

Research Paper

Growth of Microorganisms in Total Parenteral Nutrition Solutions Containing Lipid

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Abstract

Background: To identify the microorganisms that can grow rapidly in total parenteral nutrition (TPN) solutions, we investigated the growth of the major causes of catheter-related blood stream infection (*Staphylococcus aureus*, *Serratia marcescens*, *Bacillus cereus*, and *Candida albicans*) in TPN solutions containing lipid. **Methods:** The pH value of a TPN solution containing lipid (pH 6.0, containing 20 ppm of NaHSO₃) was adjusted by the addition of HCl to 5.7, 5.4, or 4.9. The pH value of another TPN solution (pH5.5, containing 400 ppm of NaHSO₃) was adjusted by the addition of NaOH to 5.9, 6.3, or 6.8. A specific number of each microorganism was added to 10 mL of each test solution and incubated at room temperature. The number of microorganisms was counted as colony forming units at 0, 24, and 48 hrs later. **Results:** *C. albicans* increased similarly at any pH values in the TPN solution. The bacterial species also increased rapidly at pH6.0 in the solution containing 20 ppm of NaHSO₃, but growth was suppressed as the pH value was reduced, with growth halted at pH4.9. However, these bacterial species did not increase, even at pH5.9, in the other solution containing 400 ppm of NaHSO₃. **Conclusions:** These results suggest that *Candida* species can grow rapidly in almost all TPN solutions regardless of the acidity, lipid, and NaHSO₃; also, some bacterial species may grow in TPN solutions containing lipid unless the pH value is 5.0 or less. Therefore, each TPN solution should be examined whether or not the bacterial species can proliferate.

Key words: CRBSI, microbial growth, TPN solution, lipid, pH, bisulfite

INTRODUCTION

Catheter-related blood stream infection (CRBSI) is one of the most common complications of intravenous catheters.¹⁻³ To reduce or prevent CRBSI, factors that enhance or inhibit microbial growth in parenteral solutions should be investigated and identified. Total parenteral nutrition (TPN) solutions are considered to be relatively good growth media for microorganisms,^{1,4} whereas a number of investigators have shown that TPN solutions are poor growth me-

dia for most microorganisms that cause CRBSI, with the exception of *Candida* species.⁵⁻⁹ Likewise, we have previously confirmed that while bacterial species do not grow in TPN solutions without lipid, *Candida* species grow rapidly.¹⁰ Our data have shown that bacterial species cannot grow due to the acidity, but *Candida* species can grow regardless of the acidity.¹⁰ In contrast, most microorganisms grow rapidly in commercial lipid emulsions,¹¹⁻¹⁵ and sporadic CRBSI and

outbreaks have been traced to contaminated lipid emulsion.^{16,17} Because lipid emulsions have been shown to contribute to the rapid growth of most microorganisms that cause CRBSI, it can be assumed that TPN solutions containing lipid promote the microbial growth compared with TPN solutions without lipid.¹⁸ Consequently, the Center for Disease Control and Prevention (CDC) has recommended that when any infusion solutions containing lipid are administered, the entire delivery system such as the administration set be replaced every 24 hours.¹⁹

As the major causes of CRBSI, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Serratia marcescens*, *Escherichia coli*, *Klebsiella pneumoniae*, *Candida albicans*, etc. were shown.^{2,3,6,11} Furthermore, blood stream infection outbreaks of *Bacillus cereus* via intravenous line were recently reported in Japan.²⁰ In the present study, we investigated the growth in TPN solutions containing lipid of the same microorganisms studied previously:¹⁰ ie, *Staphylococcus aureus* as a delegate of gram positive cocci, *Serratia marcescens* as a delegate of gram negative rods, *Bacillus cereus* as a delegate of gram positive rods, and *Candida albicans* as a delegate of fungi. To clarify whether the acidity of TPN solutions suppresses microbial growth in the presence of lipid, the growth of all strains employed were investigated at various pH values in a commercial TPN solution containing lipid in the first experiment. To identify which factors enhance or inhibit the growth of each microorganism, we supplemented a

TPN solution containing lipid with multivitamins, supplemented a TPN solution that did not contain lipid with lipid, and increased the bisulfite concentration in a TPN solution containing both lipid and multivitamins in the following experiments.

MATERIALS AND METHODS

Microorganisms employed

A standard American Type Culture Collection (ATCC) strain and 1 clinical isolate were used for each microorganism; the standard strain ATCC6538 and the clinical isolate N3 of *Staphylococcus aureus*, the standard strain ATCC13880 and the clinical isolate N4 of *Serratia marcescens*, the standard strain ATCC11778 and the clinical isolate H2 of *Bacillus cereus*, and the standard strain ATCC10231 and the clinical isolate N7 of *Candida albicans*.

Test solutions

A commercial TPN solution containing lipid (ML; MIXID-L, Otsuka Pharmaceutical Factory, Inc., Japan), a commercial TPN solution without lipid (AT1; AMINOTORIPA-1, Otsuka Pharmaceutical Factory, Inc.), multivitamins (MV; Otsuka MV Injection, Otsuka Pharmaceutical Factory, Inc.), a 20% lipid emulsion (IL; Intralipos 20%, Otsuka Pharmaceutical Factory, Inc.), and a physiological saline were used. The compositions of ML and AT1 are shown in Table 1.

Table 1. The compositions of ML and AT1

Solution (volume)	ML	AT1
Amino acids	900 mL	850 mL
Glucose	30.0 g	25.0 g
Fructose	110.0 g	79.8 g
Xylitol	-	40.2 g
Lipid	-	19.8 g
Na ⁺	15.6 g	-
K ⁺	35 mEq	35 mEq
Mg ²⁺	27 mEq	22 mEq
Ca ²⁺	5 mEq	4 mEq
Cl ⁻	8.5 mEq	4 mEq
SO ₄ ²⁻	44 mEq	35 mEq
Acetate ⁻	5 mEq	4 mEq
Gluconate ⁻	25 mEq	44 mEq
Citrate ³⁻	8.5 mEq	4 mEq
P	-	10 mEq
Zn	150 mg	154 mg
NaHSO ₃	10 μmol	8 μmol
pH	17 mg	412 mg
	6.0	5.5
Osmotic pressure ratio to physiological saline	Approx. 4	Approx. 5

Experiment 1: ML was used as the base solution. The pH value, osmotic pressure ratio to physiological saline (OPR, the osmolality of each solution/the osmolality of physiological saline), and concentration of NaHSO₃ of ML were 6.0, approximately 4, and 17 ppm (about 20 ppm), respectively. The other pH solutions of ML were adjusted to 5.7, 5.4, or 4.9 by the addition of 0.5 mol/L HCl.

Experiment 2: ML(pH6.0), ML(pH4.9), and those supplemented with MV (MLV) were used.

Experiment 3: AT1 was supplemented with MV and used as the base solution (AT1V). The pH value, OPR, and concentration of NaHSO₃ of AT1V were 5.5, approximately 5, and 412 ppm (about 400 ppm), respectively. The other pH solutions of AT1V were adjusted to 5.9, 6.3, or 6.8 by the addition of 0.5 mol/L NaOH. Each pH solution of AT1V was added with 1/10 volume of IL (L+AT1V) or physiological saline (S+AT1V).

Experiment 4: ML was supplemented with MV and used as the base solution (MLV). The other pH solutions of MLV were adjusted to 5.7, 5.4, or 5.0 by the addition of 0.5 mol/L HCl. Besides, MLV(pH5.7) and MLV(pH5.4) were added with 100 ppm (MLV+100 ppm) or 200 ppm of NaHSO₃ (MLV+200 ppm).

Addition of microorganism, incubation and sampling

A specific number of each test microorganism was added to each 10 mL of test solutions in sterile plastic tubes, and all tubes were allowed to stand at room temperature (23-26°C). An aliquot of test solution was sampled at 0, 24, and 48 hours after the addition of microorganisms.

Measurement of viable microorganisms

Each aliquot of test solution sampled was inoculated in a Soybean Casein Digest (SCD) agar plate in duplicate. When necessary, the aliquot of test solution was diluted 10-fold to 10⁷-fold with physiological saline before inoculation. After a 24-hour incubation at 37°C, the number of colony forming units (CFU) of each microorganism was counted for each plate. The mean CFU of duplicate data was calculated for each aliquot, and number of each microorganism per mL was calculated by using the number of CFU per plate, aliquot volume, and diluting ratio. The results are shown as values of CFU/mL in semi-logarithmic graphs. As other experimental studies of microbial growth,^{5,12,13,21,22} the data obtained in this study were not analyzed statistically, because biological significance of these kinds of data is considered to be assessable without statistical analysis.

RESULTS

Experiment I

S. aureus increased in ML at the original pH of 6.0, but growth was suppressed as the pH value was reduced, coming to a halt at a pH of 4.9 (Figure 1). *S. marcescens* increased rapidly in ML at a pH of 6.0, also increased but with suppression at a pH of 5.7, and did not increase at pH values of 5.4 and 4.9 (Figure 2). *B. cereus* increased at a pH of 6.0. Its growth was suppressed as the pH value was reduced, decreasing at a pH of 4.9 (Figure 3). However, both strains of *C. albicans* increased rapidly and similarly at any pH value (Figure 4).

Staphylococcus aureus

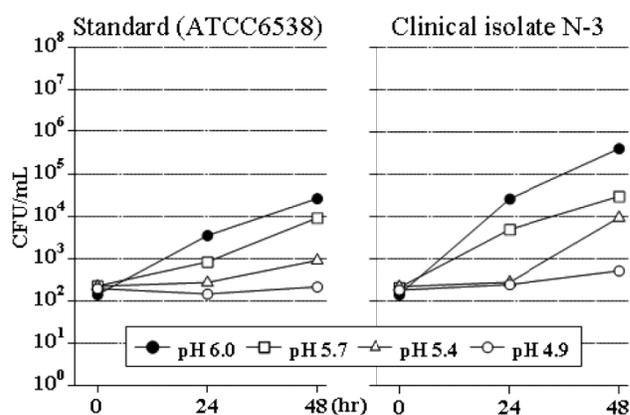


Figure 1. Effect of pH on the growth of *Staphylococcus aureus* in ML (pH6.0; NaHSO₃, 20 ppm). The pH value was adjusted by addition of 0.5 mol/L HCl.

Serratia marcescens

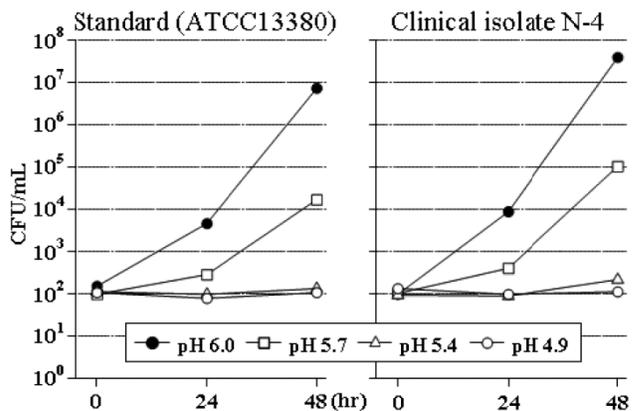


Figure 2. Effect of pH on the growth of *Serratia marcescens* in ML (pH6.0; NaHSO₃, 20 ppm). The pH value was adjusted by addition of 0.5 mol/L HCl.

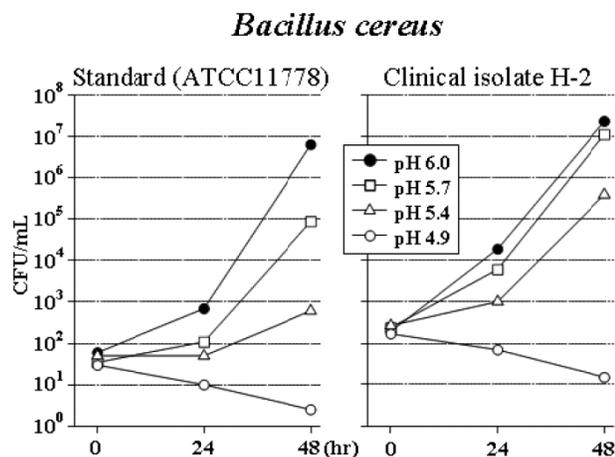


Figure 3. Effect of pH on the growth of *Bacillus cereus* in ML (pH6.0; NaHSO₃, 20 ppm). The pH value was adjusted by addition of 0.5 mol/L HCl.

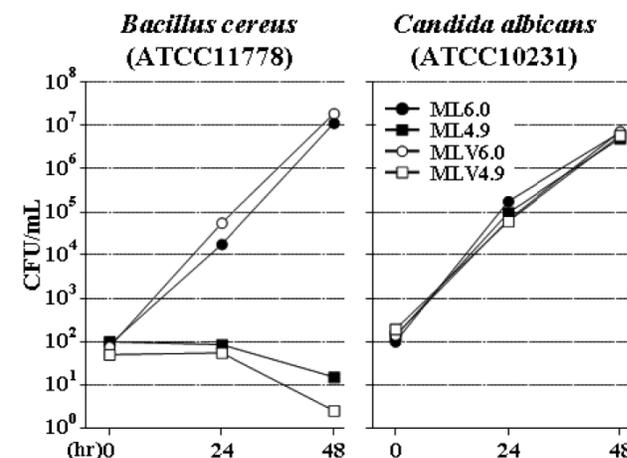
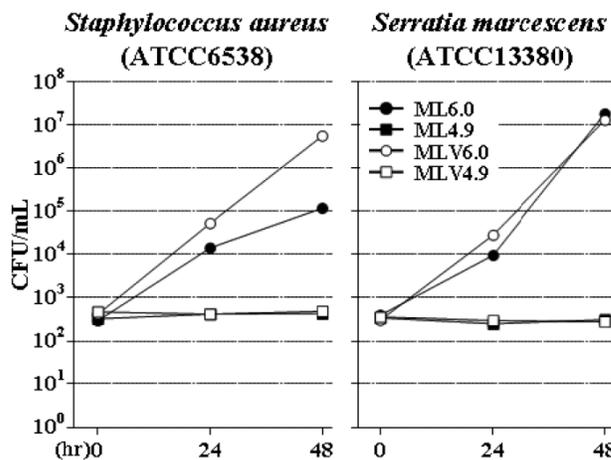


Figure 5. Growth of *Staphylococcus aureus*, *Serratia marcescens*, *Bacillus cereus*, and *Candida albicans* at pH6.0 and pH4.9 in ML and in MLV (ML supplemented with multivitamins).

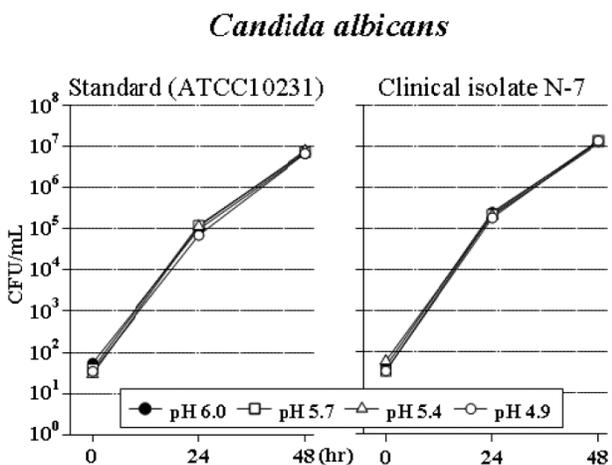


Figure 4. Effect of pH on the growth of *Candida albicans* in ML (pH6.0; NaHSO₃, 20 ppm). The pH value was adjusted by addition of 0.5 mol/L HCl.

Experiment 2

Supplementation with MV to ML enhanced only the growth of *S. aureus* at a pH of 6.0 but did not resume the growth at a pH of 4.9 (Figure 5). Concerning *S. marcescens*, *B. cereus* and *C. albicans*, MV did not affect them both at pH 6.0 and at pH 4.9 (Figure 5).

Experiment 3

The OPR of AT1V was 4.6, and the OPR of L+AT1V (or S+AT1V) was 4.3 (or 4.2) as a result of the diluting effect from the addition of the isotonic solution.

In AT1V, the standard strains of *S. aureus*, *S. marcescens* and *B. cereus* did not increase at pH 5.9 as well as at the original pH of 5.5 but increased slowly at pH 6.3 and increased rapidly at pH 6.8 (Figures 6-8), similar to the results of our previous study.¹⁰

Surprisingly, none of *S. aureus*, *S. marcescens* and *B. cereus* increased in L+AT1V at either pH 5.5 or pH 5.9, despite the presence of lipid (Figures 6-8). These results conflicted with those of Experiment 1. At pH 6.3 and pH 6.8, however, supplementation with 1/10 volume of IL to AT1V (L+AT1V) enhanced mildly the growth of these bacterial species (Figures 6-8).

Addition of 1/10 volume of physiological saline

to AT1V (S+AT1V) contributed to the reduction of OPR from 4.6 to 4.2, enhancing the growth of the bacteria less slightly than did supplementation with IL (Figures 6-8).

Experiment 4

The growths of 2 strains of *S. aureus* in MLV were halted only at pH 5.0 (Figure 9). The addition of 100 ppm of NaHSO₃ was not enough to stop the growth of *S. aureus*. However, when 200 ppm of NaHSO₃ was added, the growth of the standard strain was halted at pH 5.7 and the clinical isolate was halted at pH 5.4 (Figure 9).

The growth of 2 strains of *S. marcescens* in MLV were halted at pH 5.0 and pH 5.4 (Figure 10), corresponding with the results of Experiment 1. The addition of 200 ppm of NaHSO₃ halted the growth of both strains of *S. marcescens* at pH 5.7 (Figure 10).

The growth of the standard strain of *B. cereus* in MLV was halted both at pH 5.0 and pH 5.4, and the growth of the clinical isolate was halted at pH 5.0 (Figure 11). The addition of 200 ppm of NaHSO₃ halted the growth of both strains of *B. cereus* at pH 5.7, and the addition of 100 ppm of NaHSO₃ halted the growth of the clinical isolate at pH 5.4 (Figure 11).

Staphylococcus aureus (ATCC6538)

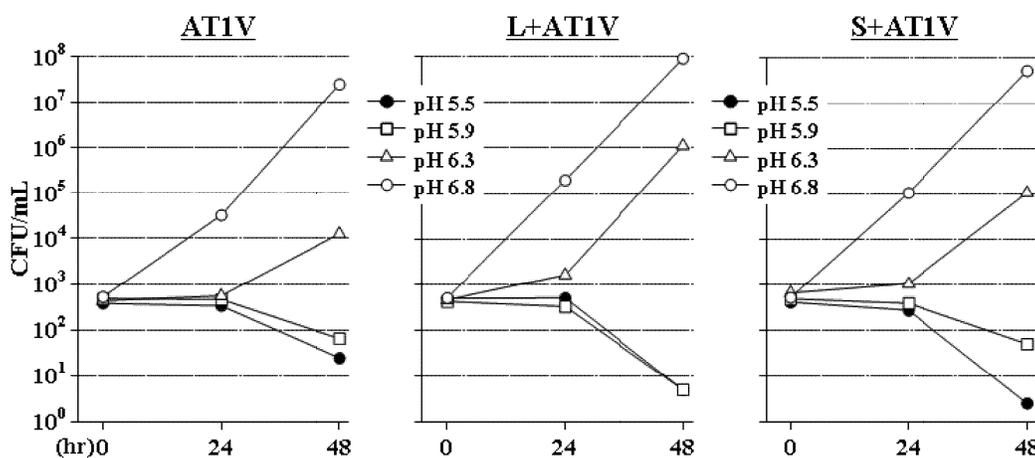


Figure 6. Effect of lipid on the growth of *Staphylococcus aureus* in AT1V (pH5.5; NaHSO₃, 400 ppm). The pH value was adjusted by addition of 0.5 mol/L NaOH. To AT1V was added 1/10 volume of 20% lipid emulsion (L+AT1V) or physiological saline (S+AT1V).

Serratia marcescens (ATCC13380)

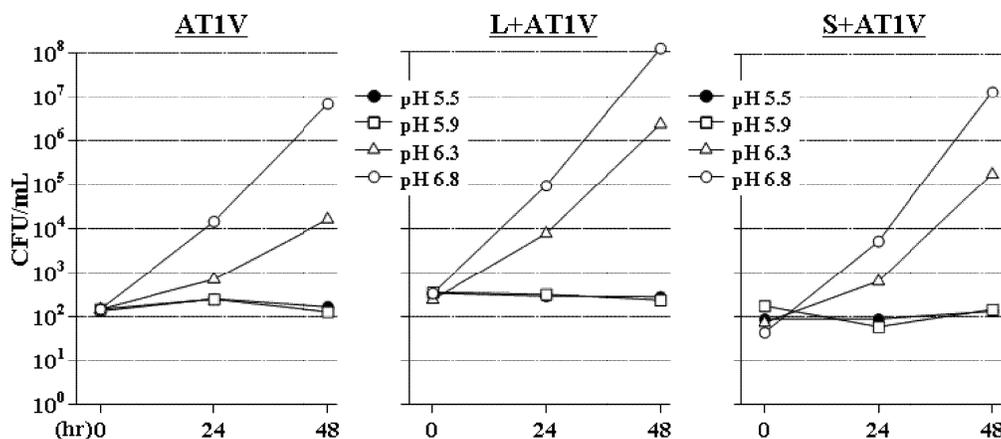


Figure 7. Effect of lipid on the growth of *Serratia marcescens* in AT1V (pH5.5; NaHSO₃, 400 ppm). The pH value was adjusted by addition of 0.5 mol/L NaOH. To AT1V was added 1/10 volume of 20% lipid emulsion (L+AT1V) or physiological saline (S+AT1V).

Bacillus cereus (ATCC11778)

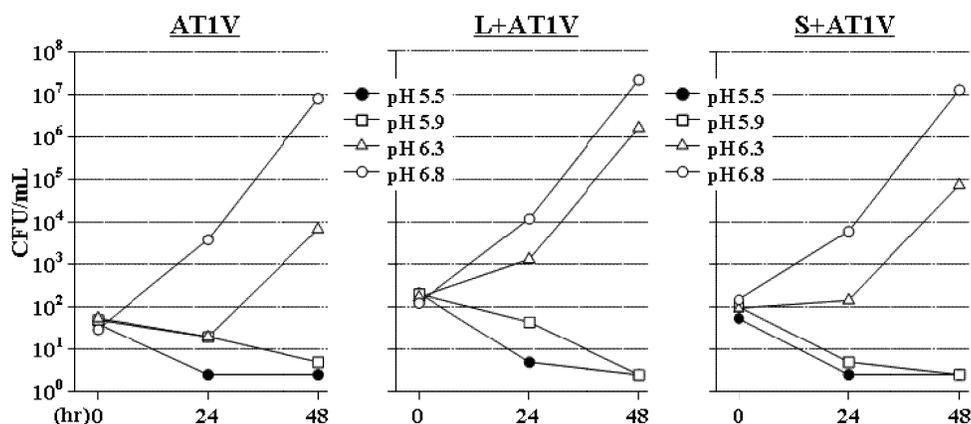


Figure 8. Effect of lipid on the growth of *Bacillus cereus* in ATIV (pH5.5; NaHSO₃, 400 ppm). The pH value was adjusted by addition of 0.5 mol/L NaOH. To ATIV was added 1/10 volume of 20% lipid emulsion (L+ATIV) or physiological saline (S+ATIV).

Staphylococcus aureus

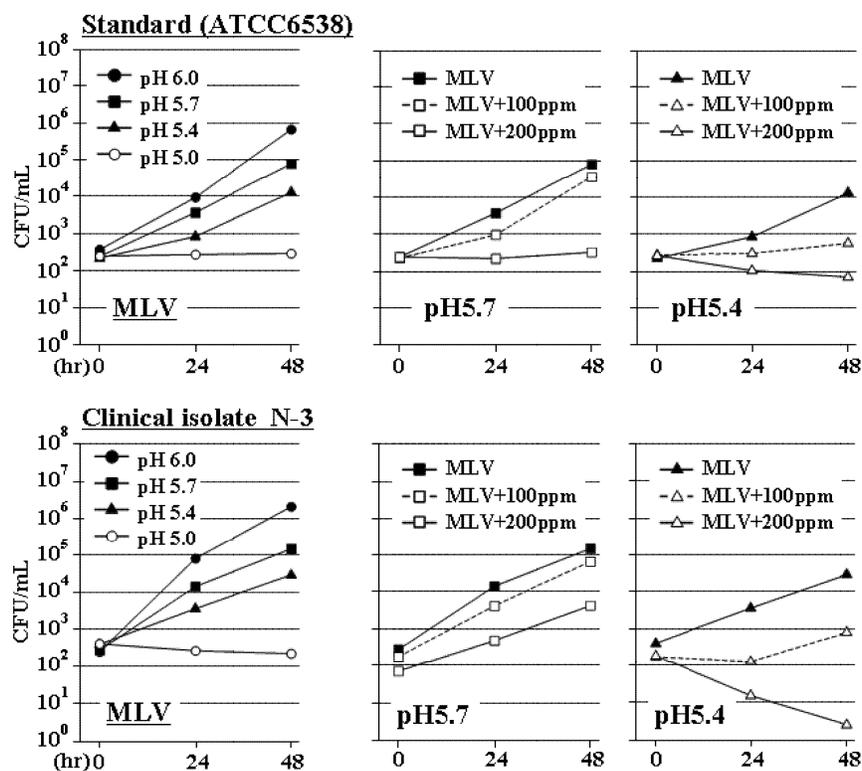


Figure 9. Effect of bisulfite concentration on the growth of *Staphylococcus aureus* in MLV (ML supplemented with multivitamins). The pH value was adjusted by addition of 0.5 mol/L HCl. To MLV was added 100 ppm or 200 ppm of NaHSO₃ (+100ppm or +200ppm).

Serratia marcescens

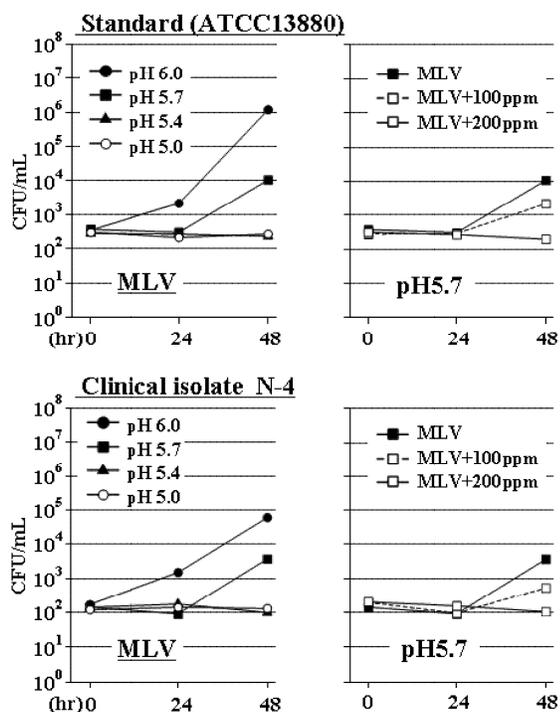


Figure 10. Effect of bisulfite concentration on the growth of *Serratia marcescens* in MLV (ML supplemented with multivitamins). The pH value was adjusted by addition of 0.5 mol/L HCl. To MLV was added 100 ppm or 200 ppm of NaHSO₃ (+100ppm or +200ppm).

Bacillus cereus

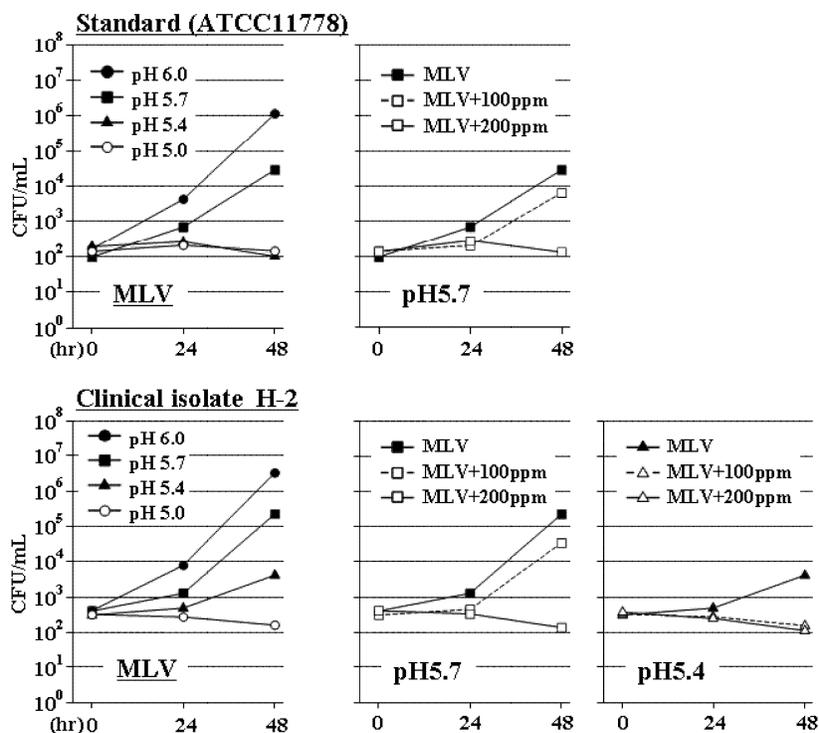


Figure 11. Effect of bisulfite concentration on the growth of *Bacillus cereus* in MLV (ML supplemented with multivitamins). The pH value was adjusted by addition of 0.5 mol/L HCl. To MLV was added 100 ppm or 200 ppm of NaHSO₃ (+100ppm or +200ppm).

DISCUSSION

To reduce or prevent catheter-related blood stream infection (CRBSI), we have to understand the growth properties of the microorganisms that cause this condition. We have previously investigated the growth of the microorganisms that are known as the major causes of CRBSI (*Staphylococcus aureus*, *Serratia marcescens*, *Bacillus cereus* and *Candida albicans*) in total parenteral nutrition (TPN) solutions without lipid.¹⁰ Therefore, we investigated the growth of the same microorganisms in TPN solutions containing lipid in the present study.

In a commercial TPN solution containing lipid (ML), both standard strains and clinical isolates of all microorganisms (*S. aureus*, *S. marcescens*, *B. cereus* and *C. albicans*) increased rapidly at the original pH of 6.0, even without multivitamins. Although only *C. albicans* increased equally at any pH value, the growth of *S. aureus*, *S. marcescens* and *B. cereus* was suppressed as the pH value was reduced, with growth halted at pH4.9 (Experiment 1). However, these 3 bacterial species did not increase in another TPN solution containing lipid (L+AT1V) even at pH5.5 and pH5.9 (Experiment 3), which is the same result as obtained in the solution without lipid (AT1V or S+AT1V); this finding conflicts with the results of Experiment 1. The conflicting results from these 2 TPN solutions may be attributable to the difference in the bisulfite concentrations (ML contains NaHSO₃ at a very low concentration [20 ppm], but L+AT1V contains NaHSO₃ at a relatively high concentration [400 ppm]) because the bactericidal effect of bisulfite is enhanced in acidic conditions.²¹ Therefore, the additional experiment (Experiment 4) was performed to investigate the effect of bisulfite concentration in the TPN solution containing both lipid and multivitamins (MLV). As a result, the growth of the 3 bacterial species was suppressed or halted at the same pH (5.4 or 5.7) as the concentration of NaHSO₃ increased (20 ppm, 100 ppm, and 200 ppm). These results suggest that the concentration of bisulfite in TPN solutions is an important factor for suppressing bacterial growth, especially between pH5.0 and pH6.0: the bacterial species cannot increase at pH5.9 with 400 ppm of NaHSO₃, at pH5.7 or pH5.4 with 200 ppm of NaHSO₃, and at pH5.0 with 20 ppm of NaHSO₃.

Other findings in the present study are as follows: 1) even if lipid is contained, the acidity of TPN solution is the critical factor suppressing the bacterial growth; 2) the addition of lipid enhances mildly the growth of the bacterial species in TPN solutions but does not affect the growth substantially; 3) the addition of multivitamins further enhances the growth of

S. aureus but does not affect the growth of *S. marcescens*, *B. cereus*, and *C. albicans* in TPN solutions containing lipid; 4) *C. albicans* can grow regardless of acidity, bisulfite, and lipid.

Because *C. albicans* could grow at pH5.5 with 400 ppm of NaHSO₃ (AT1V) in our previous study,¹⁰ the effect of bisulfite concentration on the growth of *C. albicans* was not investigated in the present study. However, it has been reported that *C. albicans* could not increase in a TPN solution at pH4.4 with 500 ppm of NaHSO₃, whereas *C. albicans* increased in the same TPN solution at pH4.4 with 40 ppm of NaHSO₃ or at pH5.0 with 500 ppm of NaHSO₃.²³ Practically, *Candida* species can grow rapidly in almost all TPN solutions.

The pH values of most of the recent TPN solutions are within 5.0 and 6.0, similar to the old TPN solutions. On the other hand, the old TPN solutions contain bisulfite at relatively high concentrations, but the recent TPN solutions contain very low concentrations of bisulfite or are bisulfite-free. To investigate bacterial growth in the recent TPN solutions, referring to results from the studies that used the old TPN solutions that contained high concentration of bisulfite is not appropriate, even at the same pH range. In the recent TPN solutions containing lipid, some bacterial species may proliferate unless the pH value is 5.0 or less. Although the TPN solutions containing lipid can be theoretically improved to be bacteriostatic by reducing the pH value and/or increasing the bisulfite concentration, more studies seem needed to improve the solution because lipid emulsions become unstable as the pH value reduces or as the concentration of bisulfite increases.

In conclusion, *Candida* species can grow rapidly in almost all TPN solutions regardless of the acidity and the presence of lipid; also, some bacterial species may grow in TPN solutions containing lipid unless the pH value is 5.0 or less. Therefore, each TPN solution should be investigated to determine whether or not the bacterial species can proliferate.

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Conflict of Interest

We declare that there are no conflicts of interest for all of us.

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