The influence of chitin may result from an increase in the number of mycolytic micro-organisms. Chitin, however, is known to be one of the few substances which markedly increase the actinomycete population[4]. Because actinomycetes are particularly active in antibiotic production, in culture media at least, the content of antifungal substances in chitin-amended soil was determined by the method described previously[5]. The results obtained indicate that the toxicity of aqueous extracts of chitin-treated soils is far greater than that in unamended controls (K. C. Lu and J. E. Dawson, personal communication). Further work is in progress on the mechanism of the chitin-induced suppression.

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Biological Characteristics of Colicine *X*

The name colicine *X* was given[1] to a colicine produced by the strain of *Escherichia coli* used industrially by Hageda, AG., Berlin-Köln, for the preparation of the emulsion 'Mutaflor'. This colicine can be distinguished from other types of colicines by the following characteristics:

(1) *Specificity of mutants resistant to colicine X.* Specificity to colicines is determined by receptors, which are specific for each colicine[2]. The sensitive bacteria can lose the specific receptors one after another by mutation. By successive mutations a sensitive strain may become resistant towards many colicines. In a series of experiments I used two strains sensitive to colicines *X*, *V*, *B*, *E*, *I*, *K*, S_3+I, *D*, S_4, $J+I$, *A* and *C*, and by successive mutations I was able to isolate 200 mutants resistant to one or more colicines. Loss of receptor for colicine *X* was never accompanied by a loss of receptor for another type of colicine, and cross-resistance between colicine *X* and one of the other 12 colicines was not observed. The experiments prove that mutants resistant to colicine *X* are specific.

(2) *Spectrum of activity.* Colicine *X* is very active against some groups of Enterobacteriaceae; but like the other colicines does not inhibit growth of *Staphylococcus aureus*, *Bacterium anitratum* or *Pseudomonas* (Table 1). The *Escherichia coli*, *Citrobacter* and *Klebsiella-Cloaca* group are more frequently sensitive to colicine *X* than to other colicines, while *Salmonella* and *Proteus* are generally resistant. Strains of *Escherichieae* and *Klebsielleae* isolated from animal fæces are more sensitive to colicine *X* than strains isolated from human fæces; but it is still impossible to explain this ecological difference[3].

Table 1. Activity Spectrum of Colicine *X*

Organism	No. of strains examined	No. of strains sensitive	Percentage
Staphylococcus aureus	30	0	0
Bacterium anitratum	20	0	0
Pseudomonas	27	0	0
Enterobacteriaceae			
Salmonella	40	1	2·5
Proteus	20	0	0
Escherichia coli	130	100	76·9
Citrobacter	16	13	81·2
Klebsiella-Cloaca	40	27	67·5

The sensitivity of *E. coli* isolated from animal fæces towards 13 colicines is reported in Table 2. The results show clearly that colicine *X* is more active against *E. coli* than any other colicine used in this work.

Table 2. Sensitivity of 80 Strains of *Escherichia coli* towards Colicine *X* and 12 other Colicines

Type of colicine	No. of sensitive strains	Percentage
X	74	92·5
D	60	75
F	30	37·5
V	29	36·2
B	25	31·2
S_3+I	24	30
E	11	13·7
$J+I$	10	12·5
I	4	5
C	4	5
K	3	3·7
S_4	3	3·7
A	3	3·7

(3) *Other characteristics.* (*a*) The production of colicine *X* is not influenced by growth of the colicinogenic strain in synthetic medium[1].

(*b*) The diameters of the inhibition zones in nutrient agar are usually 20–26 mm. The morphology of the inhibition zone is not characteristic, but resembles that of colicines *D* and *K*.

(*c*) It has been difficult to study the heat resistance of colicine *X*, because suspensions of this colicine with a high titre cannot be prepared by the usual methods (see *d*). Undiluted colicinogenic cultures in broth were used for heat-resistance experiments, and it was found that colicine *X* is destroyed after boiling for 15 min.

(*d*) Colicine *X* is rapidly destroyed by the addition of 2–4 ml. chloroform to 100 ml. of bacterial culture in 'Oxoid' nutrient broth. The strain 'Mutaflor' is sensitive to streptomycin but the addition of streptomycin to the broth culture does not offer any advantages for the preparation of suspensions of colicine, as the concentration of colicine *X* in broth is low. It is possible that colicine *X* does not diffuse easily outside the bacterial cell.

(*e*) Colicine *X* is resistant to the proteolytic enzymes, which are produced by some strains of *Bacilli* or *Proteus*.

The foregoing characteristics permit the distinction of colicine *X* from the other 12 colicines produced by the type cultures of P. Fredericq.

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