Antimicrobial Activities and Antagonists of Bacilysin and Anticapsin

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SUMMARY

The dipeptide antibiotic bacilysin is active against a wide range of bacteria and against *Candida albicans*. Its C-terminal amino acid, anticapsin, is a very poor antibacterial agent. The activities of both substances are strongly dependent on the nature of the culture medium. In a minimal medium the minimum inhibitory concentration for bacilysin with *E. coli* B is 10^{-3} $\mu\text{g ml}^{-1}$. The action of bacilysin is antagonized by a variety of dipeptides and that of anticapsin by a number of amino acids. With several bacteria, bacilysin-resistant mutants are found in unusually large numbers. It is suggested that peptide and amino acid transport systems play a role in these phenomena. The antimicrobial action of bacilysin is also inhibited by glucosamine and *N*-acetylglucosamine. This antibiotic may therefore interfere with glucosamine synthesis and thus with the synthesis of microbial cell walls.

INTRODUCTION

An antibiotic produced by a strain of *Bacillus subtilis* was found by Abraham, Callow & Gilliver (1946) to cause partial lysis of growing cultures of *Staphylococcus aureus* (NCTC6571). This substance, named bacilysin (Abraham & Florey, 1949), was later isolated from culture filtrates of *B. subtilis* A14 (Rogers, Newton & Abraham, 1965; Walker & Abraham, 1970*a*) and shown (Walker & Abraham, 1970*b*) to be a dipeptide with the structure I (Fig. 1). The culture filtrates also contained a new amino acid (II; Fig. 1), named AA1, which was identical with the C-terminal amino acid of bacilysin but did not show the antistaphylococcal activity of the latter (Walker & Abraham 1970*b*). Whitney *et al.* (1970) detected in the culture fluid of a strain of *Streptomyces griseoplanus* NRRL3507 a substance which they named anticapsin because it inhibited the formation of the hyaluronic acid capsule of group A streptococci. Anticapsin was isolated by Shah *et al.* (1970) and shown by Neuss *et al.* (1970) to have the structure II. Anticapsin was thus identical with the C-terminal amino acid of bacilysin.

This paper is concerned with the antimicrobial activities of bacilysin and anticapsin and with the action of antagonists, some of which may inhibit the transport of these antibiotics into microbial cells.

METHODS

Organisms. *Staphylococcus epidermidis*, *Serratia marcescens* and *Escherichia coli* type I (a rough laboratory strain) were obtained from Dr Vollum, Radcliffe Infirmary, Oxford. *Escherichia coli* B was obtained from Sir Paul Fildes and came originally from the Pasteur Institute, Paris. *Salmonella gallinarum* was from the Lilly Research Laboratories, Indianapolis, Indiana, U.S.A. *Salmonella typhi* (Mrs S.) was the strain described by

Fig. 1. Structure of bacilysin, I, and amino acid AA1 (anticapsin), II.

Felix & Pitt (1935). *Candida albicans* was strain NCYC597. Other organisms used are described in the text.

Reagents. D-Glucosamine hydrochloride and *N*-acetyl-D-glucosamine were from BDH; amino acids were also from BDH, except L-leucine, L-phenylalanine, D-phenylalanine and D-methionine which were from Sigma. Dipeptides and tripeptides were from Sigma except L-alanylglycine (Koch-Light) and triglycine (BDH). Nicotinic acid and thiamine pyrophosphate chloride were from BDH and D-biotin from Sigma.

Preparation and assay of bacilysin. Bacilysin was prepared from culture filtrates of *B. subtilis* A14 by the method of Walker & Abraham (1970*a*). The antibiotic was assayed by the hole-plate method (Brownlee *et al.*, 1948) with *Staphylococcus aureus* (Oxford) NCTC6571 grown on Lab-Lemco agar as the test organism. The bacilysin concentration in a solution is expressed in units/ml; these units are the units of cephalosporin C sodium salt (8 units per mg) required per ml to give a zone of inhibition of the same diameter. The specific activity of pure bacilysin was 250 units/mg (Walker & Abraham, 1970*a*).

Determination of sensitivity of organisms. The sensitivity of organisms to bacilysin and anticapsin and the reversal of their action by antagonists was determined by the hole-plate method (Brownlee *et al.*, 1948).

Chemically-defined media. Minimal medium contained (g/l distilled water): $\text{NaNH}_4\text{HPO}_4$ (microcosmic salt), 1.5; KH_2PO_4 , 1; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2; glucose, 2. For minimal agar, Oxoid No. 1 or No. 3 agar (12 g l⁻¹) was added. For *Candida albicans* the minimal medium contained biotin (2.5 mg l⁻¹) and (unless otherwise stated) sodium glutamate (1.5 g l⁻¹) instead of microcosmic salt as the source of nitrogen.

Dulbecco agar was prepared by the addition of 100 ml of ten times concentrated Dulbecco's minimum essential medium (Bio-cult Laboratories, Glasgow) to 900 ml liquid (1.4 %, w/v) Oxoid No. 3 agar containing 0.196 % NaHCO_3 at 48 °C.

Undefined media. Lab-Lemco agar was prepared from (g/l distilled water): Oxoid Lab-Lemco extract, 10; peptone (Evans Medical Ltd, Speke, Liverpool), 10; NaCl, 5; Oxoid No. 3 agar, 20. The pH was adjusted to 6.8. The agar was melted, and a solution of phenol red (0.5 %, 5 ml) and phosphate buffer pH 6.8 was added.

Nutrient broth was Oxoid No. 2.

Sabouraud agar contained (g l⁻¹): Oxoid mycological peptone, 10; glucose, 40; D-biotin, 0.0025; Oxoid No. 3 agar, 20. Sabouraud liquid medium was identical except that it contained no agar.

Growth in liquid media. Bacteria were grown aerobically, with shaking at 37 °C in inverted T-tubes (Kay & Fildes 1950). *Candida albicans* was grown similarly at 30 °C. The extinction of the culture was measured (after dilution if necessary) in a Spekker absorptiometer (Adam Hilger Ltd) with a neutral-grey filter (H508) and a 1.8 cm light path.

Minimum inhibitory concentrations (m.i.c. values). The m.i.c. values of bacilysin and anticapsin for *E. coli* were measured by use of serial twofold dilutions of the antibiotic. The inoculum was prepared from an overnight brain-heart infusion agar slope culture by dilution in minimal medium. The mixture in minimal medium contained about 10^5 to 10^6 bacteria/ml, plus bacilysin at concentrations which ranged from 0.0313 to 0.000125 $\mu\text{g ml}^{-1}$. These tubes, together with controls, were incubated for 48 h at 37 °C. The lowest concentration of bacilysin which prevented visible growth was taken as the m.i.c.

The proportion of bacilysin-resistant bacteria in cultures of Staph. aureus. Dilutions of the culture were spread over the surface of Lab-Lemco agar plates with bacilysin (40 $\mu\text{g ml}^{-1}$) or without it. The plates were incubated for 20 h at 37 °C and the colonies counted.

RESULTS

Antimicrobial activities of bacilysin and anticapsin on solid media

On Lab-Lemco agar, bacilysin (300 $\mu\text{g ml}^{-1}$) gave inhibition zones (often > 30 mm diam) against the following organisms: *Staph. aureus* (Oxford), *Staph. epidermidis*, *Micrococcus tetragenus* NCTC7501, *Corynebacterium xerosis* NCTC7243, *Bacillus megatherium* de Bary, *Sarcina lutea* NCTC8340, *Salm. typhi*, *Salm. gallinarum*, *Ser. marcescens* and *Proteus vulgaris* NCTC4636. On minimal agar *E. coli* B and *E. coli* type I were highly sensitive to bacilysin, but *B. subtilis* A14 (by which it is produced) and *Pseudomonas aeruginosa* NCTC8203 were resistant.

In contrast with bacilysin, anticapsin had no detectable activity at a concentration of 1 mg ml^{-1} against *Staph. aureus*, *Salm. typhi* and *Salm. gallinarum*, organisms which could only be grown in a relatively rich medium, but showed some activity against *E. coli* B (Table 1) and *E. coli* type I, growing in minimal medium. With *E. coli* B, 2×10^3 times as much anticapsin as bacilysin was required to produce a zone of the same diameter (Table 1).

When tested on Sabouraud agar against *C. albicans*, bacilysin produced a large zone of inhibition (diameter 43 mm) at 80 $\mu\text{g ml}^{-1}$, but on minimal agar its activity was much higher (Table 1). Anticapsin on minimal agar was about one-sixth as active as bacilysin (Table 1).

In contrast to many other antibiotics, bacilysin produced zones of inhibition against several organisms which contained numerous isolated colonies. With *Staph. aureus* (in Lab-Lemco agar), *Salm. typhi* (Lab-Lemco) and *C. albicans* (in Sabouraud and minimal agar), colonies which grew within zones of inhibition due to bacilysin gave cultures which were completely resistant to the same concentrations of bacilysin. Determination of the proportion of resistant bacteria in two resting-phase cultures of *Staph. aureus* at bacilysin concentrations of 40 $\mu\text{g ml}^{-1}$ gave values of 1.8×10^{-6} and 8.5×10^{-6} . In contrast, no resistant colonies were obtained with *E. coli* B or *E. coli* type I on minimal agar with either bacilysin or anticapsin. Bacilysin-resistant colonies of *C. albicans* on minimal agar were resistant to anticapsin, and vice versa.

Lysis and emergence of resistant organisms in liquid media

Following the addition of bacilysin (final concentration 40 $\mu\text{g ml}^{-1}$) to a culture of *Staph. aureus* or *Salm. typhi* the extinction of both cultures continued to increase for about 1.5 h and then fell, due to lysis. A renewed increase in extinction was observed after 8 h for *Staph. aureus* and 10 h for *Salm. typhi*. The changes in extinction of *Staph. aureus* in the presence of bacilysin at 2.4 $\mu\text{g ml}^{-1}$ (8.9 μM) and 24 $\mu\text{g ml}^{-1}$ (89 μM) are shown in Fig. 2. Similar changes were observed when bacilysin at 40 $\mu\text{g ml}^{-1}$ was added to *C. albicans* growing in Sabouraud's medium, or when either bacilysin (1 $\mu\text{g ml}^{-1}$) or anticapsin (10 $\mu\text{g ml}^{-1}$) was added to this organism growing in minimal medium. On reaching the stationary phase the cultures

Table 1. *Activity of bacilysin and anticapsin against E. coli B and C. albicans* NCYC597 on minimal agar

Escherichia coli zones of inhibition were measured after incubation at 37 °C overnight; *C. albicans* incubation was at 28 °C for 48 h.

	Concentration		Diameter of inhibition zone (mm)	
	$\mu\text{g ml}^{-1}$	μM	<i>E. coli</i>	<i>C. albicans</i>
Bacilysin	1.0	3.7		45
	0.5	1.85		38
	0.25	0.92	24	32
	0.125	0.46	19	26
	0.062	0.23	16	
Anticapsin	500	2500	28	
	250	1250	23	
	125	625	16	
	4	20		46
	2	10		37
	1	5		28
	0.5	2.5		23

consisted largely, if not entirely, of bacilysin-resistant organisms when tested by the hole-plate method.

In contrast, the partial lysis of a culture of *E. coli* B which occurred in minimal medium after the addition of bacilysin was not followed by observable growth during more than 24 h (Fig. 2). Hence, m.i.c. values could be determined in liquid culture. After 48 h the m.i.c. values for bacilysin were 0.008 and 0.001 $\mu\text{g ml}^{-1}$ with inocula of 8×10^5 and 8×10^4 bacilli/ml respectively. Minimum inhibitory concentrations for anticapsin were 250 and 62 $\mu\text{g ml}^{-1}$ with inocula of 7×10^5 and 7×10^4 bacilli/ml respectively.

Antagonists of the action of bacilysin and anticapsin

Peptides. Bacilysin appeared to be at least five times as active against *Staph. aureus* NCTC6571 on Dulbecco agar as on Lab-Lemco agar. Since the Dulbecco medium contains free amino acids but no peptides, it seemed possible that the presence of peptides in the Lab-Lemco medium was responsible for the lower activity of the dipeptide bacilysin in the latter.

A number of dipeptides inhibited, to different degrees, the activity of bacilysin against *Staph. aureus* on Dulbecco agar and *E. coli* B on minimal agar (Table 2). When *E. coli* B was grown in the presence of 3.7 μM -bacilysin and the concentration of L-alanyl-L-phenylalanine was increased stepwise from 0.125 to 4 mM, inhibition was first detected when the molar ratio of the dipeptide to bacilysin was about 50:1 and approached completion when it was about 150:1. L-Alanyl-L-phenylalanine also inhibited the action of anticapsin (5 mM) against *E. coli* B, although here the maximum inhibition observed (with 4 mM-dipeptide) was 57 %. In a concentration of 15 mM it completely inhibited the activity of both bacilysin (0.37 μM) and anticapsin (125 μM) against *C. albicans*.

Amino acids. In contrast to the results obtained with dipeptides, none of 17 L-amino acids (5 mM) caused a substantial reduction in the activity of bacilysin (7.4 μM) against *E. coli* B (Table 3), but more than half caused a major decrease in the activity of anticapsin (5 mM). The three D-amino acids were much less effective as antagonists than were the corresponding L-forms. In minimal glutamate medium, L-phenylalanine (15 mM) eliminated the action of anticapsin (125 μM) against *C. albicans*, but had no effect on the action of bacilysin (0.37 μM).

Fig. 2

Fig. 3

Fig. 2. Effect of bacilysin on growing cultures of *Staph. aureus* NCTC6571 in nutrient broth and of *E. coli* B in minimal medium at 37 °C. Bacilysin was added at the points indicated by the arrows. ●, Control culture of *Staph. aureus*; ■, *Staph. aureus* with 8.9 µM-bacilysin; ▲, *Staph. aureus* with 89 µM-bacilysin; ○, control culture of *E. coli*; △, *E. coli* with 74 µM-bacilysin.

Fig. 3. Inhibition by glucosamine of the effect of bacilysin on *Staph. aureus* NCTC6571 in nutrient broth at 37 °C. Bacilysin (final concentration 81 µM) and glucosamine were added at the time indicated by the arrow. ●, 400 µM-glucosamine; ○, 100 µM-glucosamine; ▲, 10 µM-glucosamine.

Table 2. Reversal of the action of bacilysin against
Staph. aureus and *E. coli* by peptides

Dulbecco agar plates were seeded with *Staph. aureus* NCTC6571 and minimal agar plates with *E. coli* B. For determination of activities against *Staph. aureus* the concentration of bacilysin was 29.6 µM and that of each peptide was 6 mM. With *E. coli* B the corresponding concentrations were 3.7 µM and 1 mM. Zone diameters were read after incubation overnight at 37 °C and compared with those produced by standard solutions of bacilysin alone.

Peptide	Decrease in bacilysin activity (%) in the presence of peptide against:	
	<i>Staph. aureus</i>	<i>E. coli</i>
L-Alanyl-L-phenylalanine	> 75	> 91
L-Alanylglycine	49	> 93
L-Alanyl-L-alanine	> 73	> 95
L-Alanyl-L-serine	36	89
L-Alanyl-L-methionine	> 75	91
L-Alanyl-L-leucine	> 75	> 93
Glycylglycine	< 14	82
Glycyl-L-leucine	66	> 93
L-Leucyl-L-phenylalanine	> 73	> 94
L-Phenylalanyl-L-alanine	41	> 94
L-Alanyl-L-tyrosine	71	
L-Tyrosyl-L-tyrosine	60	
Triglycine	14	29
Tri-L-phenylalanine		65

Table 3. *Reversal of the action of bacilysin and anticapsin against E. coli by L- and D-amino acids*

Activities were determined by the hole-plate method on minimal agar plates seeded with *E. coli* B. The solutions tested contained bacilysin alone, anticapsin alone, the amino acid alone, and mixtures of bacilysin ($7.4 \mu\text{M}$) or anticapsin (5 mM) with each of the amino acids. The amino acid concentration was 5 mM , except that of L-cystine which was 2.5 mM .

Amino acid	Decrease in activity (%)	
	Bacilysin	Anticapsin
L-Alanine	< 16	43
D-Alanine		24
L-Arginine-HCl	< 16	< 14
L-Aspartic acid	16	< 14
L-Cystine	< 16	14
L-Sodium glutamate	16	34
Glycine	16	24
L-Histidine-HCl. H_2O	< 16	< 14
L-Leucine	< 16	89
L-Isoleucine	< 16	84
L-Lysine-HCl	< 16	< 14
L-Methionine	< 16	87
D-Methionine		12
L-Phenylalanine	16	87
D-Phenylalanine		14
L-Proline	< 16	14
L-Serine	< 16	62
L-Threonine	< 16	86
L-Tryptophan	< 16	< 14
L-Valine	< 16	81

Ammonium ions. When sodium glutamate replaced $\text{NaNH}_4\text{HPO}_4$ as the nitrogen source for the growth of *C. albicans* in minimal agar, the inhibition zones produced by bacilysin ($0.2 \mu\text{g ml}^{-1}$) and anticapsin ($50 \mu\text{g ml}^{-1}$) increased in diameter from 25 to 35 mm and from 35 to 41 mm, respectively.

Glucosamine and N-acetylglucosamine. When bacilysin ($81 \mu\text{M}$) and glucosamine ($400 \mu\text{M}$) were added together to a culture of *Staph. aureus*, no lysis was observed and, after growth had come to an end, the culture was still bacilysin-sensitive; with $100 \mu\text{M}$ - and $10 \mu\text{M}$ -glucosamine there was partial lysis and the final cultures were bacilysin-resistant (Fig. 3). Addition of glucosamine (1.0 mM) 2 h after bacilysin ($81 \mu\text{M}$), when lysis had already begun, was followed by further lysis and then by growth to give a culture containing a higher proportion of bacilysin-resistant organisms than the original culture.

The activity of bacilysin against *Staph. aureus*, *Salm. typhi*, and *C. albicans* on Lab-Lemco agar was reduced by more than 95 % by the incorporation of glucosamine (4.6 mM) into the agar. The presence of $0.5 \mu\text{M}$ -*N*-acetylglucosamine in minimal agar caused a decrease in bacilysin activity against *E. coli* B of about 65 %.

Glucosamine and *N*-acetylglucosamine produced zones of growth on Lab-Lemco and minimal agar plates seeded with *Staph. aureus* or *E. coli* type 1 and containing bacilysin in inhibitory concentrations. The diameters of such zones indicated that *N*-acetylglucosamine was about twice as effective as glucosamine in reversing the action of bacilysin against *Staph. aureus*, and more than 1000 times as effective as glucosamine in reversing it against *E. coli* type 1. *N*-acetylglucosamine was also much more effective than glucosamine in reversing the action of anticapsin in minimal agar seeded with *E. coli* B.

DISCUSSION

Bacilysin is active against a wide range of bacteria and against *C. albicans*, but its activity is strongly dependent on the composition of the culture medium. Against *E. coli* B in a minimal medium its activity is very high (m.i.c. 10^{-3} $\mu\text{g ml}^{-1}$). Anticapsin is a poor antibacterial agent, but shows detectable activity against *E. coli* and *C. albicans* in simple media. It is inactive against organisms which require a relatively rich medium for growth.

The findings that a variety of dipeptides and amino acids antagonize the action of bacilysin and anticapsin raise the question whether the antagonism is due to a competition for transport systems. In *E. coli* there appear to be separate transport systems for dipeptides and oligopeptides (Payne & Gilvarg, 1968; Barak & Gilvarg, 1975) and for amino acids and peptides (Cohen & Rickenberg, 1956). Some amino acids appear to share an uptake system of rather broad specificity, and it is of interest that there is a similarity between the ability of certain monoamino monocarboxylic acids to inhibit the action of anticapsin (Table 3) against *E. coli* B and to inhibit the uptake of [^{14}C]homoserine and [^{14}C]threonine by this organism (Templeton & Savageau, 1974). The fact that anticapsin shows a higher activity against *C. albicans* in a medium containing glutamate than it does in one containing ammonium ions as a source of nitrogen is consistent with reports that ammonium ions inhibit the uptake of amino acids by fungi (Grenson, Hou & Crabeel, 1970; Hunter & Segal, 1973; Robinson, Anthony & Drabble, 1973). Inhibition of the action of anticapsin, as well as of bacilysin, against *E. coli* and *C. albicans* by L-alanyl-L-phenylalanine raises the question whether the dipeptide can use an amino acid transport system in these organisms. Magill *et al.* (1973) found that histidylglycine inhibited the uptake of histidine by *Neurospora crassa*.

Bacilysin has a much higher activity than anticapsin against *E. coli* in a medium containing only ammonium ions as a source of nitrogen, suggesting that the dipeptide, bacilysin, is transported into the organism more efficiently than its C-terminal amino acid, anticapsin. Peptides may be taken up more readily than their constituent amino acids (for references see Barak & Gilvarg, 1975).

Analogy with other studies of peptide transport and peptide-resistant organisms suggests that bacilysin-resistant strains of *Staph. aureus*, *Salm. typhi* and *C. albicans* may arise by mutations in genes concerned with peptide transport. De Felice *et al.* (1973) observed that triornithine-resistant colonies of *E. coli* were defective in their oligopeptide transport system. Barak & Gilvarg (1974) concluded that the frequency of mutation at the locus in *E. coli* for the genes concerned with oligopeptide transport is approximately 100 times higher than the normal frequency of mutation. A possible explanation of the failure to obtain bacilysin-resistant mutants of *E. coli* may lie in the ability of dipeptides to use the oligopeptide transport system of this organism (Payne, 1968).

Inhibition of the action of bacilysin against *Staph. aureus* by glucosamine does not appear to be due to interference with bacilysin transport (Kenig, Vandamme & Abraham, 1976) and, in conjunction with the fact that anticapsin inhibits the glucosamine synthetase of *Strep. pyogenes* and the formation of its hyaluronic acid capsule (Whitney & Funderburk, 1970), raises the possibility that the antimicrobial activity of bacilysin is a consequence of a failure in glucosamine synthesis. The finding that *N*-acetylglucosamine is somewhat more effective than glucosamine as a bacilysin antagonist with *Staph. aureus* as the test organism and very much more effective with *E. coli*, may reflect differences in the uptake of these two substances by the bacteria concerned (Dobrogosz, 1968; Suzuki *et al.*, 1972).

Walton & Rickes (1962) showed that the antibacterial activity of bacillin, an antibiotic of unknown structure produced by *B. subtilis* (Foster & Woodruff, 1946), was reversed by

N-acetylglucosamine. Recent work has indicated that bacilysin, a dipeptide antibiotic tetaine, and bacillin are identical (Kaminski & Sokolowska, 1973; Atsumi, Oiwa & Omura, 1975).

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REFERENCES

- ABRAHAM, E. P., CALLOW, D. & GILLIVER, K. (1946). Adaptation of *Staphylococcus aureus* to growth in the presence of certain antibiotics. *Nature, London* **158**, 818-821.
- ABRAHAM, E. P. & FLOREY, H. W. (1949). Antibiotics from bacteria in the genus *Bacillus*. In *Antibiotics*, vol. 1, p. 457. Edited by H. W. Florey, E. Chain, N. G. Heatley, M. A. Jennings, A. G. Sanders, E. P. Abraham and M. E. Florey. Oxford: Oxford University Press.
- ATSUMI, K., OIWA, R. & OMURA, S. (1975). Production of bacillin by *Bacillus subtilis* sp. strain no. KM-208 and its identity with tetaine (bacilysin). *Journal of Antibiotics* **28**, 77-78.
- BARAK, Z. & GILVARG, C. (1974). Triornithine-resistant strain of *Escherichia coli*: isolation, definition and genetic studies. *Journal of Biological Chemistry* **249**, 143-148.
- BARAK, Z. & GILVARG, C. (1975). Peptide transport. In *Biomembranes*, vol. 7, pp. 167-218. Edited by L. A. Manson. London: Plenum Press.
- BROWNLEE, K. A., DELVES, C. S., DORMAN, M., GREEN, C. A., GRENFELL, E., JOHNSON, J. D. A. & SMITH, N. (1948). The biological assay of streptomycin by a modified cylinder plate method. *Journal of General Microbiology* **2**, 40-53.
- COHEN, G. N. & RICKENBERG, H. V. (1956). Concentration spécifique reversible des amino acides chez *Escherichia coli*. *Annales de l'Institut Pasteur* **91**, 693-720.
- DE FELICE, M., GUARDIOLA, J., LAMBERTI, A. & IACCARINO, M. (1973). *Escherichia coli* K-12 mutants altered in the transport systems for oligo- and dipeptides. *Journal of Bacteriology* **116**, 751-756.
- DOBROGOSZ, W. J. (1968). *N*-Acetylglucosamine assimilation in *Escherichia coli* and its relation to catabolite repression. *Journal of Bacteriology* **95**, 585-591.
- FELIX, A. & PITT, R. M. (1935). Virulence and immunogenic activities of *B. typhosus* in relation to its antigenic constituents. *Journal of Hygiene* **35**, 428-436.
- FOSTER, J. W. & WOODRUFF, H. B. (1946). Bacillin, a new antibiotic substance from a soil isolate of *Bacillus subtilis*. *Journal of Bacteriology* **51**, 363-369.
- GRENSON, M., HOU, C. & CRABEEL, M. (1970). Multiplicity of the amino acid permeases in *Saccharomyces cerevisiae*. *Journal of Bacteriology* **103**, 770-777.
- HUNTER, D. R. & SEGAL, I. H. (1973). Effect of weak acids on amino acid transport by *Penicillium chrysogenum*. *Journal of Bacteriology* **113**, 1184-1192.
- KAMINSKI, K. & SOKOLOWSKA, T. (1973). The probable identity of bacilysin and tetaine. *Journal of Antibiotics* **26**, 184-185.
- KAY, D. & FILDES, P. (1950). The calcium requirement of a typhoid bacteriophage. *British Journal of Experimental Pathology* **31**, 338-348.
- KENIG, M., VANDAMME, E. & ABRAHAM, E. P. (1976). The mode of action of bacilysin and anticapsin and biochemical properties of bacilysin-resistant mutants. *Journal of General Microbiology* **94**, 46-54.
- MAGILL, C. W., NELSON, S. O., D'AMBROSIO, S. M. & GLOVER, G. I. (1973). Histidine uptake in mutant strains of *Neurospora crassa* via the general transport system for amino acids. *Journal of Bacteriology* **113**, 1320-1325.
- NEUSS, N., MOLLOY, B. B., SHAH, R., & DE LA HIGUERA, N. (1970). The structure of anticapsin, a new biologically active metabolite of *Streptomyces griseoplanus*. *Biochemical Journal* **118**, 571-575.
- PAYNE, J. W. (1968). Oligopeptide transport in *Escherichia coli*. *Journal of Biological Chemistry* **243**, 3395-3403.
- PAYNE, J. W. & GILVARG, C. (1968). The role of terminal carboxyl group in peptide transport in *Escherichia coli*. *Journal of Biological Chemistry* **243**, 335-340.
- ROBINSON, J. H., ANTHONY, C. & DRABBLE, W. T. (1973). Regulation of the acidic amino-acid permease of *Aspergillus nidulans*. *Journal of General Microbiology* **79**, 65-80.
- ROGERS, H. J., NEWTON, G. G. F. & ABRAHAM, E. P. (1965). Production and purification of bacilysin. *Biochemical Journal* **97**, 573-578.
- SHAH, R., NEUSS, N., GORMAN, M. & BOECK, L. D. (1970). Isolation, purification and characterisation of anticapsin. *Journal of Antibiotics* **23**, 613-617.
- SUZUKI, J., HORI, M., SAEKI, T. & UMEZAWA, H. (1972). Macarbomycin, an inhibitor of peptidoglycan synthesis. *Journal of Antibiotics* **25**, 94-104.
- TEMPLETON, B. A. & SAVAGEAU, M. A. (1974). Transport of biosynthetic intermediates. *Journal of Bacteriology* **117**, 1002-1009.

- WALKER, J. E. & ABRAHAM, E. P. (1970*a*). Isolation of bacilysin and a new amino acid from culture filtrates of *Bacillus subtilis*. *Biochemical Journal* **118**, 557–561.
- WALKER, J. E. & ABRAHAM, E. P. (1970*b*). The structure of bacilysin and other products of *Bacillus subtilis*. *Biochemical Journal* **118**, 563–570.
- WALTON, R. B. & RICKES, E. L. (1962). Reversal of the antibiotic, bacillin, by *N*-acetylglucosamine. *Journal of Bacteriology* **84**, 1148–1151.
- WHITNEY, J. G. & FUNDERBURK, S. S. (1970). Anticapsin, a new biologically active metabolite. IV. Mechanism of action. *Abstracts for the X International Congress for Microbiology, Mexico City*, p. 101.
- WHITNEY, J. G., FUNDERBURK, S. S., WESTHEAD, J. E., LIVELY, D. H., SOLENBURG, J. M. & DENNEY, J. W. (1970). Anticapsin, a new biologically active metabolite. I. Anticapsular screen and assay. *Bacteriological Proceedings*, 7.