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Antifungal activity of bacitracin and its interaction with metals

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Abstract. Bacitracin was more growth-inhibitory to *Neurospora crassa* on a minimal magnesium medium than on a normal magnesium-medium. Both magnesium and manganese were able to counteract the growth inhibition. The antifungal activity of bacitracin was potentiated by zinc. Potassium could not counteract the growth inhibition by this antibiotic. The mycelial magnesium levels were low in bacitracin-inhibited cultures.

Keywords. *Neurospora crassa;* bacitracin; peptide antibiotics.

Introduction

Metals like zinc, nickel, manganese and cobalt chelate with the antibiotic, bacitracin (Anker *et* al., 1948; Gross *et al.*, 1954; Weinberg, 1958; Garbutt *et al.*, 1961). The antibacterial activity of bacitracin was enhanced by cadmium and zinc both singly and in combination and occasionally by manganese (Adler and Snoke, 1962). It was also reported that chelation with copper inactivated bacitracin (Sharp *et al.*, 1949). These studies indicated that chelation with metals by bacitracin was related to its anti-bacterial activity. The primary mode of action of bacitracin is probably by the impairment of permeability of the membranes of sensitive cells, resulting in the leakage of potassium ions (Hancock and Fitz-James, 1964). This paper reports the antifungal activity of bacitracin and the effect of metals on it.

Methods

Organism, media and growth conditions

A wild-strain of *Neurospora crassa* Em5297a maintained by weekly subcultures on 2% agar slants, was grown in 50 ml Corning conical flasks containing 10 ml of basal medium (Sivarama Sastry *et al.*, 1962). Where necessary, bacitracin was aseptically added to the medium before inoculation with spores. The organism was allowed to grow at 30 ± 1 °C for 72 h and the dry weights of the mycelial pads were obtained after drying them at 70-80°C for 8 h.

Estimation of magnesium and bacitracin

The mycelial magnesium content was determined by a microbiological procedure (Sivarama Sastry *et al*., 1963). Bacitracin was estimated by Pauly's diazo method (Block *et al*., 1966) in order to determine the antibiotic either bound to the mycelia or taken up by the organism.

Chemicals

Bacitracin (Calbiochem) was a gift sample from Professor L. K. Ramachandran. Analytical grade sulphate salts of magnesium, manganese and zinc were employed in these studies.

Results

Effect of bacitracin on the growth of N. crassa

The antifungal activity of bacitracin tested with *N. crassa* is depicted in figure 1. The growth inhibition by the antibiotic was examined using two types of media:

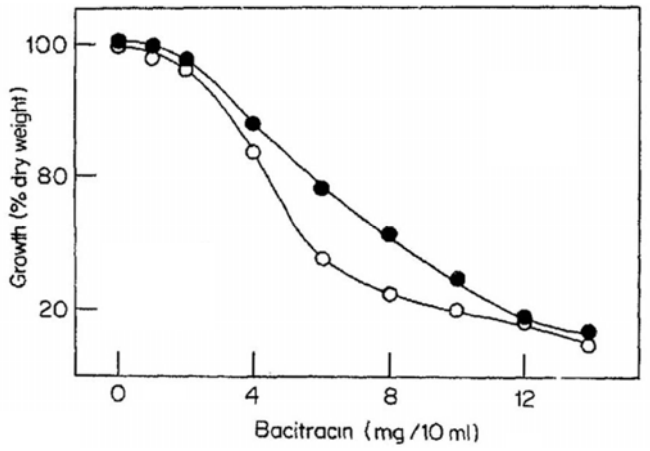

Figure 1. The effect of bacitracin on the growth of *N. crassa* Em5297a. Toxicity on normal magnesium medium(●); toxicity o n minimal magnesium medium (O).

(i) a minimal magnesium medium (50 µg Mg^{2+} /10 ml) and (ii) a normal magnesium medium (500 µgMg_{2+} /l0 ml) (Sivarama Sastry *et al*., 1962). The antibiotic appeared to be more inhibitory on the minimal magnesium medium, requiring only 5 mg/10 ml to produce 50% inhibition of growth as compared to 7.5 mg/10 ml to produce the same inhibition on the latter medium.

Effect of metals on the antifungal activity of bacitracin in N. crassa on minimal magnesium medium

The results presented in figure 2 indicate that both excess magnesium and manganese could counteract the toxicity of bacitracin. Magnesium was able to protect the organism at a molar ratio of bacitracin to magnesium of 1:2, whereas the ratio for manganese was 20:1. (to obtain 100% growth restoration, only 250 µg

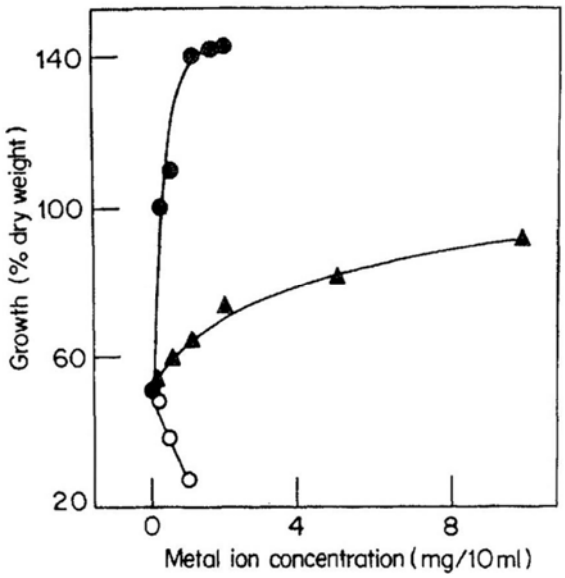

Figure 2. The effect of metals on the growth inhibition of *N. crassa* Em5297a by bacitracin on minimal magnesium/zinc medium, manganese (●), zinc (O), magnesium (▲)

manganese/10 ml was required to counteract the growth inhibition by 5 mg of bacitracin). This observation highlighted that manganese was much more efficient in reversing the growth inhibition as compared to magnesium. On the other hand, zinc could not alleviate the growth inhibition but also potentiated the antifungal activity of bacitracin. This metal., at a concentration of 1000 µg/10 ml, further reduced the growth of the organism (roughly 20% growth) in the presence of bacitracin (5 mg/10 ml medium).

Table 1. The uptake of magnesium and its effect on bacitracin content of mycelia

Bacitracin (mg/10 ml medium)	Mg^{2+} or Mn^{2+} (µg/10 ml medium)	Uptake (mg/100 mg dry wt)	
		Mg^{2+}	Bacitracin
Nil	Nil	0.085	Nil
5.0	Nil	0.040	17.0
5.0	2000 Mg^{2+}	0.080	9.1
5.0	250 Mn^{2+}	–	8.3

The organism was grown for 72h on minimal medium. Magnesium or manganese were added to the medium where necessary along with bacitracin.

Effect of bacitracin on mycelial magnesium uptake

Magnesium was antagonistic to the action of bacitracin. The content of this metal in the antibiotic-inhibited mycelia was reduced to nearly 52%. However, the mycelial magnesium content was restored to the normal level when the inhibition was relieved by this divalent cation (table 1).

The results presented in table 1 also indicate that nearly 72% of the bacitracin added to the medium was either bound to the organism or entered the cells. But such a binding or entry of the antibiotic was drastically lowered (50% of that of toxic cultures) in the presence of excess magnesium or manganese.

Discussion

The antifungal activity of peptide antibiotics has been reported by Chalmers (1974) and Al-Saqur (1975). Bacitracin is toxic to *N. crassa*; a 50% growth inhibition being produced at a concentration of 5 mg/10 ml on a minimal magnesium medium. The inhibitory concentration obtained in this study is in close agreement with the value of Al-Saqur (1975) who reported that about 0.5 mg bacitracin/ml could inhibit the growth of N. crassa by 55%. The present studies also prove that more bacitracin is required to produce the same inhibition of growth on a normal magnesium medium. This antagonistic relationship between bacitracin and magnesium is more evident on a minimal magnesium medium. A comparison was made by Haavik (1976) between the toxicities of certain divalent metals and bacitracin in bacteria, where both types of toxicities were counteracted by excess magnesium. Such an antagonism between magnesium and other toxic metals like cobalt, nickel, etc. is also well-documented in *N. crassa* (Sivarama Sastry *et al.*, 1962; Venkateswerlu and Sivarama Sastry, 1973).

Divalent metals like zinc, cadmium and manganese were reported to enhance the antibacterial activity of bacitracin (Adler and Snoke, 1962). The present studies indicated that zinc enhanced the antifungal activity of bacitracin like its antibacterial activity. This metal ion is known to produce a conditioned deficiency of magnesium in fungi (Adiga *et al*., 1961; Sivarama Sastry *et al.*, 1962); obviously the increased toxicity of bacitracin to *N. crassa* in the presence of zinc was probably due to increased magnesium deficiency. Excess supplementation of phosphate in the medium was found to be ineffective either in the reversal or in the enhancement of the toxicity (results not presented). It was confirmed that in the presence of relatively high but non-toxic amounts of manganese or cobalt, small amounts of bacitracin significantly inhibited the growth of *Bacillus leicheniformis* (Haavik, 1976). Earlier Adler and Snoke (1962) observed that manganese also enhanced the potency of bacitracin. However, the results of the present investigation are not in complete conformity with the above observation, since, the antifungal activity of this antibiotic is enhanced by zinc but not by manganese; on the other hand manganese behaved like magnesium in antagonizing the toxicity. The mechanism of reversal by manganese of the antibiotic toxicity in this mold is not clear at present. One possibility is that it may be substituting for magnesium as in many other cases.

Hancock and Fitz-James (1964) reported that bacitracin interferes with cell-membrane permeability resulting in the leakage of potassium ions. However, in preliminary studies no such interference was found to occur since excess potassium (checked upto 4000 µg K^+ /10 ml) supplementation could not protect the organism from the bacitracin toxicity.

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