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# The potential of vancomycin-resistant enterococci to persist in fermented and pasteurised meat products

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**Abstract**

Experiments with 148 isolates of vancomycin-resistant enterococci (VRE) were performed to assess their potential to persist and grow in fermented sausages and pasteurised meat products. All strains were meat isolates and Van-type A, except a single VanC1 strain. In total, 143 strains of *Enterococcus faecium* were involved.

Eight selected strains were examined for their potential to grow at high salt and nitrite levels and at reduced pH. The same isolates were used in experiments with fermented sausages. All available strains were subjected to heating tests in meat suspensions with added curing ingredients.

All but one of the eight tested isolates grew at pH 4.0 in tryptone soya broth (TSB). With the combination of 8% w/w NaCl, 400 ppm $NaNO_2$ and 0.5% w/w glucose in the meat suspension, all isolates grew at 37 °C, whereas none grew at 7 °C even after 56 days. With the addition of 10% w/w NaCl, 200 ppm $NaNO_2$ and 0.5% w/w glucose, still one *E. faecium* isolate grew at 37 °C, although very slowly. Overall, the strains tolerated high salt and nitrite concentrations and reduced pH very well, even beyond levels applied in the regular production of fermented and/or pasteurised meat products.

The tested strains could be isolated after the fermentation and further ripening of "boerenmetworst" and "snijworst". Overall, their colony counts decreased on average about 1 log-unit over a period of 60 days after batter manufacture.

All 148 isolates demonstrated a relatively weak thermal resistance compared to results for selected vancomycin-sensitive enterococci strains reported in the literature and to results collected under identical experimental conditions in this laboratory. None of the strains (log inoculation level about 5–6 $ml^{-1}$ for each isolate) could be cultured after heating at 70 °C for 10 min.


*Keywords:* Enterococci; Vancomycin-resistant; Fermentation; Thermal resistance; Meat

## 1. Introduction

Over the last decade, enterococci have become recognised as one of the leading causes of nosocomial infections (Clifford McDonald et al., 1997; Huycke et al., 1998; Rice, 2001). Most of these infections are caused by *Enterococcus faecalis* and only a small number are caused by *Enterococcus faecium* (Witte et al., 1999). However, in recent years, the number of infections caused by *E. faecium* has progressively increased (Wade, 1997). From a clinical perspective, multi-resistant enterococci or vancomycin-resistant enterococci (VRE) are a major problem.

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As reviewed by Barna and Williams (1984), vancomycin and teicoplanin belong to the group of glycopeptide antibiotics. Glycopeptides are relatively large water-soluble molecules that cannot penetrate the lipid outer membrane of Gram-negative bacteria. In contrast, almost all Gram-positive bacteria are susceptible to the activity of glycopeptides. Glycopeptide antibiotics interact with the terminal D-alanyl-D-alanine group of the pentapeptide side chains of peptidoglycan precursors. Due to this interaction, the cell wall synthesis is inhibited.

Resistance to glycopeptides is due to the synthesis of modified cell-wall precursors that show decreased affinity for vancomycin and teicoplanin. Resistance can be intrinsic (with low-level resistance to both vancomycin and teicoplanin; e.g. VanC1, VanC2 and VanC3) or acquired (with high-level resistance to both vancomycin and teicoplanin [VanA], intermediate level resistance to both glycopeptides [VanD], or variable level of resistance to vancomycin only [VanB]) (Arthur and Courvalin, 1993; Perichon et al., 1997). A new VanE-type resistance has recently been described (Fines et al., 1999). Resistance to VanA and VanB is mainly encoded by homologous transposons named *Tn1546* and *Tn1547*, respectively. These transposons are located on self-transferable plasmids and are transferred by conjugation. VanA-mediated resistance has been most extensively studied.

Vancomycin-resistant strains have been isolated in The Netherlands from (healthy) humans, farm animals, meat and dairy products and sewage (Endtz et al., 1997; Schouten et al., 1997; Braak et al., 1997, 1998; Bogaard, 2000; Braak, 2001). After the avoparcin ban in animal husbandry in the EC in 1997, the carriage of VRE decreased in Germany, Italy, Denmark and The Netherlands (Bogaard et al., 2000).

In the meat processing industry, enterococci are generally considered rather robust organisms. These bacteria frequently survive pasteurisation processes (Houben, 1980), can tolerate sodium chloride and nitrite and can even multiply in cured meat products at refrigeration temperatures (Houben, 1980; Franz et al., 1999; Teuber et al., 1999). The organisms are frequently isolated from fermented meat (Teuber et al., 1999) and dairy products (Holzapfel and Franz, 2000).

Little is known about the survival and subsequent ability to grow of VRE in cured meat products. However, in the medical literature some attention has been paid to their thermal resistance and their persistence under dry conditions. For example, Panagea and Chadwick (1996) studied the heat resistance of 27 *E. faecium* isolates suspended in water. Ten strains (VanA/VanB) were obtained from patients, eight (VanA) from meat and the remaining nine (all vancomycin-sensitive) were from patients. No difference in thermal resistance was observed between vancomycin-sensitive enterococci and VRE, or between isolates from patients and meat. Kearns et al. (1995) studied the resistance to heat and hypochlorite of 12 enterococci strains that caused nosocomial infections. Only two of the strains were vancomycin resistant. These VRE isolates were not different from the other strains in terms of their resistance to heat and hypochlorite. Wendt et al. (1998) found the survival of 4 VRE and of 15 vancomycin-sensitive enterococci strains on dry surfaces to be the same.

The present study was performed with 148 VRE strains isolated from meat products. All isolates were subjected to thermal resistance tests. The ability of eight strains to grow at high salt and nitrite levels, and at reduced pH was investigated. These strains were also inoculated in sausage batters, which were then processed into two Dutch types of fermented sausage.

## 2. Materials and methods

### 2.1. Test strains

All but 2 of the 148 strains were isolated from poultry products (Braak et al., 1998). The remaining two were isolated during summer 2000 from fermented sausages. All strains were VanA, except for an *Enterococcus gallinarum* strain (VanC1). Overall, 143 strains of *E. faecium* were studied; however, genetically identical isolates may have been involved.

Eight strains were selected (Table 1) for investigating their ability to grow at high salt and nitrite levels, and at reduced pH. Selection criteria were to test VanA isolates preferably and to include various *Enterococcus* species.

Table 1
Codes and description of the eight selected strains involved in the growth/survival testing in the model experiments and the fermented sausages

| | Code |
|---|---|
| *E. faecalis* (Nijmegen 125)[a] | Efa 1 |
| *E. faecalis*[b] (Weert) | Efa 2 |
| *E. faecium* PFGE-type A (Zutphen 197) | Efi 1 |
| *E. faecium* PFGE-type B (Zutphen 198) | Efi 2 |
| *E. faecium*[b] (Roermond) | Efi 3 |
| *E. durans* (Den Bosch 242) | Edu |
| *E. hirae* (Zutphen 213) | Ehi |
| *E. gallinarum* (Zutphen 199) | Ega |

[a] Reference to codes of Braak et al. (1998).
[b] Strains recently isolated from fermented sausages (see Section 2.1).

The strains were received in a lyophilised condition. After culturing the individual strains for 48 h at 37 °C in Tryptone Dextrose Yeast extract Meat extract Peptonised milk (TDYMP), sterile glass beads (diameter, 3 mm) were brought in the medium. Beads with attached bacteria were collected and preserved at −80 °C. TDYMP is a rich medium (Mossel and Krugers Dagneaux, 1959) which has been reported to encourage enterococci growth (Houben, 1980). The ingredients for this medium were purchased from Oxoid (Unipath, Basingstoke, England).

### 2.2. Tests for sensitivity to sodium chloride, sodium nitrite and a reduced pH

The sensitivity of the selected strains (Table 1) to NaCl, $NaNO_2$ and a reduced pH was initially tested in tryptone soya broth (TSB; Oxoid CM129). The applied concentrations of NaCl and $NaNO_2$ and tested pH levels (adjustment with diluted HCl) are shown in Table 2. NaCl was added before the medium was sterilised; $NaNO_2$ was added after sterilisation as a filter-sterilised aqueous solution. The tubes were inoculated at a log inoculation level $ml^{-1}$ of 4.30–4.70 with fresh 48-h TSB cultures, initially seeded with a few glass beads bearing the preserved culture (see Section 2.1). After an incubation of the modified TSB for 48 h at 37 °C, the growth of the organisms was assessed by measuring absorbance at 520 nm. Uninoculated tubes were used as blanks.

In a subsequent series of experiments, the strains were inoculated in meat suspensions. Lean pork (pH 5.7–6.0) was blended in a Stephanal TA-20 chopper (Stephan, Diessen am Ammersee, Germany). Thereafter portions of 100 g were vacuum-packaged and stored at −20 °C. Before use, the packages were thawed in lukewarm water and then mixed with 200 ml distilled water in a Waring Blender vessel (Waring Products Division, New Hartford, USA). After being hermetically sealed, the vessel was sterilised for 20

Table 2
Growth of the eight selected strains in modified tryptone soya broth during an incubation of 48 h at 37 °C (inoculation level: $2.0–5.0 \times 10^4$ $ml^{-1}$)

| | Strains examined (codes shown in Table 1) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Efa 1 | Efa 2 | Efi 1 | Efi 2 | Efi 3 | Edu | Eri | Ega |
| Additions | | | | | | | | |
| 2% w/w NaCl | +++[a] | ++ | ++ | +++ | +++ | +++ | ++ | ++ |
| 4% w/w NaCl | +++ | ++ | ++ | ++ | +++ | ++ | + | ++ |
| 6% w/w NaCl | +++ | ++ | ++ | +++ | +++ | ++ | + | ++ |
| 8% w/w NaCl | +++ | ++ | ++ | ++ | ++ | + | ++ | ++ |
| 2% w/w NaCl + 200 ppm $NaNO_2$ | ++ | ++ | ++ | ++ | ++ | ++ | + | ++ |
| 2% w/w NaCl + 400 ppm $NaNO_2$ | + | ++ | ++ | ++ | ++ | + | + | + |
| 2% w/w NaCl + 600 ppm $NaNO_2$ | + | ++ | ++ | ++ | ++ | + | + | + |
| 2% w/w NaCl + 800 ppm $NaNO_2$ | + | + | + | ++ | + | + | + | + |
| pH 5.5 | +++ | +++ | ++ | ++ | +++ | ++ | ++ | ++ |
| pH 5.0 | ++ | ++ | ++ | ++ | +++ | ++ | ++ | ++ |
| pH 4.5 | ++ | ++ | ++ | ++ | ++ | + | ++ | ++ |
| pH 4.0[b] | + | + | + | + | ++ | ++ | + | neg |

[a] Absorbance ≈ 0 (no multiplication) = neg; 0.1–0.3 = +:0.3–0.6 = ++ and 0.6–0.9 = +++.
[b] Growth observed after a 9-day incubation at 37 °C; after a 48-h growth was not detected.

min at 112 °C. The effectiveness of sterilisation of meat suspensions at this level was previously tested (Houben, 1980). After chilling and renewed blending, a suspension was obtained which no longer coagulated on heating. Glucose (0.5% w/w) was added as a filter-sterilised aqueous solution. Salt, nitrite and the test strains were added as described above for the modified TSB. Tubes were kept at 7 and 37 °C. Upon growth of enterococci primarily lactic acid will be produced from glucose. Growth of the strains was monitored here by measuring pH with a Schott pH meter (type CG818) (Schott, Mainz, Germany) in replicate tubes, which were discarded. Uninoculated tubes were used as blanks.

### 2.3. Survival of VRE in fermented sausages

The selected strains (Table 1) were dispersed over a slowly revolving chopper bowl in the final batter manufacturing stage of two types of fermented sausage: ''boerenmetworst'' a traditionally made dry, fermented pork sausage, stuffed in pork casings and ''snijworst'' a modern style, semi-dry, Dutch-type cervelat sausage, containing pork and beef, stuffed in cellulose casings of 90-mm diameter. Product compositions and manufacturing procedures were as formulated by TNO (Anon, 1996). Batters of ''boerenmetworst'' and ''snijworst'' contained 0.3% and 0.7% w/w glucose, respectively. Fermentation was performed at 25 °C for 1 and 2 days for ''boerenmetworst'' and ''snijworst'', respectively. Products were further ripened at 17 °C. A *Lactobacillus curvatus* starter strain (inoculation level $10^6$ $g^{-1}$) was used which rapidly converts available glucose primarily into lactic acid.

The six VRE isolates from chicken products were added as a cocktail; all other strains were applied separately. The inoculum was from 48 h, 37 °C TDYMP broth cultures, initially seeded with a few glass beads bearing the preserved culture (see Section 2.1).

The persistence and growth of enterococci were monitored by plating in KF Streptococcus Agar (KF Oxoid CM701), with or without the inclusion of 8-mg vancomycin $l^{-1}$ medium. The medium was incubated for 48 h at 37 °C. The number of lactic acid bacteria was counted on De Man Rogosa Sharpe Agar (MRS Oxoid CM361; pour plates and incubated for 3 days at 30 °C under anaerobic conditions, BBL GasPak Plus™ system (Becton Dickinson, Sparks, USA) delivering $H_2$ and $CO_2$ in an anaerobic jar). Acidification of the products was monitored by measuring pH. Product drying was assessed by measuring the moisture content (ISO/1442, 1973).

### 2.4. Determination of the thermal resistance of VRE in meat suspensions

Heating tests were done with all isolates in meat suspensions (see Section 2.2) to which 18% w/w of an injection-type pasteurised ham brine was added. The brine contained (in % w/w): NaCl (13.22); $NaNO_2$ (0.08); $Na_2H_2P_2O_7$ (0.30); $Na_5P_3O_{10}$ (3.0); glucose (3.30); sodium-L-glutamate (0.60); ascorbic acid (0.30); distilled water (79.20).

The brines were freshly prepared and filter-sterilised before use. The meat suspension with the curing ingredients (MSI) contained per kg: 20.2 g NaCl and about 120 mg $NaNO_2$; pH was 6.2–6.3; $a_w$ (25 °C) was 0.97. Water activity was determined with a Novasina AWC 500 instrument (Novasina, Pfäffikon, Switzerland). To perform heating tests, the strains were first cultured for 48 h at 37 °C in TDYMP broth. The cells were collected from the medium by centrifugation and washed twice with 0.1% w/w peptone physiological saline and finally suspended in this medium.

MSI was inoculated at a level of $5 \times 10^6$ $ml^{-1}$ with a freshly prepared cell suspension, and after thorough mixing was pipetted in 5-ml amounts into sterile polyethylene pouches (Whirl pak, 6 oz, Nasco, Ft. Atkinson, USA); thickness after filling was 2 mm. These were heat-sealed and heated in duplicate for pre-determined times at 60 ± 0.1 and 70 ± 0.1 °C in a Colora precision water bath NB/DS 1056 (Colora, Frankenthal, Germany). Sampling intervals were set at 10- and 5-min heating at 60 and 70 °C, respectively, and maximum heating times for the respective temperatures were 90 and 30 min. The pouches were heated in a suspended position, completely immersed in the water. The internal pouch temperature reached the water bath temperature in 30 s. After heating, the pouches were rapidly chilled in ice water. Surviving cells were plated in TDYMP-agar, and colonies were counted after an incubation of 3 days at 37 °C. Survivor curves were calculated by linear regression

and Decimal Reduction Times (*D*-values) were recorded. The selected test strains (Table 1) were subjected to detailed *D*-value determinations; the remaining isolates were screened for heat resistance only. For the latter, cocktails of 10–11 different isolates at approximately the same inoculation level of $10^5$–$10^6$ $ml^{-1}$ for each strain were inoculated in MSI and heated, in duplicate, at 70 °C. The numbers of colonies were counted before and after heating as described above. The maximal possible *D*-values were tentatively calculated from the numbers of decimal reductions, assuming that the enumerated surviving organisms were all originating from one single strain.

## 3. Results and discussion

### 3.1. Sensitivity to sodium chloride, sodium nitrite and a reduced pH

The eight selected strains all grew at 37 °C, though at variable rates, in the modified TSB to which 8% w/w NaCl was added (Table 2). For the combination 2% w/w NaCl and 800 ppm $NaNO_2$, weak to moderate growth was observed for all strains. At pH 4.0 all isolates, except for the *E. gallinarum* strain, demonstrated weak to moderate growth.

As expected, multiplication of the eight strains in the meat suspension at the optimum temperature of 37 °C was clearly better than at the refrigerated storage temperature of 7 °C (Table 3). In the presence of 8% w/w NaCl, 400 ppm $NaNO_2$ and 0.5% w/w glucose, all strains grew at 37 °C within <3–10 days. Increasing the salt level from 8% to 10% w/w clearly had a negative effect: one isolate (Efi 1) grew but not within a period of 10 days at 37 °C. At this salt concentration no growth was seen after 56 days at 7 °C.

Overall, the strains tolerated high salt and nitrite concentrations and reduced pH well, even beyond levels applied in the regular production of fermented and/or pasteurised meat products.

### 3.2. Survival of the VRE strains in fermented sausages

Table 4 (isolates from chicken products) and Table 5 (isolates from fermented sausages) present the data obtained after fermentation and ripening of the fermented sausages. Significant growth of the VRE, inoculated in the batter at levels ranging from $1.3 \times 10^5$ to $2.1 \times 10^6$ $g^{-1}$, was not observed in any of the products. Their potential to compete with the *L. curvatus* starter strain was apparently too weak. During fermentation, the numbers of VRE remained constant or decreased slightly. The average reduction (log units) was 0.20 (range +0.11 to −0.75). During a ripening period of 60–90 days, the numbers of VRE gradually decreased: about 60 days after batter manufacture, the average decrease was 0.99 (range 0.30 to 1.80). The numbers of lactic acid bacteria hardly

Table 3
Growth[a] of the eight selected strains at 7 and 37 °C in modified meat suspensions[b] (inoculation level: $4.0–6.0 \times 10^4$ $ml^{-1}$)

| | Strains examined (codes shown in Table 1) | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Efa 1 | | Efa 2 | | Efi 1 | | Efi 2 | | Efi 3 | | Edu | | Ehi | | Ega | |
| Incubation temperature (°C) | 7 | 37 | 7 | 37 | 7 | 37 | 7 | 37 | 7 | 37 | 7 | 37 | 7 | 37 | 7 | 37 |
| Additions | | | | | | | | | | | | | | | | |
| 8% w/w NaCl + 200 ppm | 14–50[c] | | 14–50 | | 14–50 | | 14–50 | | 14–50 | | 14–50 | | 14–50 | | 14–50 | |
| $NaNO_2$ + 0.5% w/w glucose | | <3 | | <3 | | <3 | | <3 | | <3 | | <3 | | <3 | | <3 |
| 6% w/w NaCl + 400 ppm | 14–50 | | 14–50 | | 14–50 | | 14–50 | | 14–50 | | 14–50 | | 14–50 | | 14–50 | |
| $NaNO_2$ + 0.5% w/w glucose | | <3 | | <3 | | <3 | | <3 | | <3 | | <3 | | <3 | | <3 |
| 8% w/w NaCl + 400 ppm | >56[d] | | >56 | | >56 | | >56 | | >56 | | >56 | | >56 | | >56 | |
| $NaNO_2$ + 0.5% w/w glucose | | 3–10 | | <3 | | <3 | | <3 | | <3 | | 3–10 | | 3–10 | | 3–10 |
| 10% w/w NaCl + 200 ppm | >56 | | >56 | | >56 | | >56 | | >56 | | >56 | | >56 | | >56 | |
| $NaNO_2$ + 0.5% w/w glucose | | >45 | | >45 | | 10–45 | | >45 | | >45 | | >45 | | >45 | | >45 |

[a] The table presents the number of days of incubation required to obtain a decrease in pH of ≥0.5.
[b] The initial pH of the suspensions ranged from 5.86 to 6.02.
[c] After the highest recorded number of days, growth was observed only. No pH measurements were performed in the interim period.
[d] > means that no growth was observed after the recorded number of days.

Table 4
Survival of the six selected vancomycin-resistant strains isolated from poultry products (see Table 1), inoculated as cocktail, and lactic acid bacteria in three batches of fermented sausages (average numbers of colony forming units expressed as log values ΔG = weight loss. KF + Van = KF to which 8 mg vancomycin $l^{-1}$ was added)

| | pH | ΔG (%) | KF[a]+ Van | KF | MRS[b] |
|---|---|---|---|---|---|
| *A. Boerenmetworst* | | | | | |
| Batter ($t=0$) | 5.71 | n.a.[c] | 5.43 | 5.42 | –[d] |
| After fermentation ($t=1$ day) | 5.11 | – | 5.22 | 5.34 | – |
| Sausage (after 28 days) | 5.30 | 43 | 4.84 | 5.10 | 8.57 |
| Sausage (after 62 days) | 5.40 | – | 4.15 | 5.36 | 7.76 |
| Sausage (after 89 days) | 5.40 | 45 | 4.26 | 4.43 | 7.76 |
| *B. Boerenmetworst (batter with additional 0.4% w/w glucose)* | | | | | |
| Batter ($t=0$) | 5.70 | n.a. | 5.11 | 5.38 | – |
| After fermentation ($t=1$ day) | 5.20 | – | 5.22 | 5.30 | – |
| Sausage (after 28 days) | 5.05 | 44 | 4.79 | 4.90 | 6.15 |
| Sausage (after 62 days) | 5.20 | – | 4.49 | 4.66 | 7.18 |
| Sausage (after 89 days) | 5.22 | 47 | 3.75 | 3.84 | 5.08 |
| *C. Snijworst* | | | | | |
| Batter ($t=0$) | 5.71 | n.a. | 5.40 | 5.41 | – |
| After fermentation ($t=2$ days) | 5.12 | – | 5.40 | 5.55 | – |
| Sausage (after 28 days) | 4.80 | 25 | 5.42 | 5.57 | 8.16 |
| Sausage (after 62 days) | 4.90 | – | 5.10 | 5.18 | 7.67 |
| Sausage (after 89 days) | 4.90 | 44 | 5.05 | 5.22 | 7.90 |

[a] KF: K–F Streptococcus agar.
[b] MRS: De Man, Rogosa, Sharpe agar.
[c] n.a. = not applicable.
[d] Not measured.

Table 5
Survival of two field isolates from fermented sausages (see Table 1) and lactic acid bacteria in two batches of fermented sausages (average numbers of colony forming units expressed as log values. ΔG = weight loss. KF + Van = KF to which 8 mg vancomycin $l^{-1}$ was added)

| | pH | ΔG (%) | KF[a] + Van | KF | KF + Van | KF | MRS[b] |
|---|---|---|---|---|---|---|---|
| | | | Efa 2 | Efa 2 | Efi 3 | Efi 3 | |
| *A. Boerenmetworst* [c] | | | | | | | |
| Batter ($t=0$) | 5.60–5.70 | n.a.[d] | 6.18 | 6.40 | 6.30 | 6.38 | –[b] |
| After fermentation ($t=1$ day) | 5.25–5.29 | – | 6.15 | 6.43 | 6.10 | 6.46 | 8.46–8.48 |
| Sausage (after 35 days) | 5.07–5.10 | – | 4.85 | 4.89 | 5.70 | 5.82 | 8.42–8.45 |
| Sausage (after 60 days) | 5.06–5.12 | 37 | 4.66 | 5.08 | LA[e] | LA | 8.71 |
| *B. Snijworst* [c] | | | | | | | |
| Batter ($t=0$) | 5.54–5.61 | n.a. | 6.32 | 6.60 | 6.90 | 6.90 | – |
| After fermentation ($t=2$ days) | 5.07–5.17 | – | 5.57 | 5.52 | 6.60 | 6.70 | 8.48–8.49 |
| Sausage (after 35 days) | 4.83–4.91 | – | 4.56 | 4.51 | 6.51 | 6.46 | 8.23–8.26 |
| Sausage (after 60 days) | 4.87–5.10 | 24 | 4.52 | 4.95 | LA | LA | 8.00–8.71 |

[a] See footnotes to Table 4.
[b] See footnotes to Table 4.
[c] Products were vacuum packaged after 7 days of ripening to stop desiccation.
[d] See footnotes to Table 4.
[e] Laboratory accident.

Table 6
Thermal resistance of the eight selected strains (average *D*-values in min; measured in the meat suspension containing curing ingredients)

| | 60 °C | 70 °C |
|---|---|---|
| Strains examined (codes shown in Table 1) | | |
| Efa 1 | 9.9 | –[a] |
| Efa 2 | 14.1 | – |
| Efi 1 | 39.7 | 0.36 |
| Efi 2 | 18.3 | – |
| Efi 3 | 20.3 | 0.45 |
| Edu | 12.4 | – |
| Ehi | 24.0 | – |
| Ega | 9.2 | – |

[a] Not determined.

decreased with time; directly after fermentation (Table 5), there were about (log units) 8.50 $g^{-1}$; after approximately 60 days numbers ranged between 8.00 and 8.71 $g^{-1}$.

Overall, the VRE strains showed their potential to persist in these fermented sausages.

### 3.3. Thermal resistance of VRE in meat suspensions

Table 6 shows the *D*-values obtained at 60 and 70 °C for the eight selected strains. The highest thermal resistance was observed with an *E. faecium* strain (Efi 1). *E. faecium* strains often show the highest thermal resistance amongst the vancomycin-sensitive enterococci (Houben, 1980).

The screening tests performed with the remaining 140 strains (results not shown) revealed that the strains generally had an estimated $D_{70\ °C}$ in MSI lower than 1.0 min. Estimates showed that the maximum $D_{70\ °C}$ value for a single strain might approximate 1.6 min.

Measurements performed under identical conditions in this laboratory recently showed $D_{70\ °C}$ values in MSI for *E. faecium* (E20) of up to about 50 min (unpublished results). Other literature sources report maximum $D_{70\ °C}$ values obtained in meat-containing substrates ranging from 13.6 (*E. faecium* RR1; Pedrazzoni et al., 1995) to ±3 min (*E. faecalis*: Reichert et al., 1988 and with another *E. faecalis* isolate: Ba-Dle and Zuber, 1996). The heat resistance of the studied VRE was generally weak compared to that of vancomycin-sensitive enterococci selected for their heat resistance.

In the Dutch meat industry, $P^{70}{}_{10}$ values of about 50 min and higher are usually applied. $P^{70}{}_{10}$ values can be interpreted as the time required at the reference temperature 70 °C that would be equivalent to the actual process with respect to the killing of micro-organisms with a *Z*-value of 10 °C. The *Z*-value of *E. faecium* E20 in comminuted ham tissue containing added brine was 8.8 and 11.8 °C for 24- and 48-h cultures, respectively (Houben, 1982). Assuming that the *Z*-values of the studied VRE strains are about 10 °C, in practice their chance of surviving pasteurisation appears negligibly small.

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