Journal of Industrial Microbiology, 4 (1989) 275–278
Elsevier

SIM 00184

Altered bacterial culture density following exposure to aflatoxins

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Received 3 June 1988
Revised 27 September 1988
Accepted 14 November 1988

Key words: Aflatoxin; Bioassay; Cell growth; Bacterium; Density

SUMMARY

Eight species of bacteria were incubated in culture media containing 10 μg/ml aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), or aflatoxin G_2 (AFG_2). Their culture density at 20°C was determined at four and eight days (d) after inoculation. In all species of bacteria studied (*Bacillus cereus, Proteus mirabilis, Erysipylothrix rusiopathie (insidiosa), Streptococcus fecalis, Staphylococcus epidermis, Klebsiella pneumoniae, Micrococcus* spp., and *Escherichia coli*), AFB_1, AFB_2, and AFG_2 substantially decreased culture sizes at 4 d, but not at 8 d. In *B. cereus* and *P. mirabilis*, culture sizes were increased by AFB_1, AFB_2, and AFG_2 at 8 d post inoculation. These results indicate that AFB_1, AFB_2, and AFG_2 suppressed initial growth of these species in vitro, while later growth in some species was either unaltered or enhanced.

INTRODUCTION

Several methods have been developed to determine the toxicity of mycotoxins. These include animal, plant, and microbial studies. Commonly, organisms are exposed to various concentrations or mixtures of mycotoxins and LD_{50}, LC_{50}, LT_{50}, changes in morphology/development, and reproductive effects are observed. The effects of mycotoxins on microbial cultures have been observed under various experimental conditions. These include change in colony size [1], lysogenic induction [8], antimicrobial activity [5], mutational specificity [11], antibacterial and genotoxic properties [2], blood lymphocyte binding capability [9], bacteria proteolytic activity [10], and toxin metabolism [6].

Changes in bacterial colony characteristics due to sensitivity of specific organisms to mycotoxins are utilized to indicate toxic effects of mycotoxins. These assays appear to be reliable as well as sensitive [3,7,12]. Here, culture density patterns of specific bacteria (all obtained from animal sources) re-

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Table 1

Percent change in relative culture population numbers for microorganisms treated with aflatoxins[1]

Organism	Treatments[2]							
	Control		AFB_1		AFB_2		AFG_2	
	Day 4	Day 8	Day 4	Day 8	Day 4	Day 8	Day 4	Day 8
Bacillus cereus	164.8 ± 2.3	138.7 ± 12.2	128.6 ± 2.4	153.9 ± 1.0	119.6 ± 4.2	166.8 ± 11.9	113.1 ± 1.8	164.7 ± 11.6
Proteus mirabilis	163.8 ± 3.8	138.7 ± 3.3	128.9 ± 2.3	153.9 ± 1.7	114.9 ± 1.3	156.8 ± 1.5	115.4 ± 2.9	160.0 ± 5.4
Erysipelothrix rusiopathie (insidiosa)	161.5 ± 3.4	157.3 ± 9.5	130.7 ± 8.5	155.9 ± 3.1	117.0 ± 0.7	156.8 ± 1.4	111.7 ± 1.6	164.5 ± 10.4
Streptococcus fecalis	155.1 ± 1.8	149.7 ± 1.3	125.5 ± 4.7	155.4 ± 0.7	120.9 ± 8.6	156.1 ± 2.8	111.9 ± 4.8	157.8 ± 0.6
Staphylococcus epidermis	155.9 ± 7.0	154.1 ± 3.8	124.0 ± 1.1	154.2 ± 0.2	116.3 ± 3.1	156.9 ± 1.4	108.1 ± 1.7	156.2 ± 0.3
Klebsiella pneumoniae	145.6 ± 4.8	152.0 ± 0.6	122.4 ± 5.7	157.4 ± 1.9	112.3 ± 3.4	157.8 ± 0.7	107.2 ± 2.6	156.8 ± 1.0
Micrococcus spp.	136.2 ± 6.9	154.8 ± 5.6	119.7 ± 1.0	156.0 ± 0.7	111.0 ± 2.1	155.8 ± 1.8	113.7 ± 7.7	162.7 ± 11.2
Escherichia coli	133.0 ± 2.3	151.5 ± 1.7	118.0 ± 3.7	155.1 ± 2.4	117.8 ± 7.1	157.2 ± 1.8	104.9 ± 2.8	163.9 ± 11.6

[1] Each table value represents the mean ± S.D. ($n = 6$) percent change in initial culture size (each initial culture inoculated on day 1 with 10 μg/ml of respective aflatoxin; culture size on day 1 = 100%).

[2] The relative rate of culture growth was determined by the difference in optical density between pure culture and the cell-free supernatant at 4 days and 8 days post-inoculation.

sulting from exposure to aflatoxins (AFTs) are discussed.

METHODS AND MATERIALS

Bacterial species used in this study were isolated from various animal sources. Pure cultures from nutrient agar plates were inoculated into thioglycollate media (Becton Dickinson and Co., Cockeysville, MD) to maintain a stock culture that was incubated at 20°C.

For the bacterial culture density experiments, inocula from the stock cultures were placed into separate 10% solutions of thioglycollate medium that contained 10 μg/ml AFB_1, AFB_2, or AFG_2. The volume of the inoculum from the stock solution was adjusted to provide an approximate starting culture density of 10^3 cells/ml. Cultures were grown in darkness at 20°C. Culture density was determined by changes in turbidity (660 nm) of a suspended culture on a Bausch and Lomb Model 21 Spectrophotometer and measured against a cell-free supernatant to account for removal of nutrients and release of cellular metabolites which also absorb at 660 nm. The spectrophotometer was calibrated with sterile culture media each day and culture sizes were determined. Within each treatment group, the culture size was expressed as a per cent of initial density.

Data concerning culture density were collected on days one, four, and eight (inoculation of cultures was on day one). This method was used to avoid the more rapid log-phase growth periods at higher temperatures which would obscure all but the most marked differences in culture size in the presence of AFTs.

Data were subjected to statistical analysis utilizing the Standardized Student's t-test. Levels of significance were determined at the $P < 0.05$ level.

RESULTS AND DISCUSSION

All experimental bacterial cultures had some characteristics in common, when their percent change in density in AFT-containing media were compared to those of the control media. In all cases, the percent increase in culture density was significantly lower ($P < 0.001$) in the experimental cultures exposed to aflatoxins (AFTs), when compared to those values found in the controls at four days (Table 1). However, many of these results were reversed at eight days. Table 1 indicates that *B. cereus, P. mirabilis, S. fecalis, K. pneumoniae,* and *E. coli* populations were significantly higher ($P < 0.001$) at eight days than other experimental and control groups with respect to all AFTs tested. *S. epidermis* had significantly higher ($P < 0.01$) populations at eight days when exposed to AFB_2 and AFG_2, but showed no difference in population numbers when exposed to AFB_1. Also, *Micrococcus* spp. showed a significantly higher ($P < 0.001$) population response at eight days to AFG_2, but no difference in response to either AFB_1 or AFB_2 treatments. In addition, no significant differences were observed at eight days with any of the AFT treatments in experimental groups of *E. rusiopathie.*

These experiments indicated that aflatoxins B_1, B_2, and G_2, the major AFT metabolites produced naturally by *Aspergillus flavus* and *A. parasiticus* [4], depressed initial growth rates in all species of bacteria tested at four days and, in general, stimulated growth rates by eight days in most species studied. The bacterial species studied provide a good cross-section of biochemical and morphological characteristics in animal pathogens and commensals. The toxins do not appear to selectively alter any species or particular biochemical or morphological group, as indicated by growth rates in vitro. For example, both *B. cereus,* a Gram-positive rod, and *P. mirabilis,* a Gram-negative enterobacterium, showed increased colony size in the presence of these AFTs at eight days. The causal mechanism for this phenomenon is not known.

The initial reduced density of all species in the presence of AFTs may reflect an adjustment of the bacteria to these conditions. It is possible that these bacteria eventually degraded the AFTs, resulting in normal bacterial colony density. However, it seems unlikely that all species of bacteria tested would degrade these AFTs at similar rates. Periods greater

than four days do not appear to provide useful bioassay information, because the sensitivity of these AFTs appears to diminish with time. Therefore, it appears that AFTs used in this study would not provide acceptable, sensitive, and long-term bioassay results with these bacteria. Indeed, this study indicates that more intensive work needs to be completed before the relationship of bacterial growth responses can be strongly correlated to AFT effects over extended periods.

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