FURTHER STUDIES ON INHIBITION OF GROWTH OF SPORES OF *PENICILLIUM* SP. AND *ASPERGILLUS* SP. ISOLATED FROM THE WHITE MOLDS OF SILAGES[1]

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ABSTRACT

Finely powdered commercial sulfur does not inhibit appreciably the growth of spores of the white mold, *Penicillium* sp. and *Aspergillus* sp.

Diethyl sulfite in concentration of 7.38 grams/liter is found to be toxic to the spores of the white mold, and its toxicity is markedly increased by addition of lactic acid. Infrared absorption spectra studies of mixtures of sodium metabisulfite and sodium lactate indicate no interaction between the sulfite and the lactate ions.

The use of a solution of lactic acid and sodium bisulfite on heating silage in the silo gave considerable reduction in the temperature.

The present paper is the continuation of previous studies carried out on the spores of white mold isolated from silages (Pasiut and DeMarinis, 1958, 1959). This study includes: a) the toxic action of finely powdered commercial sulfur; b) the toxic action of ethyl ester of sulfurous acid or diethyl sulfite; c) the infrared absorption spectra of the mixture of 2.5 per cent sodium metabisulfite and 2.5 per cent sodium lactate; and d) results obtained in field experiments on ensiled grass and corn silage.

PROCEDURES AND RESULTS

The Action of Fine Pulverized Commercial Sulfur

The molds used for the toxicity tests were isolated from moldy silage by standard methods and grown on Sabouraud's agar media. Because water does not wet the finely pulverized commercial sulfur, a gum arabic dispersion was tried.

TABLE 1

The combined effect of a 3 per cent suspension of pulverized commercial sulfur in broth and a 5 per cent solution of lactic acid on the growth of Penicillium *and* Aspergillus *sp.*

Ml of 3% sulfur-suspension added to 15 ml agar media	Ml of 5% lactic acid added to 15 ml agar media	Ratio of concn of sulfur-suspension to lactic acid in g/liter in agar media	Effect on thegrowth of molds
3	4	4.09/9.10	Inhibited growth for 8 days
3	3	4.29/7.15	Complete growth in 36 hr
3	2	4.50/5.00	"
3	1.5	4.62/3.85	"
3	1.0	4.73/2.63	"
3	0.75	4.80/2.00	"
3	0.5	4.87/1.35	"
0	1.5	4.53	"
0	2.0	5.88	"
0	3.0	8.34	Inhibited growth for 8 days

A 3 per cent sulfur suspension was prepared in a 3 per cent gum arabic aqueous medium. From this suspension, samples ranging from 0.1 ml to 4 ml were added to each 15 ml agar medium. Each tube was then inoculated with 0.2 ml of spores suspended in broth, then poured into petri dishes. The mold colonies completely

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covered the dishes within 48 hr of incubation. In these experiments it was found that the toxic effect of the pulverized commercial sulfur was relatively low. A possible explanation for this might be that the sulfur was in a very low oxidized state.

Next were prepared a 3 per cent suspension of pulverized commercial sulfur in standard broth medium, and a 5 per cent lactic acid solution. The suspension and the solution were mixed in varying proportions, as indicated in table 1. Commercial sulfur did not show any inhibitory action of mold growth except for the positive effect of the first and last concentrations listed. In both cases, the effect is mainly due to the inhibitory action of high concentrations of lactic acid as reported earlier by Pasiut and DeMarinis (1958). These results are not contradictory with the findings of Williams and Young (1929) and Young and Williams (1928) because they prepared hydrophilic sulfur, while here, finely ground commercial sulfur was used.

The Inhibiting Effect of Diethyl Sulfite

Diethyl sulfite was selected for its property of slow decomposition in an aqueous solution into ethyl alcohol and sulfurous acid. A 2.44 per cent aqueous solution

TABLE 2

The effect of diethyl sulfite, $(C_2H_5)_2SO_3$*, on the growth of molds of* Penicillium *sp. and* Aspergillus *sp.*

Ml of 2.44% sol. of $(C_2H_5)_2SO_3$ added to 15 ml agar media	Grams/liter of diethyl sulfite in agar media	Effect on growth of molds (approx no. of colonies per 65 cm²)					pH
		24 hr	48 hr	56 hr	72 hr	8 days	
6.5	7.38	——	——	——	——	——	3.8
6.0	6.98	——	——	——	complete growth (30 colonies)		3.8
5.5	6.53	——	——	——	complete growth (110 colonies)		4.0
5.0	6.10	——	——	——	complete growth (530 colonies)		4.0
4.5	5.63	——	——	complete growth (approx 800 colonies)			4.2
4.0	5.14	——	——	complete growth (approx 800 colonies)			4.2
3.5	4.62	——	——	complete growth (approx 800 colonies)			4.4
3.0	4.07	——	——	complete growth (approx 800 colonies)			4.6
2.5	3.48	complete growth (approx 800 colonies)					4.8
2.0	2.87	—— complete growth (approx 800 colonies)					4.8

of diethyl sulfite is approximately a saturated solution at room temperature (21 C). Cultures were prepared in the same manner as above and results were obtained as shown in table 2. A concentration of 7.38 g/liter of diethyl sulfite in nutrient agar media was found to be the most toxic. The media remained free of mold for 8 days. Lower concentrations of diethyl sulfite reduced the number of colonies, but at the same time enhanced the size of these colonies, indicating that some sort of selective process was going on (see table 3).

Tests were made to determine the combined effect of diethyl sulfite and lactic acid on the growth of these molds. Aqueous solutions of 2.44 per cent diethyl sulfite and 0.94 per cent lactic acid were prepared and mixed in varying proportions in agar media in the same manner as above. Results are listed in table 3. A

concentration ratio of 3.48 g/liter diethyl sulfite to 1.34 g/liter lactic acid was sufficiently toxic to inhibit the growth of these molds for 8 days. Similar inhibitory effect required a concentration of 7.38 g/liter of diethyl sulfite, which is approximately twice the concentration of diethyl sulfite used in the preceeding mixture (table 2). Equivalent inhibitory effect requires a concentration of 8.34 g/liter of lactic acid (see table 1), which is approximately six times the concentration of lactic acid as that used in combination with diethyl sulfite. These results seem to indicate that the addition of the lactate ion to these inhibitory reagents markedly increased their toxicity. This effect was also observed in the earlier experiments (Pasiut and DeMarinis, 1958, 1959), where the addition of sodium lactate (0.78 g/liter) to sodium metabisulfite (0.78 g/liter) markedly increased the toxic action

TABLE 3

The combined effect of 2.44 per cent diethyl sulfite, $(C_2H_5)_2SO_3$, and 0.94 per cent lactic acid concentrations on the growth of the molds Penicillium *sp. and* Aspergillus *sp.*

Ml of 2.44% sol. $(C_2H_5)_2SO_3$ added to 15 ml agar media	Ml of 0.94% sol. of lactic acid added to 15 ml agar media	Ratio of concn of $(C_2H_5)_2SO_3$ and lactic acid in g/liter in agar media	Effect on growth of molds during 8 days of incubation	pH
6.0	6.0	5.42/2.08	No growth	2.8
5.5	5.5	5.16/1.98	"	2.8
5.0	5.0	4.88/1.88	"	2.9
4.5	4.5	4.57/1.76	"	3.0
4.0	4.0	4.24/1.63	"	3.0
3.5	3.5	3.88/1.49	"	3.2
3.0	3.0	3.48/1.34	"	3.4
2.5	2.5	3.05/1.17	growth (approx 210 colonies/65 cm²)	3.6
2.0	2.0	2.57/0.99	growth (approx 600 colonies/65 cm²)	3.8

of the latter. It occurred to us that perhaps sodium metabisulfite and sodium lactate might interact in solution to form an addition compound which is more toxic than either sodium metabisulfite or sodium lactate. This possibility was tested by examining a mixture of sodium metabisulfite and sodium lactate by means of infrared absorption spectra.

The Infrared Absorption Spectra of the Mixture of a 2.5 per cent Sodium Metabisulfite and 2.5 per cent Lactic Acid

The infrared absorption spectra of sodium metabisulfite as ion was given by Miller and Wilkins (1952).

Frequency (CM–1)	973, absorbance very strong 10.4 μ
Frequency (CM–1)	1060, absorbance very strong 9.45 μ

The infrared absorption spectra of sodium lactate as lactate ion was not at hand so it was run on a 50 per cent sodium lactate solution and gave the following absorbances for the ion.

Frequency (CM–1)	3500, absorbance medium 3.1 μ
Frequency (CM–1)	1590, absorbance very strong 6.3 μ
Frequency (CM–1)	1060, absorbance medium 8.9 μ
Frequency (CM–1)	850, absorbance weak 11.75 μ

The characteristic absorbances are presented in figures 1 and 2. In figure 1, the heavy line represents the absorbance of sodium lactate solution transposed over the dotted line of the absorbance of a mixture of sodium lactate and sodium meta-

bisulfite between (CM–1) 1050 and 775. From this, the unchanged characteristics of sodium bisulfite ion in the mixture can be observed, with its very strong absorbance in 10.4 μ.

In figure 2, the heavy line represents the absorbance of sodium lactate between 4000 and 1400 (CM–1), indicating its medium absorbance at 3500 (CM–1) with 3.1 μ and a very strong absorbance at 1590 (CM–1) with 6.3 μ. The line of absorbance of the mixture of sodium lactate and sodium metabisulfite follows the absorbance line of sodium lactate. It can be stated that, in figure 2, the dominating

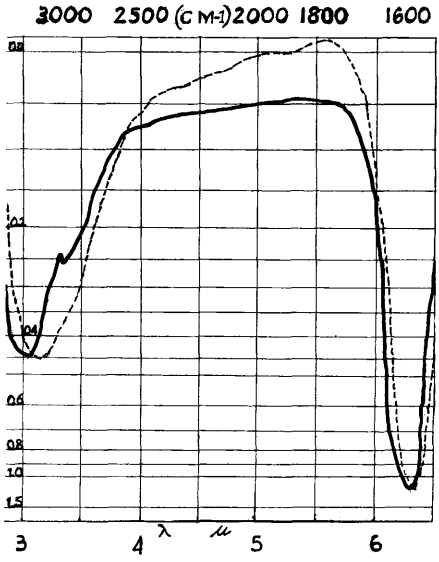

FIG. 1

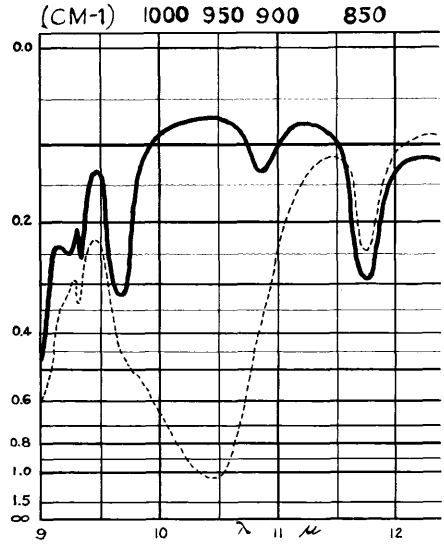

FIG. 2

FIGURE 1. The heavy line represents the absorbance of sodium lactate, the dotted line the absorbance of the mixture of sodium lactate and sodium meta bisulfite. Between (CM–1) 1050 and 775 frequency, strong absorbance of bisulfite at 10.4 μ

FIGURE 2. The heavy line represents the absorbance of sodium lactate, the dotted line the absorbance of a mixture of sodium lactate and sodium meta-bisulfite. Between (CM–1) 4000–1400 frequency, strong absorbance of bisulfite and lactate ion at 6.3 μ.

characteristic was the lactate ion, while in figure 1, the bisulfite ion was most important. This analysis indicates that the two components of the mixture are present as ions in the solution seemingly unaltered.

A Note on the Field Tests

Some field tests were made by applying a mixture of lactic acid and meta-bisulfite solution to fluffy silage (grass and corn) which had begun to show signs of overheating. Overheating is an indication of spoilage caused by unwanted molds and generating ammonia, rendering the silage unpalatable to ruminants. This loss cannot always be controlled by removing the silage more rapidly, as is often done in practice. Field tests have indicated that there is a definite reduction in temperature when applying lactic acid-metabisulfite solution to the surface of the silage, in the ratio of 0.60/0.60 g/liter as determined by earlier laboratory test by Pasiut and DeMarinis (1958). A reduction of approximately 10 F was obtained with this application, from an average of 112 F to an average of 102 F. Bender (1948) proposed a temperature range of 80 to 100 F for suitable silage, but we were not able to achieve this range in these tests.

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