Influence of the Yeast *Debaryomyces hansenii* on dry Sausage Fermentation

K. H. GEHLEN[1], C. MEISEL[2], A. FISCHER[3] and W. P. HAMMES[3]

1: Nordreco AB, Box 520, S-26700 Bjuv, Sweden*

2: Südzucker AG, W-6718 Grünstadt 1, Germany

3: Institut für Lebensmitteltechnologie, Universität Hohenheim, W-7000 Stuttgart 70, Germany

SUMMARY: The effect of *Debaryomyces hansenii* on the quality of fermented sausages was studied. In all batches with *D. hansenii*, the cell counts of the fortuitous staphylococci were reduced to a greater extent during ripening than in the correspondent batches without yeast. Therefore, in the batches without a nitrate reducing starter organism, *D. hansenii* caused a retarted nitrate reduction. Generally, the yeast caused an increased ammonia concentration and a higher pH in the sausages, and decreased concentrations of acetic acid and lactic acid. Upon inoculation with $5x10^5$ cfu/g, the yeast exhibited growth in all batches within the first five days of ripening. *D. hansenii* was inhibited in growth, when *Lactobacillus curvatus* and *Micrococcus varians* were used in addition and, furthermore, an increase in ammonia concentrations was not detected in the sausages. Our results show that *D. hansenii* has a marked influence on the microbiology and the chemical composition of dry sausages. Thus, to prevent complications, the application of *D. hansenii* requires that the formula and the starters have to be adapted, according to the special effects of yeast.

INTRODUCTION: *Debaryomyces hansenii* requires oxygen for reproduction, destroys peroxides (CORETTI, 1973), is salt tolerant, does not reduce nitrate and occurs frequently in cured meat products (LEISTNER und BEM, 1970). Some starter preparations on the German market contain *D. hansenii* as single strain or in combination with lactic acid bacteria and staphylococci (HAMMES et al., 1985). The aim of this work was to investigate the influence of *D. hansenii* on the characteristics of dried sausages during fermentation.

MATERIALS AND METHODS

Organisms: *Lactobacillus curvatus* Lc2 is the same as described by MEISEL et al. (1989). *Micrococcus varians* M28 (MEISEL, 1989) was isolated from dry sausages, and *D. hansenii* Dh1 from a commercial starter culture.

Production of dry sausages: The sausage mix was composed of 30 % lean beef (fat content approx. 8 %; frozen), 20 % lean pork (approx. 5 % fat; frozen), 15 % lean beef (approx. 4 % fat; ground) and 35 % pork back fat (approx. 90 % fat; frozen). Further ingredients were (%): sodium chloride, 2.8; potassium nitrate, 0.03; dextrose monohydrate, 0.3; black pepper, 0.2; caraway, 0.03; mace, 0.03 and coriander, 0.07. The starter cultures were added as shown in table 1. The inoculum was $1.7x10^7$ (*M. varians* M28), $5.2x10^5$ (*D. hansenii*) and $1.2x10^7$ cfu/g (*L. curvatus* Lc2). A batch without starters served as control.

Table 1. Experimental design.

Batch	Starter organisms		
	L. curvatus Lc2	*M. varians* M28	*D. hansenii* Dh1
1	-	-	-
2	+	-	-
3	-	+	-
4	-	-	+
5	+	+	-
6	+	-	+
7	-	+	+
8	+	+	+

The sausage mixes were stuffed into regenerated collagen casings (NATURIN $R2^R$) of 60 mm in diameter and ripened in a

drying chamber for 7 days at 18° C followed by 21 days at 16° C. During the first week the relative humidity was reduced from 93 % to 87 % and during further 10 days from 87 % to 75 %. The sausages were exposed to friction-generated smoke for 30 min on the 2nd, 4th, 6th and 8th day of fermentation.

Analytical Methods: The pH of the sausages was measured from homogenates of 10 g sausage in 20 ml of destilled water. The dry matter was determined by the see-sand method (Amtliche Sammlung für Untersuchungsverfahren nach § 35 LMBG). The determination of nitrite and nitrate was performed according to SCHREINER et al. (1988) and that of ammonia according to GERHARDT and DAM QUANG (1979). Lactic and acetic acid were determined by ion chromatography (Biotronic IC 1000) with a high performance separation column (Biotronic, Nr. 5311006) and 0.1 n sulfonic acid as the eluent. The flow rate was 0.8 ml per min, the detection wave length 210 nm (range 0.08) (GEHLEN, 1989).

Microbiological methods were described by MEISEL et al. (1989). For the selective determination of the staphylococci, the SK-medium was used (SCHLEIFER and KRÄMER, 1980). For the enumeration of the yeasts, wort agar (Merck) was employed.

Sensory examinations were performed according to SINELL et al. (1984) with a trained panel, using descriptive sensory analysis for external and internal appearance, firmness, colour and taste. The examination took place after 21 and 27 days of ripening.

RESULTS AND DISCUSSIONS: In all batches with *D. hansenii* as a starter culture, growth of this yeast could be observed. The additional use of either *L. curvatus* or *M. varians* M28 as starter did not reduce the yield of *D. hansenii*. However, the yeast was inhibited in growth during ripening, when *L. curvatus* and *M. varians* M28 were used together (figure 1). In all batches growth of the fortuitous staphylococci was observed in the first days of fermentation. In the control batch without any starter, both maximum yield and residual level of the fortuitous staphylococci (figure 2) were higher than in any other lot. The fortuitous staphylococci grew up from 2.4×10^4 to 1.3×10^7 cfu/g within the first five days and declined to 1.2×10^6 cfu/g after 28 days of ripening. Regarding maximum and residual yield, the single starter strains *L. curvatus*, *D. hansenii* Dh1 or *M. varians* M28 inhibited the growth of the fortuitous staphylococci, the yeast exhibiting the strongest effect. In all batches produced with *D. hansenii*, the fortuitous staphylococci reached lower maximum levels and died off faster than in the corresponding batches without yeast. On a qualitative base *L. curvatus* caused the same effect (figure 2 and 3). The combination of *L. curvatus* with *D. hansenii* showed a stronger synergistic inhibitory effect on the fortuitous staphylococci than its combination with *M. varians* M28. This inhibition was again strengthened when all three strains were employed in combination. In this latter batch the lowest cell counts of the fortuitous staphylococci occured after the 2nd day and the lowest residual yield with 8×10^2 cfu/g was found after 28 days of fermentation (see figure 3). According to those results, MEISEL et al. (1989) found individual inhibition effects of *L. curvatus* Lc2, *M. varians* M101 and *D. hansenii* Dh1 towards *Staphylococcus aureus*. These effects were not lost in starter combinations but acted synergistically. The authors attributed the individual inhibition effects for *L. curvatus* to acidification, for *M. varians* M101 to the formation of nitrite from nitrate and for *D. hansenii* to the consumption of oxygen (MEISEL et al., 1989). The same mechanism might be responsible for the inhibition of the fortuitous staphylococci. The lowest pH (5.05 - 5.13) was observed in all batches with *L. curvatus* after 5 days of fermentation. In the batches without *L. curvatus* the lowest pH was seen after 7 days ranging between 5.15 and 5.27. In all lots the pH raised during the further ripening. *L. curvatus* caused decreased pH values in the finished products, whereas *D. hansenii* - according to the results of MEISEL et al. (1989) - gave rise to increased pH values in the finished products (table 2). This observation is consistent with lower concentrations of lactic acid and acetic acid in these sausages. The assimilation of lactic acid by yeasts in dried sausages is known (RAMIHONE et al., 1988). In addition, the application of *D. hansenii* increased the ammonia concentrations generally in the finished products which should cause increase in pH. Since *L.*

curvatus and *M. varians* M28 inhibited the growth of *D. hansenii* (figure 1), when used in combination, the ammonia formation was not increased in this lot. The control batch without any starter showed a quite good nitrate reduction (figure 4). The use of *M. varians* M28 resulted in an acceleration, the use of *D. hansenii* and of *L. curvatus*, respectively, in an inhibition of the nitrate reduction. The inhibition effect of *D. hansenii* on nitrate reduction was again strengthened when it was combined with *L. curvatus*. Obviously, the suppression of the nitrate reduction was closely related to the inhibitory effects towards the fortuitous staphylococci. This results could explain the color problems, observed by CORETTI (1972) in dried sausages which were produced with $5x10^6$ cfu/g of *D. hansenii* as a single starter culture. No inhibitory effect on the yield of *M. varians* M28 occured, when *D. hansenii* was used in addition. In spite of the suppression of the fortuitous staphylococci by *D. hansenii*, no significant differences in the nitrate concentrations were found in the sausages. (table 2). The sausages with *L. curvatus* and *M. varians* M28 had a slightly gray surface color. The sausages produced by *D. hansenii* or with all three starters had a substantial, red surface color which had already occured after two days of fermentation. The sausages with *L. curvatus* as single strain had a slight gray core area, whereas the products with *L. curvatus* and *D. hansenii* in addition were faulty products with a big, gray core area and a strong rancid taste after 27 days. For both defects the weak nitrate reduction should be responsible causing insufficient amounts of nitrite to enable the curing of the meat pigments and to protect the fat against rancidity. The sausages produced by all three starters were preferred in taste.

CONCLUSIONS: Our results show that *D. hansenii* has a strong influence on the microbiology and the chemical composition of dry sausages. Thus, *D. hansenii* can contribute to an improvement of taste and surface color in dried sausages. However, unproper use of this strain can cause severe defects.

ACKNOWLEDGEMENTS: Excellent technical assistance was provided by J. Hinrichs, H. Haller and K. Herrmann.

REFERENCES

"Amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG". Beuth Verlag GmbH Berlin/Köln, Stand 1987.

CORETTI, K. (1972): Kombinierte Anwendung von Milchsäurebakterien, Mikrokokken und Hefen als Starterkulturen bei der Rohwurstherstellung. Jahresbericht der BAFF, Kulmbach, I 48 - I 49.

CORETTI, K. (1973): Warum interessiert den Praktiker die Mikrobiologie der Rohwurstreifung? Fleischwirtschaft 53, 907-908

GEHLEN, K. H. (1989): Einfluß der Technologie auf die Rohwurstreifung mit *Lactobacillus curvatus*, *Micrococcus varians* und weiteren Starterorganismen unter besonderer Berücksichtigung der Nitratreduktion. Ph.D. Thesis, Universität Hohenheim .

GERHARDT, U. and DAM QUANG, T. (1979): Methoden zur Ammoniakbestimmung in Fleisch und Fleischerzeugnissen. Fleischwirtschaft 59, 946-948.

HAMMES, W. P., RÖLZ, I. and BANTLEON, A. (1985): Mikrobiologische Untersuchung der auf dem deutschen Markt vorhandenen Starterkulturpräparate für die Rohwurstbereitung. Fleischwirtschaft 65, 629-636 und 729-734.

LEISTNER, I. and BEM, Z. (1970): Vorkommen und Bedeutung von Hefen bei Pökelfleischwaren. Fleischwirtschaft 50, 350-351.

MEISEL, C. (1989): Mikrobiologische Aspekte der Entwicklung von nitratreduzierenden Starterkulturen für die Herstellung von Rohwurst und Rohschinken. Ph.D. Thesis, Universität Hohenheim.

MEISEL, C., GEHLEN, K. H., FISCHER, A. and HAMMES, W. P. (1989): Inhibition of the growth of *Staphylococcus aureus* in dry sausages by *Lactobacillus curvatus*, *Micrococcus varians* and *Debaryomyces hansenii*. Food Biotechnology 3, 145-168.

RAMIHONE, M., SIRAMI, J., LARPENT, J. P. and GIRARD, J. P. (1988): Excessively acid taste of dry ripened sausages. Viandes et Produits Carnes 9, 291-298 (in French).

SCHLEIFER, K. H. and KRÄMER, E. (1980): Selective medium for isolating staphylococci. Zbl. Bakteriol. Hyg., 1. Abt. Orig. C1, 270-280.

SCHREINER, G., KIESEL, K. H., GEHLEN, K. H. and FISCHER, A. (1988): Ionen-chromatographische Methode zur gleichzeitigen Bestimmung von Nitrit und Nitrat in Fleischerzeugnissen. Arch. Lebensmittelhyg. 39, 49-51.

SINELL, H.-J., WEBER, A. and HAUPTMANN, S. (1984): Sensorische Qualitätsmerkmale von Fleischerzeugnissen. Prüfschema zur Bewertung nach einer 5-Punkte-Skala. Fleischwirtschaft 64, 29-32.

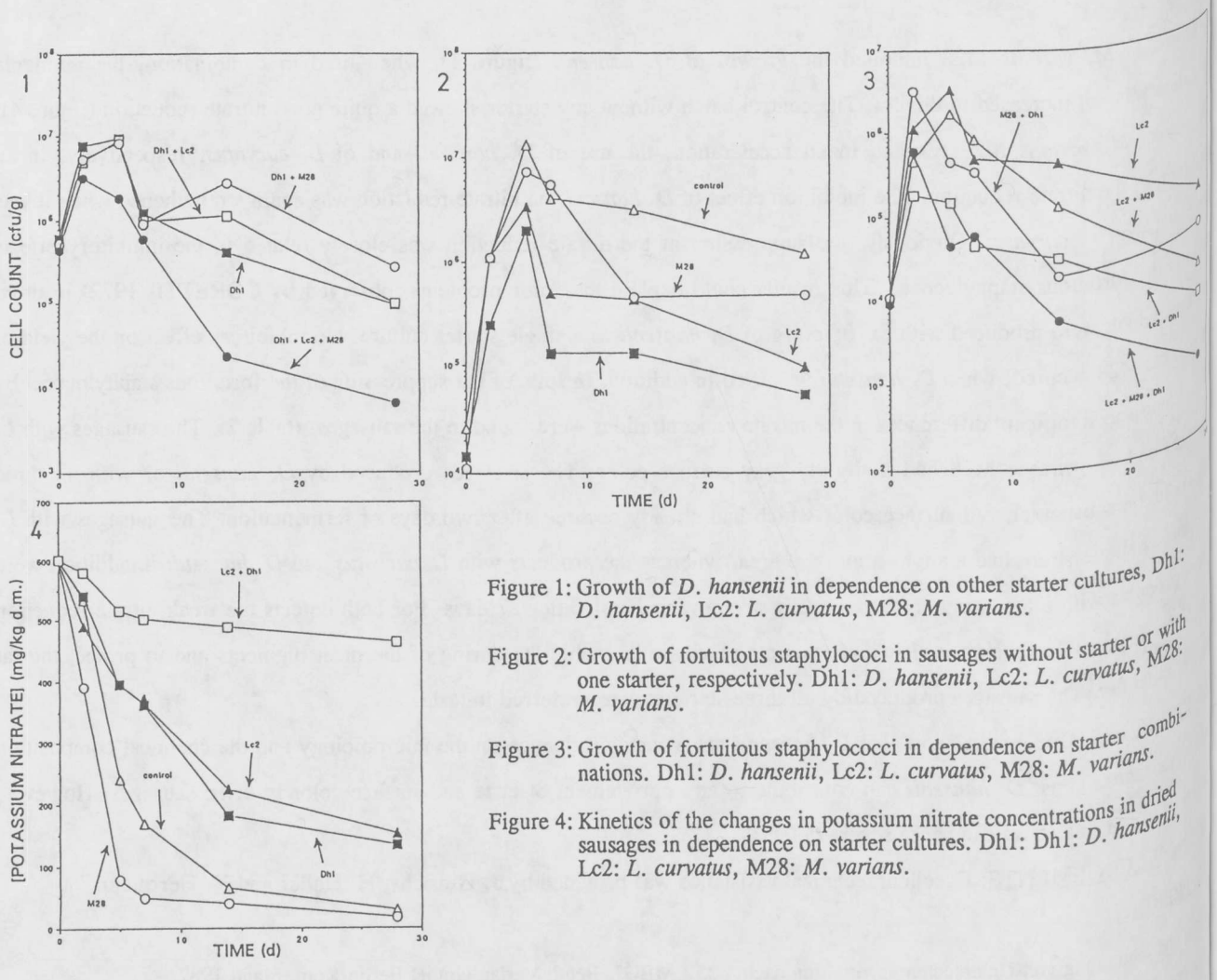

Figure 1: Growth of *D. hansenii* in dependence on other starter cultures, Dh1: *D. hansenii*, Lc2: *L. curvatus*, M28: *M. varians*.

Figure 2: Growth of fortuitous staphylococci in sausages without starter or with one starter, respectively. Dh1: *D. hansenii*, Lc2: *L. curvatus*, M28: *M. varians*.

Figure 3: Growth of fortuitous staphylococci in dependence on starter combinations. Dh1: *D. hansenii*, Lc2: *L. curvatus*, M28: *M. varians*.

Figure 4: Kinetics of the changes in potassium nitrate concentrations in dried sausages in dependence on starter cultures. Dh1: Dh1: *D. hansenii*, Lc2: *L. curvatus*, M28: *M. varians*.

Table 2: Chemical results of the dried sausages after 28 days of ripening

lot	pH	KNO_3 (ppm)	lactic acid (g/kg)	acetic acid (g/kg)	NH_3 (mg/100g)	d.m. (%)
1	5.30	20	12.2	1.4	43.1	69.7
2	5.28	105	12.5	1.1	36.8	68.8
3	5.46	11	10.7	1.3	35.6	67.1
4	5.45	90	11.3	1.1	44.4	67.9
5	5.28	21	12.6	1.2	37.8	69.1
6	5.34	316	11.9	0.9	38.3	68.0
7	5.54	17	9.9	1.2	39.3	68.9
8	5.41	25	12.0	0.8	37.6	68.9

Figure 2: Growth of fortuitous staphylococci in sausages without starter or with one starter, respectively. Dh1: *D. hansenii*, Lc2: *L. curvatus*, M28: *M. varians*.

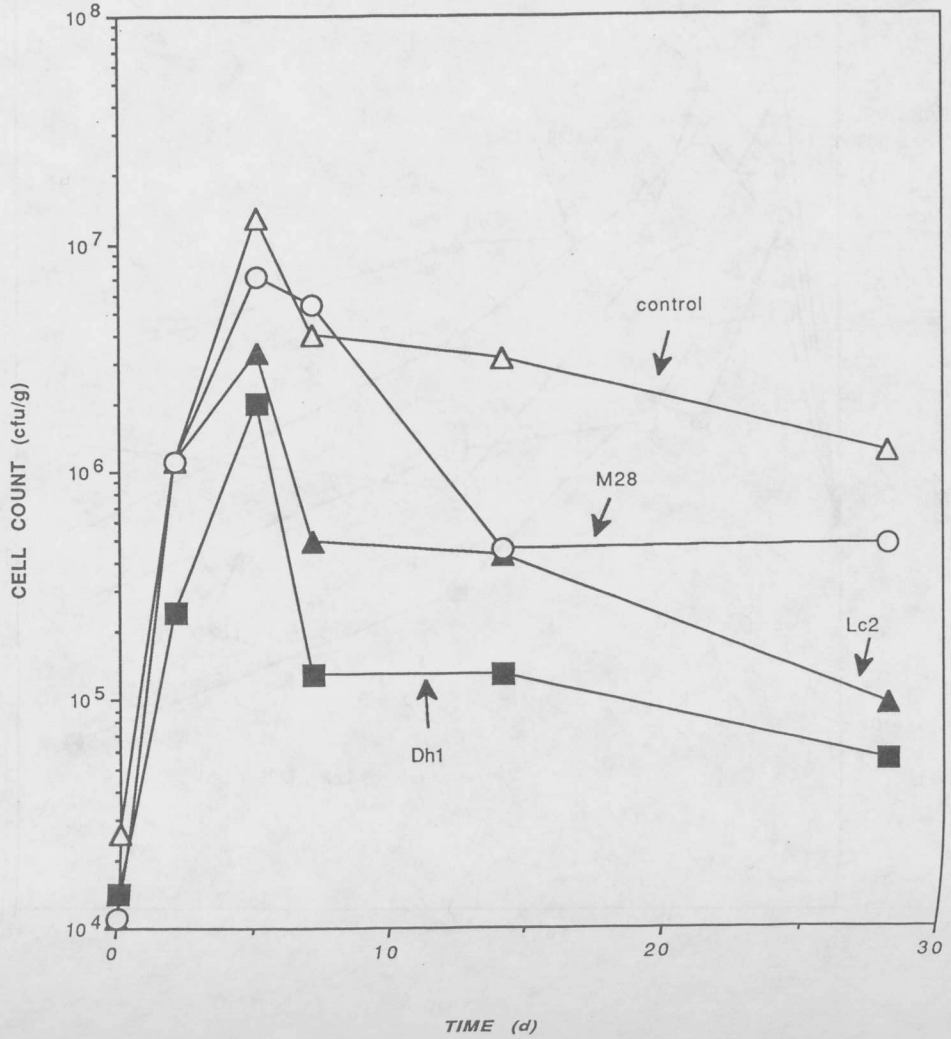

Figure 1: Growth of *D. hansenii* in dependence on other starter cultures. Dh1: *D. hansenii*, Lc2: *L. curvatus*, M28: *M. varians*.

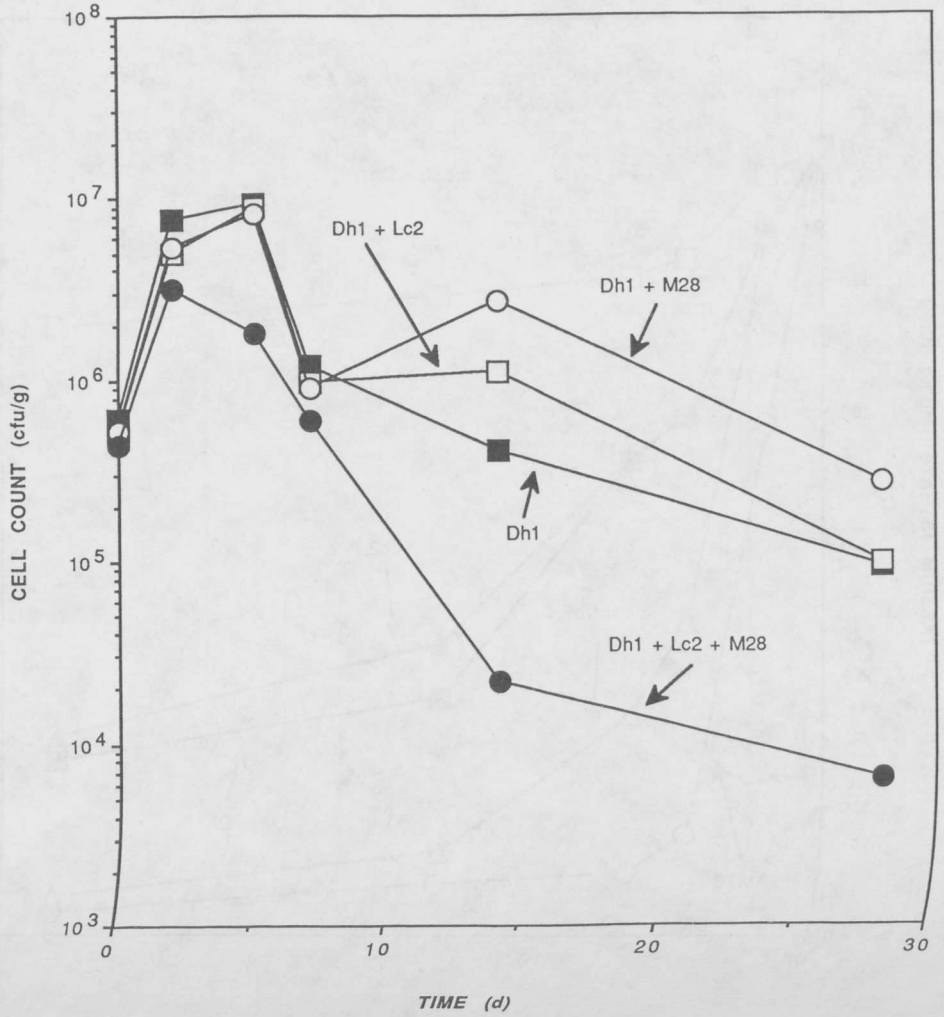

Figure 4: Kinetics of the changes in potassium nitrate concentrations in dried sausages in dependence on starter cultures. Dh1: *D. hansenii*, Lc2: *L. curvatus*, M28: *M. varians*.

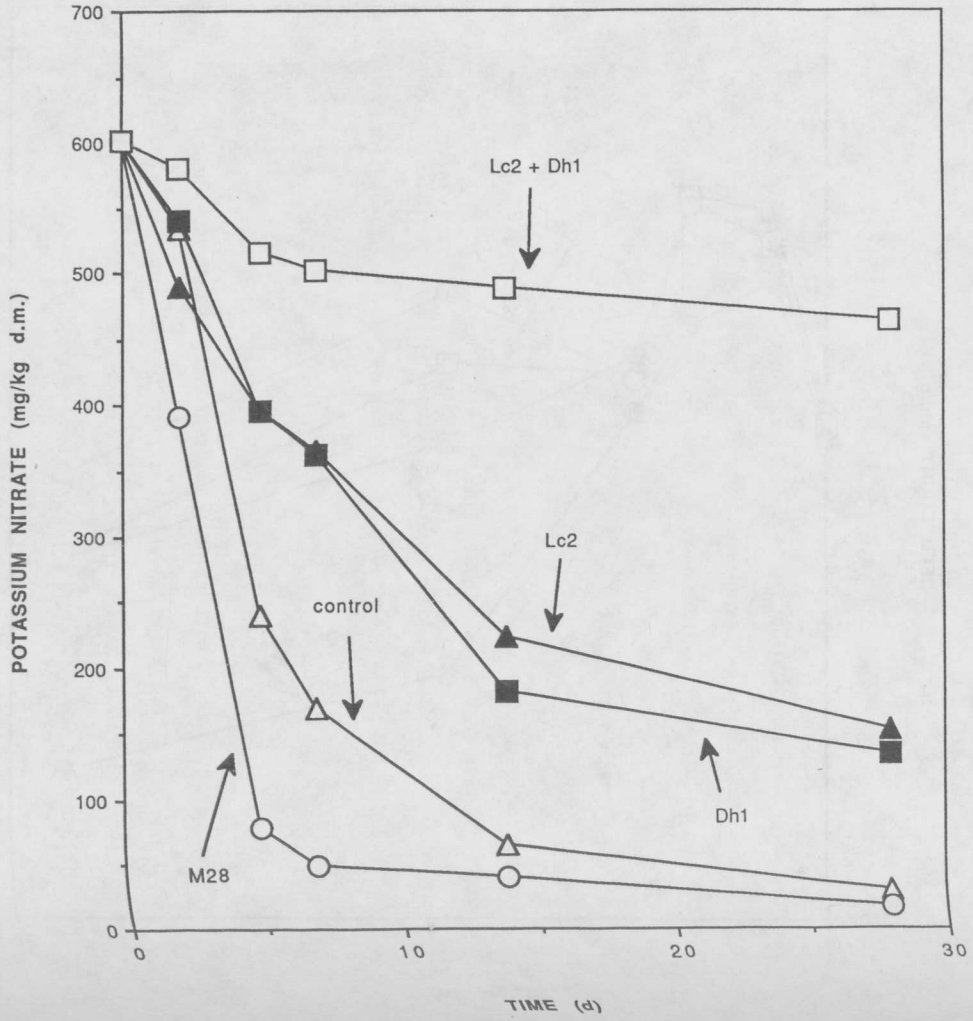

Figure 3: Growth of fortuitous staphylococci in dependence on starter combinations. Dh1: *D. hansenii*, Lc2: *L. curvatus*, M28: *M. varians*.