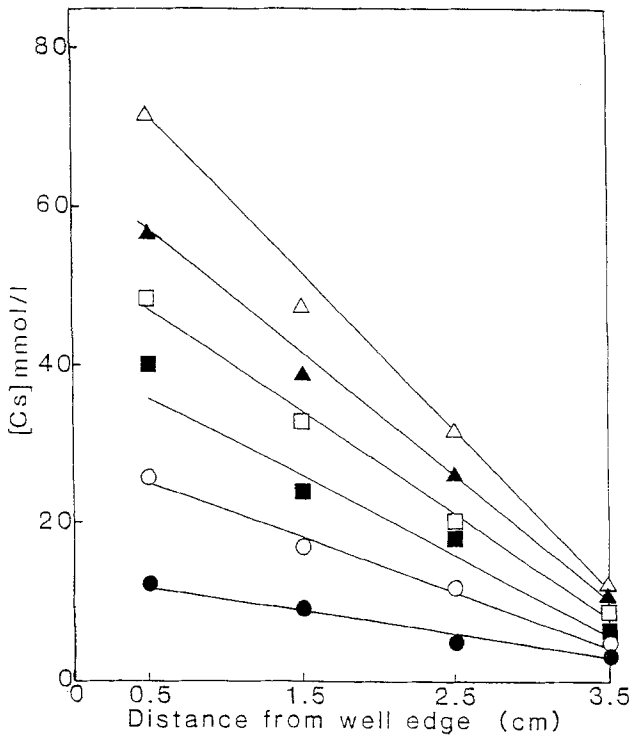

Fig. 3. Formation of gradients with caesium in nutrient agar: 50-μl aliquots of CsCl solutions at various concentrations (● 1 M; ○ 2 M; ■ 3 M; □ 4 M; ▲ 5 M; △ 6 M) were added to 10-mm diameter wells in the plate centre. Mean values from three replicate determinations are shown; SEM values were smaller than symbol dimensions.

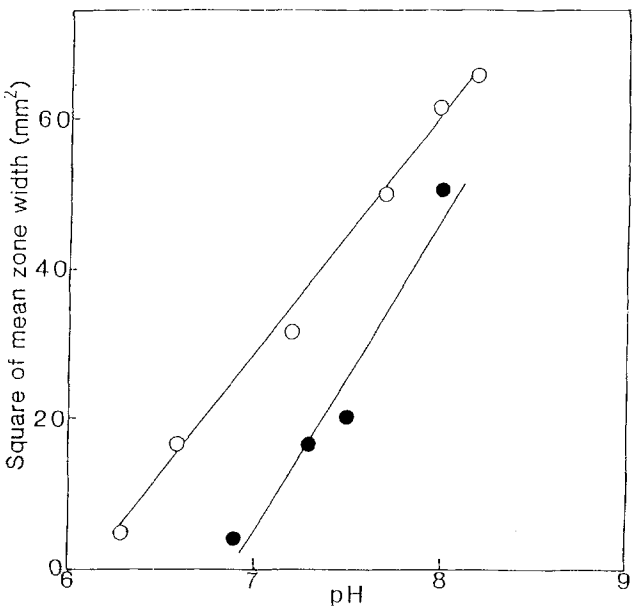

Fig. 4. Influence of pH on Cs^+ toxicity. The inhibitory effect of CsCl at various pH values (pH 6.3–8.2) towards *E. coli* NCIB 9484 (●) and *B. subtilis* NCIB 168 (○) was assessed using agar well diffusion assays. Mean values from six replicate determinations are shown; SEM values were smaller than symbol dimensions.

sensitivity over the pH range 6–9 with the latter organism being slightly more Cs^+-sensitive. It was found that the texture of the agar was altered on addition of acid or alkali becoming either brittle or semi-solid respectively. As gel strength is a critical factor in agar well diffusion assays [24] the extent of alteration in diffusional properties had to be determined. The use of a visible dye, pararosaniline chloride, showed the useful medium pH range to be from pH 6.5 to pH 8.5, observed dye zones over this range remaining constant.

Effect of other monovalent cations on Cs^+ toxicity towards Bacillus subtilis *and* Escherichia coli

Several monovalent cations (K^+, Rb^+, Na^+ and Li^+) were examined for inhibitory effects on Cs^+ toxicity towards *E. coli* and *B. subtilis*. Potassium had the most pronounced effect in reducing Cs^+ toxicity towards the four bacterial strains tested. Equimolar concentrations of both cations (K^+, Cs^+) placed in the well abolished toxicity completely (Table 1). In the *B. subtilis* strains the other monovalent cations reduced the apparent toxicity of Cs^+ in the order Na^+, Li^+ and Rb^+. For *E. coli*, the presence of all the monovalent cations (except Li^+) eliminated toxic effect(s) induced by Cs^+.

The toxicity of CsCl towards the three *B. subtilis* strains when incubated with increasing concentrations of KCl is shown in Fig. 5(A). To define such effects, we have calculated minimum inhibitory K^+: Cs^+ concentration ratios which are the ratio of the $[K^+]$ in the medium to the $[Cs^+]$ initially added to the well which abolishes the appearance of zones of inhibition after 17 h incubation at 37 °C. Using this definition, the results shown in Fig. 5(A) and (B) indicate a minimum inhibitory K^+:Cs^+ concentration ratio for the three *Bacillus* strains 007, NCIB 1650 and NCIB 168 of 1:2, 1:2.5 and 1:3 respectively. In comparison, *E. coli* exhibited a lower Cs^+ sensitivity in the presence of differing KCl concentrations, the minimum inhibitory K^+:Cs^+ concentration ratio for *E. coli* being 1:6 (Fig. 5(B)).

Interaction of K^+ and Na^+ with Cs^+ accumulation and their role in affecting Cs^+ toxicity

Accumulation of ^{137}Cs occurred in all the bacterial strains assayed (Fig. 6). Accumulation was rapid for *B. subtilis* 007, NCIB 1650 and NCIB 168 and accumulation rates of approximately 0.3 nmol min^{-1} mg dry wt^{-1} occurred in all strains; maximum accumulation occurred after approximately 0.5 h. In contrast, *E. coli* NCIB 9484 had a slower maximum rate of accumulation which levelled after 1 h. There was no relationship between the amount of ^{137}Cs accumulated and the apparent degree of toxicity exhibited towards the bacteria, e.g. *B. subtilis* NCIB 168 was the least sensitive of the *Bacillus* strains but accumulated equivalent amounts of ^{137}Cs as the most sensitive *B. subtilis* 007. Accumulation of ^{137}Cs was markedly reduced in the absence of glucose. For the *B. subtilis* strains a reduction in uptake of between 38–62% occurred: *E. coli* showed a reduction of 82%.

The presence of other essential monovalent cations (Na^+, K^+) affected the quantity of ^{137}Cs accumulated by all the bacteria. The addition of K^+ at a concentration equivalent to that found in nutrient agar (8.3 mM KCl) reduced ^{137}Cs accumu-

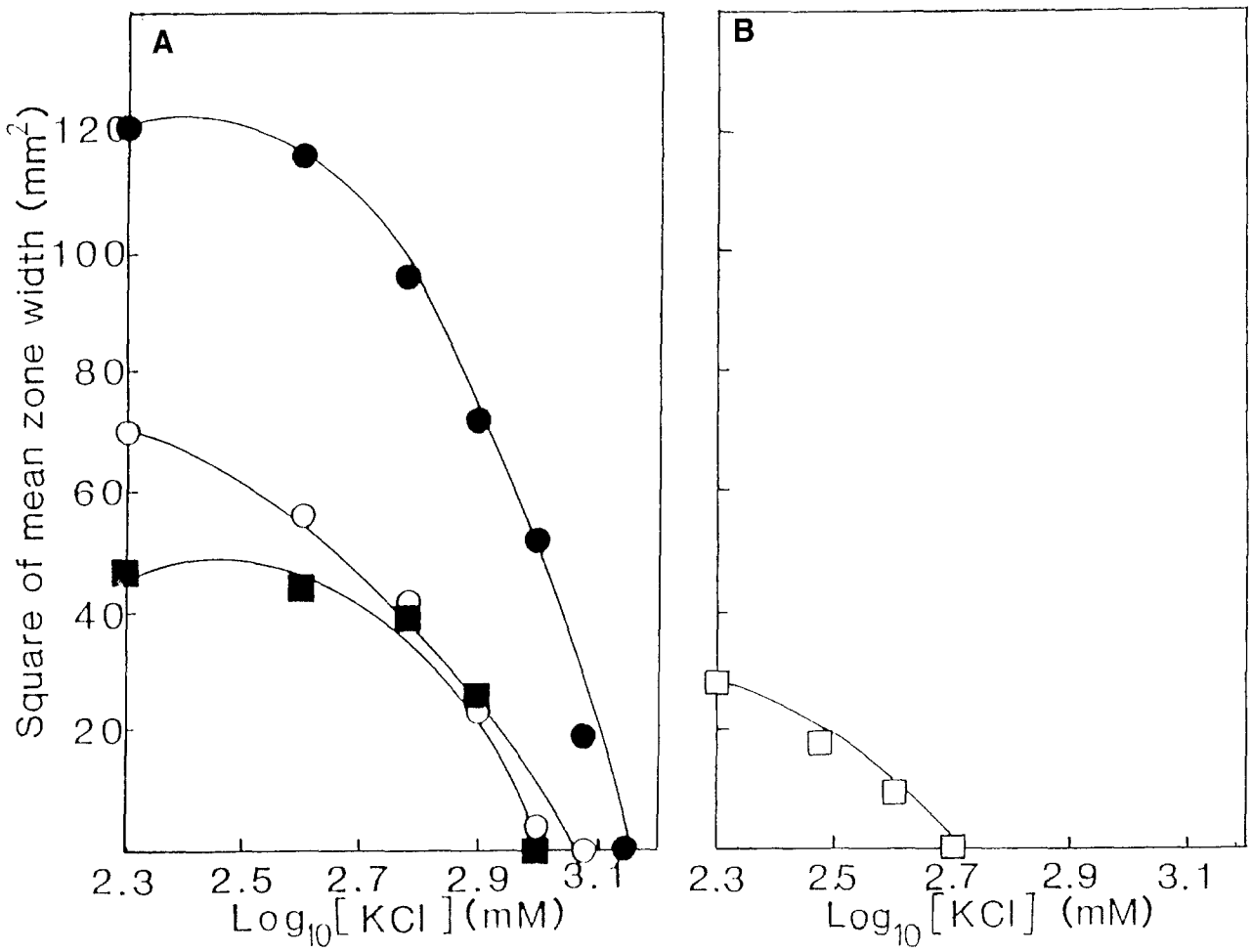

Fig. 5. Influence of KCl on Cs^+ toxicity. The inhibitory effect of KCl on Cs^+ toxicity towards four bacterial strains; (A) *Bacillus subtilis* 007 (●), *B. subtilis* NCIB 1650 (○), *B. subtilis* NCIB 168 (■) and (B) *Escherichia coli* NCIB 9484 (□) assessed using agar well diffusion assays. Aliquot volumes (50 μl) of 3 M CsCl containing various concentrations of KCl were added to 10-mm diameter wells in the plate. Each point is a mean of six replicates; SEM values were smaller than the dimensions of the symbols.

lation to a level similar to that which occurred in the absence of glucose. ^{137}Cs accumulation was also affected by the presence of Na^+ added at a concentration equivalent to that found in nutrient agar (0.13 M NaCl).

DISCUSSION

There is widespread understanding of the toxic effects of toxic divalent metal cations on microorganisms; reduction of growth rate, extension of the lag phase and changes in morphology and physiology are well documented [21]. In contrast, the toxicity of Cs^+ and other monovalent cations (except for Na^+) has received little attention to date. In this study only Cs^+ and Li^+ of the Group 1 metals inhibited growth in well diffussion assays over the concentration ranges used. Some effects of Cs^+ are known to occur because of interference with the entry of essential monovalent cations, primarily K^+, into the cell [1,2]. In some cases, this allows manipulation of the cellular cation content [31] although total exchange of K^+ for monovalent cations such as Rb^+ could not be achieved in *E. coli* [30], nor Cs^+ in *Saccharomyces cerevisiae* [32], a small residual pool of K^+ remaining. In this study, toxicity of Cs^+ and Li^+ occurred only at high concentrations for all the bacteria studied. Reasons for this could include differing transport affinities for monovalent cations other than K^+, with the presence of monovalent cations in the agar (0.13 M NaCl, 8.3 mM KCl) also lowering toxicity and accumulation. In addition there may be some binding between medium constituents and Cs^+ [19] although the chemistry of caesium may mean that this is of lower significance [25].

The toxicity of caesium towards *E. coli* was lower compared to the *Bacillus* strains; this is in agreement with a general observation that Gram-negative bacteria generally exhibit greater metal tolerance than Gram-positive bacteria [25]. The gradients formed during diffusion of CsCl into the agar give an indication of the total concentrations present at times during bacterial growth. However, as the diffusion gradients reduce with time and the concentrations eventually become equal over the whole plate [20], the actual Cs^+ concentration that prevented growth could not be determined accurately and did not adequately reflect observed differences between the strains as revealed by measurement of inhibition zones. Comparisons of

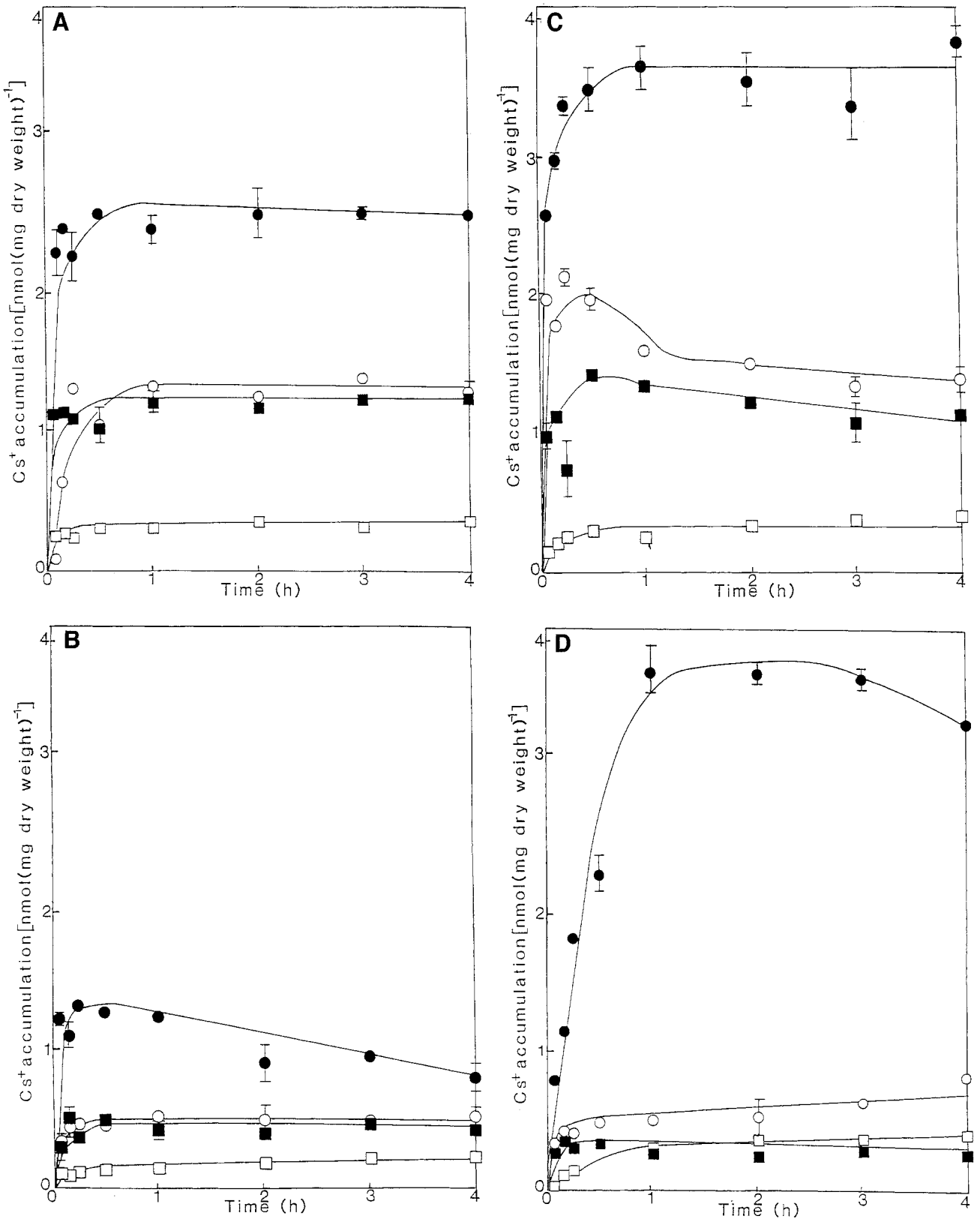

Fig. 6. Factors affecting ^{137}Cs accumulation in (A) *Bacillus subtilis* 007 (B) *B. subtilis* NCIB 1650 (C) *B. subtilis* NCIB 168 and (D) *Escherichia coli* NCIB 9484. Bacteria were suspended at an $OD_{550\,nm}$ ~5.0 in 5 mM PIPES buffer, pH 6.5, containing 25 μM CsCl + ^{137}Cs. The graphs show accumulation of ^{137}Cs in the absence (○) or presence of 50 mM glucose (●), the presence of 8.3 mM KCl (■) and the presence of 0.13 M NaCl (□). Mean values from three replicate determinations are shown ± SEM where possible.

Cs^+ toxicity between different organisms can also be complicated by media composition, gel strength and incubation temperature, factors which are fundamental to well diffusion assays [24], although these were all equalized in this study.

The actual site(s) of Cs^+ inhibition within cells have yet to be identified. The three main functions of K^+ in bacteria are the maintenance of turgor pressure [22], stabilization of intracellular structures [38] and enzyme activation [37]. The high levels of potassium within cells (~240 mM [30]) are not necessary for activation of intracellular enzymes. Many K^+-dependent enzymes are known, e.g. pyruvate kinase, but they usually require K^+ concentrations of only ~10 mM for maximum activity [37]. Higher levels of caesium may therefore be required to alter the intracellular K^+:Cs^+ ratio in order for enzymes to be affected [32]. In addition, certain interactions between K^+ and intracellular structures may be extremely specific and other cations with different radii (K^+:133 pm; Cs^+:165 pm, Li^+:78 pm, Tl^+:149 pm [16]), may not perform as efficiently. A study on *Chlorella emersonii* [2] suggested that it was not the presence of Cs^+ in cells that was growth inhibitory but the resulting loss of K^+.

Caesium toxicity was markedly influenced by external pH in both Gram-negative and Gram-positive bacteria, and well diffusion assays showed increasing Cs^+ sensitivity with increasing pH. This has been observed with the cyanobacterium *Synechocystis* PCC 6803 which also exhibits increasing Cs^+ accumulation at alkaline pH [1]. The chemiosmotic hypothesis states that cells have a transmembrane proton motive force consisting of a membrane potential and a pH gradient [18]. The magnitude of the pH gradient is largely a function of external pH because of a relatively constant internal pH [9]. Caesium accumulation may result from electroneutral Cs^+-H^+ antiport in *E. coli* [28]; increased intracellular concentration of Cs^+ at higher pH may cause increased toxicity. In addition, protonation of transport/binding sites at lower pH may also contribute to decreased Cs^+ uptake and decreased toxicity.

In common with other monovalent cation accumulation systems, Cs^+ uptake was energy-dependent [35]. However, in the absence of an energy source, *B. subtilis* had a higher binding capacity for ^{137}Cs compared to *E. coli*. Many metal cations bind to the largely anionic outer surface layers of microbial cells and *B. subtilis* binds Na^+ and K^+ in amounts that made them visible by electron scattering in thin sections [8]. The cell wall of Gram-positive bacteria is composed of several layers bearing anionic groups. Two main wall constituents, teichoic acids and teichuronic acids, covalently attached to the peptidoglycan contribute to the anionic property of the wall [33]. In contrast *E. coli* and most other Gram-negative bacteria have a lower anionic charge capacity, having less peptidoglycan, and therefore bind less metals [7]. Little knowledge exists on ^{137}Cs-binding in bacteria but the relatively high affinity shown by *B. subtilis* in this and other studies [26] may be of environmental significance although, because of the low amounts involved, is unlikely to be of relevance to the biological detoxification of Cs^+-containing effluents [27].

The presence of K^+, at an equivalent concentration to that present in the nutrient agar, considerably lowered ^{137}Cs accumulation confirming interference in the assay system by medium K^+ and also competitive inhibition of ^{137}Cs accumulation. Incubation in 0.13 M NaCl also strongly reduced ^{137}Cs accumulation in the assays and hence lowered toxicity. The combined effect of K^+ and Na^+ may be presumed to be even greater on the alleviation of Cs^+ toxicity. It is already known that osmotic stress, as a way of promoting Cs^+ accumulation and replacement of high intracellular concentrations of K^+, does not occur. The Cs^+ uptake system (TrKD) in *E. coli* is non-osmotically regulated and therefore can have little or no industrial application for effluent treatment contaminated with ^{137}Cs [27].

To conclude, well diffusion assays have been used successfully to examine Cs^+ toxicity towards Gram-positive and Gram-negative bacteria, providing a basis for subsequent physiological studies. It is clear that the high concentrations of other monovalent cations in media, e.g. K^+ and Na^+, are important and should be considered carefully as competitive inhibition may prevent Cs^+ uptake and hence lower or abolish any observed toxicity in well diffusion assays. The maintenance of a constant pH, shown by the marked influence on Cs^+ toxicity, should also be ensured. In view of the results obtained here, it seems unlikely that Cs^+ will exert significant toxic effects to bacteria in the soil environment at least in the short term. The high concentration of K^+ and Na^+ in soils and the ion adsorption capacity of soils (controlled by the aluminosilicate clay materials smectite and kaolinite) would also play an important role in limiting Cs^+ availability (13,40].

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