Bacillus subtilis; A Sensitive Bioassay for Patulin

by J. REISS
Mikrobiologisches Laboratorium
Grahamhaus Studt K.G.
655 Bad Kreuznach, West Germany

SUMMARY

The inhibition of the germination of Bacillus subtilis spores by patulin provides a sensitive and simple technique for the microbiological detection of this mycotoxin. The paper disc assay procedure is performed in standardized plastic plates containing agar and spores. Significant inhibition was obtained with as little as 1 µg patulin.

INTRODUCTION

The antibacterial action of mycotoxins (review: REISS, 1973) led to the development of simple techniques for the bioassay of these toxins: Bacillus megaterium is well suited for the detection of aflatoxins (CLEMENTS, 1968 a, b; JAYARAMAN et al., 1968; VIITASALO and GYLLENBERG, 1968; BUCKELEW et al., 1972), Spirillum serpens, Vibrio tyrogenes and Xanthomonas campestris are sensitive to the Fusarium toxin butenolide (BURMEISTER and HESSELTINE, 1970) and Bacillus cereus mycoides to ochratoxins A and B (BROCE et al., 1970). In this paper the use of Bacillus subtilis for a rapid and sensitive detection of patulin is described.

MATERIALS AND METHODS

The test is performed in a standardized, disposable plastic box with cover which is sealed in an air-tight aluminium foil until use. The test plates are obtainable from Bactiastrip S.A., 8702 Zollikon-Zürich (Switzerland) and are originally designed for the detection of antibiotics in milk and cream (KOSIKOWSKI and LEDFORD, 1960). The plate contains

Bulletin of Environmental Contamination & Toxicology,
Vol. 13, No. 6 © 1975 by Springer-Verlag New York Inc.

a layer of agar (Difco) with addition of NaCl to which a standardized "Subtilis Spore Suspension" (BBL; 1 ml/100 ml agar) has been added.

Constituents of each kit are paper discs (Schleicher & Schüll, ∅ 6 mm) which have been impregnated with a solution of 20% peptone and 20% dextrose and subsequently dried. These discs are soaked with solutions of patulin (Dr. Norstadt, Fort Collins, Colorado) in chloroform in different final concentrations (100, 10, 1 and 0.1 µg/disc). In addition the same concentrations of aflatoxin B_1 and G_1 (Serva, Heidelberg, Germany), rubratoxin B (Makor Chemicals, Jerusalem, Israel) and diacetoxyscirpenol (Dr. Stähelin, Basle, Switzerland) were tested. Control discs contain only 0.1 ml chloroform. One control disc and discs with the different toxin concentrations are placed on the agar surface and the plates are incubated for 16 - 18 hrs (overnight) at 32-35°C. Each plate with the different toxin concentrations was run in quadruple. After the incubation the diameter of the corrected inhibition zones (total diameter inhibition zone less disc diameter) are recorded.

RESULTS AND DISCUSSION

The effect of the mycotoxins tested on the growth of *B. subtilis* is shown in Table 1.

TABLE 1

Inhibitory effect of mycotoxins on *B. subtilis* spores

Toxin	Concentration (µg/disc)	Diameter of corrected inhibition zone (mm)
Control	-	0
Patulin	100	32
	10	20
	1	4
	0.1	0
Aflatoxin B_1	100	5
Aflatoxin G_1	100	0
Rubratoxin B	100	0
Diacetoxy-scirpenol	100	0

Patulin is the most toxic compound to B. subtilis. As little as 1 µg/disc can be detected by this method. Concerning this least detectable concentration the technique described here is in good agreement with the methods given of other authors using other bacteria for mycotoxin biotests. The least detectable concentrations are 1 - 2 µg for aflatoxins with B. megaterium (CLEMENTS, 1968 b; JAYARAMAN et al., 1968) and 1.5 µg for ochratoxin A with B. cereus mycoides (BROCE et al., 1970). The other toxins tested are too insensitive except a weak inhibition by 100 µg/disc aflatoxin B_1. The relative insensitivity of aflatoxin B towards B. subtilis has been reported by EKA (1972). Incubation with control discs gave no results showing that any inhibition of growth is due to the mycotoxin tested and not to the solvent.

The bioassay of patulin with B. subtilis spores can be recommended as a sensitive technique to supplement TLC identification. As it is very simple it can be performed even by untrained persons.

ACKNOWLEDGEMENTS

Author thanks Dr. F. A. Norstadt (Agricultural Research Service, Fort Collins, Colorado) and Dr. H. Stähelin (Sandoz A. G., Basle, Switzerland) for samples of patulin and diacetoxyscirpenol.

REFERENCES

BUCKELEW, A.R.jr., A. CHAKRAVARTI, W.R. BURGE, V.M. THOMAS,jr., and M. IKAWA: J. Agr. Food Chem. 20, 431 (1972).
BROCE, D., R.M. GRODNER, R.L. KILLEBREW, and F.L. BONNER: J.Ass.Off.Anal.Chem. 53,616 (1970).
BURMEISTER, H.R., and C.W. HESSELTINE: Appl. Microbiol. 20, 437 (1970).
CLEMENTS, N.L.: J.Ass.Off.Anal.Chem. 51, 611 (1968 a).
CLEMENTS, N.L.: J.Ass.Off.Anal.Chem. 51, 1192(1968 b).
EKA, O.U.: Z. Allg. Mikrobiol. 12, 593 (1972).
JAYARAMAN, A., E.J. HERBST, and M. IKAWA, J. Am. Oil Chem. Soc. 45, 700 (1968).
KOSIKOWSKI, F.V., and R.A. LEDFORD: J. Am. Vet. Med. Ass. 136, 297 (1960).
REISS, J.: Z. Lebensmittelunters. -forsch. 151, 256 (1973).
VIITASALO, L., and H.G. GYLLENBERG: Lebsnsmittel-Wiss.u. Technol. 2, 113 (1968).