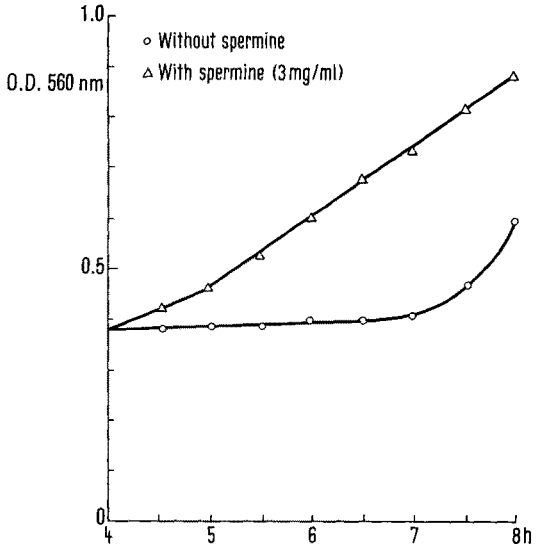
Nucleic acids were determined spectrophotometrically (reading at 260 nm) after extraction and separation according to the method of SCHNEIDER[9].

The results reported in the Table show that the level of both spermine and spermidine greatly increases during the lag time and then decreases in the first exponential period of cell growth. During all this period the level of spermidine is significantly higher than that of spermine. In the following stationary phase, the concentration of both polyamines tends to become constant and is very similar.

Polyamine and nucleic acid content in growing *S. cerevisiae*

	Time (h)						
	0	2	4	6	8	10	12
Spermine (γ/g)	389[a]	1171	281	350	398	700	841
Spermidine (γ/g)	628	1868	976	736	785	956	876
RNA (mg/g)	99.9	102.2	136.3	149.9	122.7	90.9	76.3
DNA (mg/g)	8.1	8.3	10.6	13.1	13.1	15.8	7.3

[a] The values are the means of 3 determinations.



Effect of the spermine on the growing yeast.

RNA increases rapidly in the first period of the exponential cell growth, that is when polyamines have already reached their maximum concentration, and reaches the highest level between the fourth and the sixth hour. The increase of DNA is slower and reaches its peak at the end of the cell growth period.

Parallel experiments in which the lage time of cell growth was increased by about 4 h by subsequent washings of yeast cells with distilled water, showed that the addition of spermine (3 mg/l) to the broth reduces significantly the lag time and stimulates cell growth (Figure).

The results reported indicate that the level of spermine and spermidine undergoes considerable changes during the vital cycle of yeast cells; the highest concentration is reached at the beginning of the logarithmic growth period. Moreover, when the lag time is experimentally prolonged, the presence of spermine in appropriate concentration determines an earlier appearance of the phase of cell multiplication; it is possible to suggest that, also in yeast, polyamines determine a stimulatory effect on the early phases of cell growth. The inhibitory effect observed by other authors might be due to the fact that the polyamine concentration in the culture media used was exceedingly high as compared to the physiological concentration of these compounds in yeast cells.

Riassunto. Sono state studiate le modificazioni del contenuto di spermina, spermidina ed acidi nucleici in cellule di *S. cerevisiae* durante le varie fasi di crescita. Inoltre è stato osservato un effetto di stimolo della moltiplicazione cellulare esercitato dalla spermina sul lievito in cui era stato sperimentalmente aumentato il tempo di latenza.

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Wetting of Fibrin Plate and Apparent Promotion of Fibrinolysis by Surfactin, a New Bacterial Peptidelipid Surfactant

During an investigation to search antifibrinolytic substances in microbial products using the fibrin plate method[1], a dramatic promotion of plasmin-catalyzed fibrinolysis was observed in the presence of the boiled culture filtrates of several strains of *Bacillus subtilis*.

The promoting agent present in the culture filtrate of *B. subtilis* IAM 1213 was crystallized and found further to be endowed with an ability to inhibit fibrin clot formation in the thrombin-fibrinogen system. Descriptions of its purification and characterization as well as determination of its inhibition site in fibrin clot formation have recently appeared in a communication from our laboratories[2]. The agent is a peptidelipid, with the molecular weight around 1050, composed of L-Asp, L-Glu, L-Val, L-Leu, D-Leu and a C_{15}-hydroxy *iso* acid (1:1:1:2:2:1) and from its strong surface active nature, exceeding that of sodium lauryl sulphate (SDS), it was named 'Surfactin'.

[1] T. ASTRUP and S. MÜLLERTZ, Arch. Biochem. Biophys. *40*, 346 (1952).

[2] K. ARIMA, A. KAKINUMA and G. TAMURA, Biochem. Biophys. Res. Commun. *31*, 488 (1968).

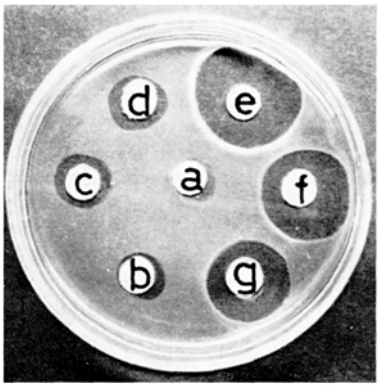

Fig. 1. Marked promotion of plasmin-catalyzed fibrinolysis by boiled culture filtrates of *B. subtilis* strains. (a) plasmin (pl) 1 arbitrary unit (a.u.), (b) pl 2 a.u., (c) pl 4 a.u., (d) pl 10 a.u., (e) pl 1 a.u. + boiled culture filtrate of *B. subtilis* IFO 3035, (f) pl 1 a.u. + boiled culture filtrate of *B. subtilis* IAM 1213, (g) pl 1 a.u. + boiled culture filtrate of *B. subtilis* IAM 1259.

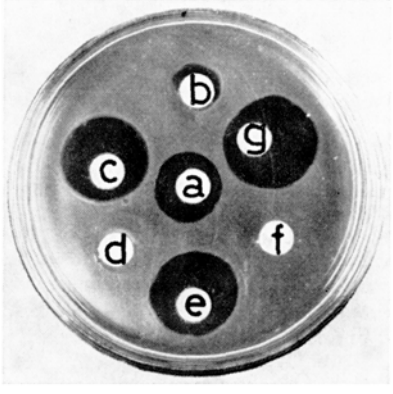

Fig. 2. Indentification of surfactin and the promoting agent originally present in boiled culture filtrate of *B. subtilis* IAM 1213. (a) plasmin, (b) culture filtrate, (c) plasmin + culture filtrate, (d) boiled (30 min) culture filtrate, (e) plasmin + boiled (30 min) culture filtrate, (f) surfactin 2.5 μg, (g) plasmin + surfactin 2.5 μg.

The marked promotion of plasmin-catalyzed fibrinolysis by the boiled culture filtrates of 3 strains of *B. subtilis* is shown in Figure 1. Fibrin plates were prepared according to the method by ASTRUP and MÜLLERTZ except that fibrinogen was Armour Bovine Fraction I. Plasmin was streptokinase-activated euglobulin. Paper discs with 8 mm diameter and with 0.025 ml capacity were dipped in solutions containing plasmin with or without samples, dried on filter papers and placed on fibrin plates. From the comparison of lysed areas in Figure 1, the degree of promotion by these boiled filtrates was calculated to be more than several 10 times. Figure 2 clearly indicates the identity of surfactin and the promoting agent present in the boiled culture filtrate of *B. subtilis* IAM 1213.

Surfactin promoted on fibrin plates the trypsin-catalyzed fibrinolysis as well and had no effect at all on caseinolysis catalyzed by either plasmin or trypsin. It did not activate plasminogen to plasmin. Therefore, the promotion by surfactin was ascribed not to the activation of fibrinolytic enzymes but to some specific effect restricted on fibrin plates. Actually, surfactin showed a peculiar

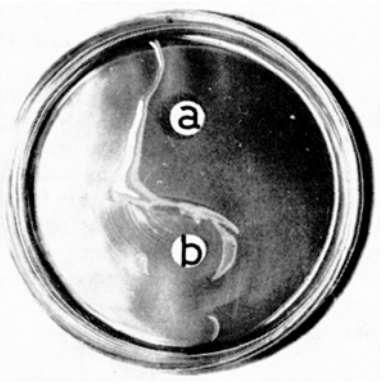

Fig. 3. Diffusion and wetting action of surfactin on fibrin plate. (a) plasmin, (b) surfactin 250 μg.

manner on fibrin plates. When a drop of an aqueous solution containing surfactin was placed on a fibrin plate, the drop diffused on the plate in a minute or two and at the same time wetted the surface of fibrin gel. Diffusion and wetting action of surfactin are demonstrated in Figure 3. In good contrast to this phenomenon, a drop without surfactin remained as it was and no detectable change occurred on the surface of the gel.

From all these experimental facts, the mechanism of the promotion by surfactin was concluded to be as follows. Surfactin by its diffusion and wetting activity carried plasmin from where the enzyme was first placed and made the plasmin-lysed area far greater than it otherwise would have been.

SDS, an anionic detergent, had neither such diffusion activity nor wetting action but denatured fibrin clot drastically. ASTRUP and ALKJAERSIG[3] reported on inhibition of plasmin and slight activation of trypsin on fibrin plates by some quaternary surface-active agents. Compared with these detergents, surfactin seems to be very unique in its behaviour against fibrin clot.

The fibrin plate method has been widely and conveniently used for the determination of activities of plasmin and other fibrinolytic enzymes. Considering, however, the existence in nature of such materials as surfactin which, without effect on enzyme activity, contributes rather physically to remarkable enlargement of lysed areas to bring an apparent promotion of fibrinolysis, we must emphasize the impropriety of estimating fibrinolytic activity by simple calculation from lysed areas.

Zusammenfassung. Aus Kulturen von *Bacillus subtilis* wurde Surfactin, eine neue Substanz, isoliert und aufgeklärt. Es handelt sich um ein Peptidlipid, welches netzende Eigenschaften besitzt und dadurch die Fibrinolyse beschleunigt.

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Department of Agricultural Chemistry, University of Tokyo (Japan), 18 July 1968.

[3] T. ASTRUP and N. ALKJAERSIG, Nature *168*, 565 (1951).

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