A Research Note

Antimicrobial Activity of Sodium Bicarbonate

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ABSTRACT

Sodium bicarbonate (SB) inhibited the growth of bacteria and yeasts in agar media model systems under certain conditions. *Escherichia coli, Lactobacillus plantarum, Staphylococcus aureus*, and *Pseudomonas aeruginosa* aerobic plate counts were reduced 10,000-fold by 0.12M (1% w/v) SB. *Saccharomyces cerevisiae* and *Hansenula wingei* were more sensitive; counts were reduced 100,000-fold by 0.06M SB. Potassium bicarbonate was equally inhibitory, but equimolar sodium chloride had no effect, ruling out osmotic- and sodium-mediated mechanisms of inhibition. The bicarbonate ion was identified as the probable cause of SB-mediated inhibition although, in some cases, pH elevation played a significant role.

INTRODUCTION

SODIUM BICARBONATE (SB) is widely used in foods at levels up to 2% for leavening, pH-control, taste, and texture development (Lindsay, 1985). Data on the antimicrobial properties of SB are limited. It is inhibitory to periodontal pathogens (Newbrun et al., 1984; Cerra and Killoy, 1982; Miyasaki et al., 1986) and is used in dental preparations. SB is lethal to *Aspergillus parasiticus* and alters aflatoxin distribution in surviving cells (Montville and Goldstein, 1987). If SB inhibits other organisms, its GRAS (generally recognized as safe) status, low cost, and lack of toxicity would favor its use as a preservative. The objective of this study was to determine if SB has antimicrobial activity against several aerobic and anaerobic bacteria and against two common yeasts.

MATERIALS & METHODS

BACTERIA were tested for sensitivity to SB in Mueller Hinton Agar (MH, Difco), a universal medium for testing anti-microbial compounds (Matson and Barry, 1974), and media appropriate for specific microorganisms, as indicated in Table 1. These were prepared at SB concentrations of 0 to 6%. Also prepared were media which contained potassium bicarbonate (KB) and NaCl in molar amounts equivalent to the Na^+ and HCO_3^- provided by inhibitory SB concentrations. Media to serve as pH controls were adjusted to pH 10 using 5N NaOH. Details of stock culture preservation and media preparation appear elsewhere (Corral, 1987). The bacteria were grown to mid-log or stationary phase, diluted to 10^4 or 10^2 CFU/mL, 1 mL added to pour plates of media tempered to 45° C, and incubated aerobically or anaerobically as indicated in Table 1. All incubations were at 37° C, except for the pseudomonads, which were at 26° C. Conditions preventing any colony formation were considered inhibitory.

To examine the inhibition of bacteria in a liquid system, Brain Heart Infusion broth (BHI, Difco Laboratories, Detroit, MI) was prepared in citrate phosphate buffer at pH 5.6, 6.0, 7.0, and Tris-HCl buffer at pH 8.6 (Costilow, 1981). SB was added to each medium at 0 to 10% and the pH readjusted with HCl. Control media, which did not contain SB, were adjusted to pH 9.4 with 5N NaOH. All media were filter-sterilized (0.45 μm) and used immediately. Cells were grown to mid-log phase, harvested, washed, and adjusted to 10^6 CFU/mL. The broths were inoculated to a final concentration of 10^5 CFU/mL, incubated aerobically without agitation, and examined for turbidity at 48 hr. Conditions completely preventing turbidity were scored as inhibitory.

Yeast Peptone Dextrose (YPD) agars containing 0 to 1.00% (w/v) SB were prepared to examine inhibition of the yeasts. Additional media were prepared to serve as controls for KB, NaCl, and pH. Twenty-four hour yeast cultures were serially diluted to inocula levels of 10^2 through 10^5 CFU/mL, plated and incubated. All experiments were conducted in duplicate and repeated once; the data represent the results of four trials.

Table 1—Influence of plating medium, inoculum level, and incubation conditions (aerobic versus anaerobic) on the concentration of sodium bicarbonate required to inhibit bacterial growth

		Sodium bicarbonate conc (%) required to inhibit growth			
		Aerobic		Anaerobic[a]	
		Inoculum level (CFU/mL)			
Species	Plating medium[a]	10,000	100	10,000	100
P. fluorescens	PCA	4	4	nd[c]	nd
	MH	4	2	nd	nd
P. aeruginosa	PCA	1	1	nd	nd
	NH	1	1	nd	nd
E. coli	PCA	1	1	nd	nd
	MH	1	1	nd	nd
C. perfringens	TSC	nd	nd	>6[d]	>6
	MH	nd	nd	>6	>6
S. mutans	BHI	nd	nd	>6	>6
	MH	nd	nd	>6	>6
S. faecalis	BHI	4	4	>6	>6
	MH	4	2	>6	>6
S. aureus	BHI	1	1	>6	6
	MH	1	1	>6	6
L. plantarum	MRS	1	1	2	1
	MH	1	1	1	1

[a] Inocula were prepared in Brain Heart Infusion (BHI) broth, except for *L. plantarum* and *C. perfringens* which were grown in Lactobacillus MRS (MRS) and Fluid Thioglycolate Medium, respectively. Other agars used were Plate Count Agar (PCA), Mueller Hinton (MH), and Tryptose Sulfite Cycloserine (TSC) without cycloserine (Harmon, 1984).

[b] GasPak Systems (BBL, Cockeysville, MD)

[c] Not done

[d] No inhibition at any sodium bicarbonate concentration tested.

RESULTS & DISCUSSION

PHASE OF GROWTH was not a factor in SB sensitivity; only data from stationary phase inocula are presented in Table 1. Aerobic growth of *E. coli, S. aureus, P. aeruginosa*, and *L. plantarum* was prevented by 1% SB in BHI or MH agars. Of the obligate and facultative anaerobes, only *L. plantarum* was sensitive to low SB concentrations under anaerobic conditions. However, twice as much SB was required to inhibit anaerobic *L. plantarum* compared with aerobic conditions when high inocula were used. Inoculum size and medium used were not major determinants of SB sensitivity, although in some cases there was increased sensitivity when low inocula were plated on MH agar. This suggests that the cell's physicochemical environment may play some role in its SB sensitivity. KB

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(0.12M) was also inhibitory to the bacteria (data not shown). Both SB and KB elevated the pH of agar media to between 9.00 and 9.87. Agar media adjusted with NaOH to a target pH value of 10 had final pH values of 9.00 to 9.76 and was also inhibitory. Growth occurred at pH ≤9.0.

When 10^5 CFU/mL of *E. coli, S. mutans, S. faecalis,* or *C. perfringens* were inoculated into BHI broth, there was little or no inhibition at pH 5.6 and 6.0 (Table 2). All four organisms were inhibited at pH 7.0; SB was more effective at pH 8.6. It was difficult to maintain stable pH values in media which contained SB. Media initially adjusted to pH 5.6, 6.0, 7.0, and 8.6 reached final values of 7.0, 8.1, 8.8, and 9.4, respectively. All four species grew in BHI (0% SB) adjusted to pH 9.4 with NaOH. The pH of BHI without SB did not drift.

Yeasts were more sensitive to SB than were the bacteria. At inoculum levels of 10^2 and 10^3 CFU/mL, 0.25% SB prevented growth; *H. wingei* was inhibited at inoculum levels of 10^5 CFU/mL. *S. cerevisiae* required 0.50% SB to inhibit growth of high inocula. Growth was not inhibited by 0.06M NaCl, but was inhibited by 0.06M KB and at NaOH-generated alkaline pH values.

In broth experiments, growth was not inhibited by alkaline pH controls. Alkalinity has also been excluded as the agent of SB inhibition against *A. parasiticus* (Montville and Goldstein, 1987) and oral anaerobes (Newburn et al., 1984). These studies, and our findings, have found KB to be equally inhibitory to SB, suggesting that HCO_3^- is the inhibitory agent. Bicarbonate:potassium ratios regulate the morphology of some streptococci (Tao et al., 1987). Bicarbonate may alter membrane permeability (Sears and Eisenberg, 1961; Jones and Greenfield, 1982). SB also uncouples oxidative phosphorylation (Daniels, et al., 1985; Newbrun, et al. 1984) by stimulating mitochondrial ATPases which are very similar to bacterial ATPases (Maloney, 1987). The increased sensitivity of facultative anaerobes when grown aerobically may be caused by the synergistic effect of H_2O_2 with SB (Miyasaki et al., 1986). Growth inhibition in solid agar media could, however, be explained solely on the basis of pH elevation. Bacterial inhibition by SB occurred only at pH > 7.0. This agrees with previous findings that SB inhibits *A. parasiticus* at pH 7.5, but not at 5.5 (Montville and Goldstein, 1987). While this would appear to limit the use of SB in foods, SB's buffering capacity can elevate the pH of hot dogs and soybeans without adversely affecting protein functionality (Bechtel et al., 1985; Lu and Jassen, 1986). Browning reactions and off flavors generated at high SB concentrations may place limits on its use. In addition, pH elevation might cause some acid foods to move into the pH range where *Clostridium botulinum* becomes a problem. Thus, while the applicability of SB inhibition to various foods might be possible, each application should be the subject of laboratory evaluations.

Table 2—Minimum concentration of sodium bicarbonate required for inhibition of bacteria inoculated at 100,000 CFU/mL into BHI broth and incubated aerobically at 37°C for 48 hr

Species	Conc (%) of sodium bicarbonate required for inhibition at pH 5.6	6.0	7.0	8.6
Escherichia coli	10	10	10	4
Streptococcus mutans	>10[a]	>10	8	6
Streptococcus faecalis	>10	>10	8	6
Clostridium perfringens	>10	>10	10	6

[a] Denotes no inhibition at any concentration tested.

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