THE BACTERIOSTATIC ACTIVITY OF CERIUM, LANTHANUM, AND THALLIUM

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The salts of cerium, lanthanum, and thallium have long been known to possess bacteriostatic properties in low concentrations, but there is little information in the literature concerning the variability of their toxicity toward different species of microorganisms. A report of the concentrations of the salts limiting development of various types of bacteria may have importance in relation to their possible use as bacteriostatic or bactericidal agents.

Bokorny (1894) found cerium compounds relatively much more toxic for bacteria than for algae. Hebert (1907) reported that cerium and lanthanum sulfates in concentrations of 5 to 10 grams per liter showed little toxicity toward *Aspergillus niger* and yeast. Sartory and Bailly (1922) reported that 0.2 per cent lanthanum sulfate depressed the growth of *Aspergillus fumigatus* in Raulin's solution and practically inhibited spore formation. Frouin (1912) observed that 0.005 grams of lanthanum sulfate per 100 ml of medium stimulated the growth of the tubercle bacillus but that higher concentrations were toxic. Frouin and Roudski (1914) studied the toxicity of lanthanum and thorium for the cholera and dysentery organisms.

Other investigators who reported bacteriostatic or lethal effects of salts of the rare earth group include Drossback (1897), Brooks (1921), Grenet and Drouin (1927), Zirpolo (1924), Frouin (1920), Simonini (1914), Doerr (1920), Eisenberg (1918), and Hotchkiss (1923). A general review of the earlier literature on this subject is found in Buchanan and Fulmer (1930). McKenzie (1941) employed thallium acetate in a medium recommended for the enrichment of the streptococci causing mastitis. The effect of cerium on enzyme activity was reported by Gould (1936). Olszewski (1932) observed no significant reduction in the bacteria of river water when 1 ppm of cerous or ceric chloride or ceric sulfate was employed. Richards (1932) reported thallium to be a growth stimulant for yeast.

The present paper reports a further investigation of the bacteriostatic activity of the salts of cerium, lanthanum, and thallium.

METHODS

Thirty-nine species of bacteria, representing 16 different genera, were employed in this study. Also, 35 species of fungi, comprising 18 genera, were used in a limited comparison of the mycostatic and bacteriostatic effects of the compounds. The salts used were cerium chloride ($CeCl_3$, cp, E. H. Sargent); cerium nitrate ($Ce(NO_3)_3 \cdot 6H_2O$, cp, General Chemical Co.); anhydrous ceric sulfate ($Ce(SO_4)_2$, G. F. Smith Chemical Co.); lanthanum chloride (cp, E. H. Sargent), and thallium nitrate (E. H. Sargent).

Other chemicals were used in some of the tests in order to determine their effect on the toxicity of the test substances. These chemicals included the sulfates and chlorides of sodium, magnesium, barium, and ammonium, and the chlorides of calcium and lithium. Stock solutions of the salts were made and the amount required for each test medium was removed by pipette. The tests were made on petri plates of solidified agar containing the specified amounts of the salts. The basal medium consisted of 1 per cent Difco peptone and 1.5 per cent Difco agar in distilled water. Inoculations of the agar plates were made by means of a 1½-mm nichrome wire loop using a 24-hour broth culture. Radial streak inoculations were made, using 8 cultures to each plate. Incubation was at 37 C except for those soil and water forms which grow better at a lower temperature and were incubated at room temperature (22 to 27 C). Observations and records were made after incubation for 1, 2, 3, and 5 days.

RESULTS

The toxicity of cerium salts for bacteria. The bacteriostatic action of three cerium salts, the trivalent cerium chloride and cerium nitrate and the tetravalent ceric sulfate, was determined against 40 different species. The results are presented in table 1. The chloride was found to be definitely less toxic than either the sulfate or nitrate of cerium. No significant difference in toxicity between the sulfate and nitrate of cerium was noted.

The reaction of the media was not adjusted after the addition of the cerium salts, and the pH values were found to vary as follows: for cerium chloride agar, 6.3 at 0.0002 M concentration to 5.95 at 0.0014 M concentration; for cerium nitrate, pH 5.8 at 0.0001 M to 5.65 at 0.0009 M; and for cerium sulfate, pH 6.7 at 0.0002 M to 5.4 at 0.0008 M.

The toxicity of cerium nitrate and cerium chloride varied relatively little among the different species of bacteria tested, and even fewer variations were observed with cerium sulfate. Of the bacterial species tried, *Aerobacter aerogenes*, *Aerobacter cloacae*, *Salmonella aertrycke*, and *Achromobacter lipolyticum* were most tolerant of cerium. The *Torula rosea* culture proved to be far more resistant to the cerium compounds than the most resistant bacteria.

The effect of pH and the presence of other salts on the toxicity of cerium compounds. In order to determine the effect of pH on cerium toxicity, the media were prepared using cerium nitrate in concentrations varying from 0.0003 M to 0.0007 M, then adjusted to pH 6.0 and 8.0. The results obtained after 2 days' incubation are presented in table 2. It was observed that at pH 8.0 all cultures except *Staphylococcus albus* developed without hindrance in 0.0007 M cerium nitrate, whereas at pH 6.0 a considerable number of cultures failed to grow in the 0.0003 M concentration of cerium.

The effect of other salts on the bacteriostatic activity of cerium compounds. The effect of various salts which are sometimes used in culture media on the bacteriostatic activity of cerium compounds was determined by adding the salts separately to the cerium-containing media and observing for bacterial growth after inoculations. It was found that sodium chloride in concentrations up to

TABLE 1

The toxicity of various cerium compounds for certain bacteria

Incubation for 2 days

CULTURE	MOLECULAR CONCENTRATION					
	$CeCl_3$		$Ce(NO_3)_3$		$Ce(SO_4)_2$	
	A	B	A	B	A	B
Salmonella paratyphi	0.0006	0.0008	0.0004	0.0005	0.0004	0.0006
Salmonella pullorum	0.0006	0.0008	0.0004	0.0005	0.0004	0.0006
Salmonella schottmuelleri	0.0008	0.0010	0.0004	0.0005	0.0004	0.0006
Salmonella enteritidis	0.0008	0.0010	0.0004	0.0005	0.0004	0.0006
Salmonella aertrycke	0.0008	0.0010	0.0004	0.0005	0.0006	0.0008
Salmonella gallinarum	0.0006	0.0008	0.0004	0.0005	0.0004	0.0006
Salmonella suipestifer	0.0008	0.0010	0.0004	0.0005	0.0004	0.0006
Eberthella typhosa	0.0008	0.0010	0.0004	0.0005	0.0004	0.0006
Shigella sonnei	0.0008	0.0010	0.0005	0.0006	0.0004	0.0006
Shigella dysenteriae	0.0008	0.0010	0.0003	0.0004	0.0004	0.0006
Aerobacter aerogenes	0.0008	0.0010	0.0005	0.0006	0.0006	0.0008
Aerobacter cloacae	†	†	0.0005	0.0006	0.0006	0.0008
Escherichia coli	0.0008	0.0010	0.0004	0.0005	0.0004	0.0006
Escherichia communior	0.0008	0.0010	0.0004	0.0005	0.0004	0.0006
Escherichia acidilactici	0.0008	0.0010	0.0004	0.0005	0.0004	0.0006
Citrobacter intermedium	0.0008	0.0010	0.0004	0.0005	0.0004	0.0006
Alcaligenes faecalis	0.0010	0.0012	0.0004	0.0005	0.0004	0.0006
Proteus vulgaris	0.0008	0.0010	0.0004	0.0005	0.0004	0.0006
Pseudomonas aeruginosa	0.0004	0.0006	0.0003	0.0004	0.0004	0.0006
Pseudomonas ovalis	0.0006	0.0008	0.0003	0.0004	0.0004	0.0006
Pseudomonas graveolens	0.0004	0.0006	0.0001	0.0002	0.0004	0.0006
Pseudomonas syncyanea	0.0006	0.0008	0.0003	0.0004	0.0004	0.0006
Pseudomonas mucedolens	0.0004	0.0006	0.0001	0.0002	0.0004	0.0006
Flavobacterium sauveolens	0.0010	0.0012	0.0005	0.0006	0.0004	0.0006
Achromobacter lipolyticum	0.0010	0.0012	0.0006	0.0007	0.0006	0.0008
Serratia marcescens	0.0010	0.0012	0.0006	0.0007	0.0004	0.0006
Bacillus subtilis	0.0010	0.0012	0.0006	0.0007	0.0004	0.0006
Bacillus mesentericus	0.0008	0.0010	0.0004	0.0005	0.0004	0.0006
Bacillus mycoides	0.0008	0.0010	0.0005	0.0006	0.0004	0.0006
Bacillus fusiformis	0.0008	0.0010	0.0004	0.0005	0.0004	0.0006
Bacillus metiens	0.0008	0.0010	0.0005	0.0006	0.0004	0.0006
Staphylococcus candidus	0.0008	0.0010	0.0005	0.0006	0.0004	0.0006
Staphylococcus flavus	0.0008	0.0010	0.0003	0.0004	0.0004	0.0006
Staphylococcus aureus	0.0008	0.0010	0.0007	0.0008	0.0004	0.0006
Staphylococcus albus	0.0010	0.0012	0.0004	0.0005	0.0004	0.0006
Sarcina lutea	0.0006	0.0008	0.0004	0.0005	0.0004	0.0006
Sarcina conjunctivae	0.0006	0.0008	0.0007	0.0008	0.0004	0.0006
Rhodococcus agilis	0.0006	0.0008	0.0003	0.0004	0.0004	0.0006
Rhodococcus rosaceous	0.0006	0.0008	0.0003	0.0004	0.0004	0.0006
Torula rosea	0.0012	*	0.0009	*	0.0008	*

A = concentration permitting growth in 2 days.

B = concentration inhibiting growth in 2 days.

* Inhibiting concentration was not determined.

† Concentration less than .0003 M was not employed.

TABLE 2

The effect of pH on the toxicity of $Ce(NO_3)_3$ for certain bacteria

CULTURE	MOLECULAR CONCENTRATION			
	pH 6		pH 8	
	A	B	A	B
S. paratyphi	0.0003	0.0004	0.0007	*
S. pullorum	0.0003	0.0004	0.0007	*
S. schottmuelleri	0.0005	0.0007	0.0007	*
S. enteritidis	0.0004	0.0005	0.0007	*
S. aertrycke	0.0004	0.0005	0.0007	*
S. gallinarum	0.0004	0.0005	0.0007	*
S. suipestifer	0.0005	0.0006	0.0007	*
E. typhosa	0.0004	0.0005	0.0007	*
S. sonnei	†	0.0003	0.0007	*
S. dysenteriae	0.0003	0.0004	0.0007	*
A. aerogenes	0.0007	*	0.0007	*
A. cloacae	0.0006	0.0007	0.0007	*
E. coli	0.0004	0.0005	0.0007	*
E. communior	0.0004	0.0005	0.0007	*
E. acidilactici	0.0003	0.0004	0.0007	*
C. intermedium	0.0006	0.0007	0.0007	*
A. faecalis	0.0003	0.0004	0.0007	*
P. vulgaris	0.0003	0.0004	0.0007	*
P. aeruginosa	†	0.0003	0.0007	*
P. ovalis	†	0.0003	0.0007	*
P. graveolens	†	0.0003	0.0007	*
P. syncyanea	†	0.0003	0.0007	*
P. mucedolens	†	0.0003	0.0007	*
F. suaveolens	0.0007	*	0.0007	*
A. lipolyticum	0.0007	*	0.0007	*
S. marcescens	0.0007	*	0.0007	*
B. subtilis	†	0.0003	0.0007	*
B. mesentericus	0.0003	0.0004	0.0007	*
B. mycoides	†	0.0003	0.0007	*
B. fusiformis	†	0.0003	0.0007	*
B. meliens	†	0.0003	0.0007	*
S. aureus	0.0006	0.0007	0.0007	*
S. candidus	0.0004	0.0005	0.0007	*
S. albus	0.0003	0.0004	0.0004	0.0005
S. flava	0.0003	0.0004	0.0007	*
S. lutea	†	0.0003	0.0007	*
S. conjunctivae	†	0.0003	0.0007	*
R. agilis	0.0003	0.0004	0.0007	*
R. rosaceous	†	0.0003	0.0007	*
Torula rosea	0.0007	*	0.0007	*

A = concentration permitting growth in 2 days.

B = concentration inhibiting growth in 2 days.

* Concentration greater than 0.0007 M was not employed.

† Concentration less than 0.0003 M was not employed.

TABLE 3

The effect of 0.4 M of NaCl and 0.1 M Na_2SO_4 on toxicity of $Ce(SO_4)_2$

CULTURE	$Ce(SO_4)_2$ CONC. (M)	CONTROLS FOR			$Ce(SO_4)_2$ PLUS	
		$Ce(SO_4)_2$	NaCl	Na_2SO_4	NaCl	Na_2SO_4
		Vigor of growth: incubation for 2 days				
S. paratyphi	0.0006	−	++	++	−	+
S. pullorum	0.0006	−	++	++	−	++
S. schottmuelleri	0.0008	−	+++	+++	−	++
S. enteritidis	0.0008	−	++	++	−	+
S. aertrycke	0.0008	+	++	++	−	+
S. gallinarum	0.0004	+	++	++	++	+
S. suipestifer	0.0008	−	++	++	−	+
E. typhosa	0.0006	−	+++	+++	−	++
S. sonnei	0.0006	−	++	++	−	+
S. dysenteriae	0.0004	++	++	++	+	+
A. aerogenes	0.0008	+	++	++	−	++
A. cloacae	0.0008	++	+++	++	−	++
E. coli	0.0006	−	+++	++	−	+
E. communior	0.0008	−	+++	+++	−	+
C. intermedium	0.0006	−	++	++	−	+
A. faecalis	0.0006	−	+++	++	−	+
P. vulgaris	0.0006	−	++	++	−	+
P. aeruginosa	0.0004	++	++	+++	−	++
P. ovalis	0.0004	++	++	++	+	++
P. graveolens	0.0004	++	++	++	−	+
P. syncyanea	0.0004	+	+++	+++	−	++
P. mucedolens	0.0004	+	++	++	−	+
F. suaveolens	0.0006	−	++	++	−	++
A. lipolyticum	0.0008	+	++	++	+	+
S. marcescens	0.0006	+	++	++	+	++
B. subtilis	0.0006	+	++	+++	−	++
B. mesentericus	0.0008	−	++	+++	−	+
B. mycoides	0.0004	++	++	+++	+	++
B. fusiformis	0.0004	++	++	+++	++	++
B. metiens	0.0004	++	++	++	++	++
S. aureus	0.0004	++	++	++	++	++
S. candidus	0.0006	−	++	++	−	+
S. albus	0.0004	++	++	++	+	++
S. flavus	0.0004	++	++	++	+	+
S. lutea	0.0004	+	++	++	−	+
S. conjunctivae	0.0004	++	++	++	+	++
R. agilis	0.0004	+	++	++	+	+
R. rosaceous	0.0004	+	++	++	+	+
Torula rosea	0.0004	++	++	++	++	++

(−) = complete inhibition of growth.
(+) = moderate growth.
(++) = good growth.
(+++) = growth better than on nutrient agar control.

TABLE 4

The bacteriostatic activity of lanthanum chloride and thallium nitrate

ORGANISM	MOLECULAR CONCENTRATION			
	Permitting growth		Preventing growth	
	$LaCl_3$	$TlNO_3$	$LaCl_3$	$TlNO_3$
S. paratyphi	0.0002	0.0006	0.0004	0.0007
S. pullorum	0.0002	0.0006	0.0004	0.0007
S. schottmuelleri	0.0004	0.0007	0.0006	0.0008
S. enteritidis	0.0004	0.0007	0.0006	0.0008
S. aertrycke	0.0004	0.0007	0.0006	0.0008
S. gallinarum	0.0004	0.0006	0.0006	0.0007
S. suipestifer	0.0004	0.0006	0.0006	0.0007
E. typhosa	0.0004	0.0006	0.0006	0.0007
S. conjunctivae	0.0006	0.0008	0.0008	0.0010
S. sonnei	0.0004	0.0007	0.0006	0.0008
S. dysenteriae	0.0004	0.0005	0.0006	0.0007
A. aerogenes	0.0004	0.0007	0.0008	0.0008
A. cloacae	0.0004	0.0007	0.0008	0.0008
E. coli	0.0002	0.0005	0.0004	0.0007
E. communior	0.0004	0.0007	0.0006	0.0008
E. acidilactia	0.0004	0.0006	0.0006	0.0008
P. aeruginosa	0.0002	*	0.0004	0.0005
P. ovalis	0.0002	0.0005	0.0004	0.0006
P. graveolens	0.0001	*	0.0002	0.0005
P. syncyanea	0.0002	*	0.0004	0.0005
P. mucedolens	0.0002	*	0.0004	0.0005
S. marcescens	0.0006	0.0007	0.0008	0.0010
R. agilis	0.0001	0.0005	0.0004	0.0006
R. rosaceous	0.0001	0.0005	0.0004	0.0006
Torula rosea	0.0020	0.0011	†	0.0080
F. sauveolens	0.0006	0.0007	0.0008	0.0008
A. lipolyticum	0.0006	0.0007	0.0008	0.0010
B. subtilis	0.0006	0.0008	0.0008	0.0010
B. mesentericus	0.0004	0.0007	0.0006	0.0008
B. mycoides	0.0004	0.0007	0.0006	0.0008
B. fusiformis	0.0002	0.0005	0.0004	0.0007
B. metiens	0.0004	0.0008	0.0006	0.0010
C. intermedium	0.0004	0.0007	0.0006	0.0008
A. faecalis	0.0004	0.0007	0.0006	0.0008
P. vulgaris x 19	0.0004	0.0007	0.0006	0.0008
S. aureus	0.0004	0.0007	0.0008	0.0008
S. candidus	0.0002	0.0007	0.0006	0.0008
S. albus	0.0002	0.0005	0.0006	0.0007
S. flava	0.0002	0.0005	0.0004	0.0006
Sarcina lutea	0.0002	0.0005	0.0004	0.0007

* No growth in lowest concentration employed.

† Growth in highest concentration employed.

0.4 M depressed slightly or had no effect on the toxicity of cerium chloride and increased slightly or had no effect on the toxicity of cerium sulfate. Sodium sulfate in 0.1 M concentration markedly reduced the toxicity of cerium sulfate (table 3), but was without significant effect when used with cerium chloride.

Magnesium chloride (0.5 M) generally diminished the toxicity of cerium chloride, but magnesium sulfate (0.5 M) was without effect. The chlorides (0.5 M) of calcium and barium slightly increased the toxicity of cerium chloride, whereas barium sulfate, lithium chloride, ammonium sulfate, and ammonium chloride were without significant effect.

The toxicity of cerium chloride for fungi. In all the preceding experiments it was observed that the *Torula* culture was far more tolerant of the cerium compounds than were the bacteria. In order to determine whether other common fungi are equally tolerant of cerium, a yeast extract glucose peptone medium was prepared with concentrations of cerium chloride sufficient to inhibit all the bacteria employed in this study. Thirty-five strains of fungi were inoculated on the media by streaking, and the results were read after incubation for 2 days at room temperature.

The following organisms were employed: *Debaromyces tyrocola*, *Endomyces hordei*, *Monilia krusei*, *Mycoderma valida*, *Pichia farinosus*, *Saccharomyces cerevisiae* Frohberg, *Saccharomyces* of Curtis, *Saccharomyces cerevisiae* Saaz, *Schizosaccharomyces mellacei*, *Torula* "Hansen" sp., *Torula humicola*, *Torula mucilaginosa*, *Torula spherica*, *Torula datilla*, *Torula colliculosa*, *Torula sanguinea*, *Torula* "pink" sp., *Torula fructicola*, *Torula liconde*, *Torula fermentati*, *Torula kefyr*, *Torula lactosa*, *Torula candida*, *Zygosaccharomyces priorianus*, *Zygosaccharomyces chevalieri*, *Vermicularia* sp., *Fusarium* sp., *Phytophthora* sp., *Neocosmospora* sp., *Dothiorella* sp., *Cunninghamella* sp., *Trichoderma* sp., *Pythium* sp., *Rhizopus* sp., and *Aspergillus* sp.

All the fungus cultures grew as well in the presence of 0.0014 M cerium chloride, the highest concentration employed, as in the control medium.

The bacteriostatic activity of lanthanum and thallium. The toxicity of lanthanum chloride and thallium nitrate for the selected bacteria was determined by the same methods employed in the preceding experiments. The results are presented in table 4. The order of toxicity of lanthanum and thallium was found to be approximately the same as that of cerium. Again, some species were observed to be relatively more resistant than others. The organisms most tolerant of the salts were found to be certain species of *Bacillus*, *Serratia marcescens*, *Sarcina conjunctivae*, *Achromobacter lipolyticum*, and *Torula rosea*. The most susceptible organisms were species of the genus *Pseudomonas*.

CONCLUSIONS

The salts of cerium, lanthanum, and thallium were found to be definitely more toxic for the bacteria than for the fungi included in this study.

The 39 species of bacteria were prevented from growth in concentrations of cerium chloride varying from 0.0006 to 0.0012 M; in cerium nitrate from 0.0004 to 0.0008 M; in cerium sulfate from 0.0006 to 0.0008 M; in lanthanum chloride from 0.0002 to 0.0008 M; and in thallium nitrate from 0.0005 to 0.0010 M.

The toxicity of cerium sulfate for most bacteria was reduced by the addition of sodium sulfate (0.05 M) to the medium, and the toxicity of cerium chloride was generally decreased by the addition of magnesium chloride (0.05 M). The addition of other salts had little effect on the toxicity of cerium.

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