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Short communication: Evaluation of amino acid consumption and necessary profiles of *Streptococcus thermophilus* T1C2 in controlled pH batch fermentations

C. Hong, Z. Shuang, X. Miao, Z. Min, L. Xin-Tong, D. Hong-Ling, M. Chun-Li, and F. Zhen[1]
Key Laboratory of Dairy Science of Ministry of Education, College of Food Science, Northeast Agricultural University, 59 Mucai Road, Harbin 150030, Heilongjiang, China

ABSTRACT

The objective of the present study was to elucidate the relationship between amino acid consumption and necessary profiles of *Streptococcus thermophilus* T1C2 to guide the design of media for high-cell-density culture. The amino acid consumption and necessary patterns of *S. thermophilus* T1C2 were investigated in the complete chemically defined medium. For amino acid consumption profiles throughout the growth of *S. thermophilus* T1C2, the most abundantly consumed amino acids were Gln and Arg, which accounted for 19 and 20% of total amino acids consumed, respectively. Asparagine, Thr, Ser, Ala, Val, Met, Leu, and Lys, consumptions of which ranged from 3 to 10% of total amino acids consumed, were the second most intensively consumed amino acids. For necessary amino acid patterns, the amount of Cys, which counted for 11% of total amino acids needed, was significantly higher than the amounts required for other amino acids in growth of *S. thermophilus* T1C2. The necessary amounts of Asp, Asn, Glu, Gln, Arg, Ala, Met, and Tyr ranked second, ranging from 5 to 8% of total amino acids needed. Compared with necessary amounts, the consumption of Asn, Thr, Ser, Gln, Arg, Ala, Val, Leu, Lys, His, and Phe exceeded the necessary amounts for growth of *S. thermophilus* T1C2 remarkably. Consumption of Gly, Met, Ile, Trp, and Pro was slightly higher than the necessary amounts. Consumption of Asp, Glu, Tyr, and Cys was lower than the necessary amounts. The overall consumption of amino acids exceeded the required amount for growth of *S. thermophilus* T1C2 almost 2.43 times, which implied a significant nitrogen wasting.

Key words: *Streptococcus thermophilus*, amino acid, consumption, necessary

Short Communication

Streptococcus thermophilus, an essential lactic acid bacterium, has been commonly used for commercial production of yogurt and cheese. It is considered the second most important industrial dairy starter after *Lactococcus lactis* (Prajapati et al., 2013). *Streptococcus thermophilus* are fastidious organisms; they require not only carbohydrates as energy and carbon source, but also AA, nucleotides, and vitamins for growth in a defined medium. Their complex nutrient requirements are usually satisfied in natural or complex growth media by the addition of undefined compounds, such as peptone, meat, and yeast extract (Hongfei et al., 2013). The production of direct-vat-set yogurt is usually carried out in complex nutrient media to obtain relatively high biomass levels. Media design for high-cell-density cultivation is based on knowledge of nutritional requirements and necessary patterns for growth of *S. thermophilus*. However, current knowledge on nutritional requirements of *S. thermophilus*, especially AA consumption and requirement, is insufficient. The lack of reliable information on consumption and necessary patterns of AA hinders the rational design of cultivation media and optimization of bioprocesses.

For high-cell-density culture of *S. thermophilus*, protein hydrolysates, peptone, peptide, and yeast extract were common nitrogen sources for growth. However, current select tests of nitrogen sources do not consider the nutritional requirements of *S. thermophilus*. Furthermore, the proliferation effect of nitrogen source is dependent on AA consumption and necessary patterns of *S. thermophilus*. The media design of high-cell density cultivation therefore required thorough understanding of the AA requirements for *S. thermophilus*.

In a previous study, *S. thermophilus* T1C2 was selected and displayed excellent properties in cheese and yogurt starter (Ma et al., 2011). The purpose of present study was to evaluate AA consumption and necessary patterns for growth of *S. thermophilus* T1C2. The ultimate goal was to pave a way for high-cell density cultivation of *S. thermophilus* T1C2.

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[1]Corresponding author: NEAU_FengZhen@163.com

Streptococcus thermophilus T1C2 was cultivated from our previous study (Ma et al., 2011). Cultures were stored at −70°C in 15% sterile reconstituted skim milk containing 10% glycerol. Complete chemically defined medium (**CDM**) was prepared as described in detail by Lahtvee et al. (2011). Batch experiments were performed in a 5-L Biotech-7000 bioreactor (Shanghai Baoxing, Shanghai, China). The reactor was filled with 1.5 L of culture medium. Temperature and rotation speed were fixed at 40°C and 80 rpm, respectively. The pH was maintained at 6.5 by automatic addition of 0.1 mol/L of NaOH.

Streptococcus thermophilus T1C2 was precultured at 37°C in M17 broth for 16 h. The culture was centrifuged (10,000 × *g*, 10 min, 4°C), and cells were washed twice with PBS buffer (50 mmol/L, pH 6.5). Inoculation of growth medium was carried out at 0.1% (vol/vol); growth rate was measured by spectrophotometric measurement at 650 nm. Biomass dry weight (**DW**) was determined gravimetrically. Each culture condition was repeated 5 times.

Free AA concentrations of supernatants were determined by an AA analyzer (Acquity UPLC; Waters Corp., Milford, MA). For measuring whole-cell AA composition of *S. thermophilus* T1C2, biomass was hydrolysed with 6 mol/L of HCl for 20 h at 120°C. Tryptophan was measured by alkaline hydrolysis with 5 mol/L of NaOH for 17 h at 100°C. Determination of Cys was carried out according to method of Hoogerheide and Campbell (1992). Determination of Gln and Asn was performed according to methods of Kuhn et al. (1996) and Zhang et al. (2012). After hydrolyzing, AA were determined as free AA as described previously.

Based on AA concentrations in the cultivation broth, consumption patterns of the AA were calculated with average growth rate ($\boldsymbol{\mu}$; Figure 1). The overall consumption of AA decreased from 13.82 to 11.09 mmol/g of DW with increasing μ. The results indicated that the most abundantly consumed AA were Gln (19% of total AA consumed) and Arg (20% of total AA consumed) throughout the growth of *S. thermophilus* T1C2, respectively, followed by the consumptions of Asn, Thr, Ser, Ala, Val, Met, Leu, and Lys, which ranged from 3 to 10% of total AA consumed; other AA had lower consumption amounts.

Based on AA concentrations in the whole-cell hydrolysate, necessary patterns of the AA were calculated with μ (Figure 2). The overall necessary amounts of AA had no significant change (from 5.23 to 5.03 mmol/g of DW) with increasing μ. Cysteine was significantly high in necessary amounts of AA of *S. thermophilus* T1C2, which covered 11% of total AA needed, followed by necessary amounts of Asp, Asn, Glu, Gln, Arg, Ala, Met, and Tyr, which covered 5 to 8% of total AA needed; other AA had lower necessary amounts.

Compared with necessary amount, the consumption of Asn, Thr, Ser, Gln, Arg, Ala, Val, Leu, Lys, His, and Phe notably exceeded the necessary amounts for growth of *S. thermophilus* T1C2. Among which, Gln, Arg, Asn, Thr, Ser, Ala, Val, Leu, and Lys were intensively consumed, whereas the consumption amounts of His and Phe were relatively small. Serine, Gln, Arg, Val, Ala, Leu, Lys, His, and Phe were essential AA, whereas Asn and Thr were nonessential AA for *S. thermophilus* T1C2 (Figure 3). Furthermore, Gly, Met, Ile, Trp, and Pro were consumed slightly higher than the necessary amounts. Among which, Met was an intensively consumed AA, followed by the consumption amounts of Gly, Ile, Trp, and Pro. In addition, Met and Trp were essential AA, whereas Gly, Ile, and Pro were nonessential AA for *S. thermophilus* T1C2. Consumption of Asp, Glu, Tyr, and Cys was lower than needed for growth of *S. thermophilus* T1C2; they were among the lowest consumption amounts of AA. In addition, Glu and Tyr were essential AA, whereas Asp and Cys were nonessential AA for *S. thermophilus* T1C2.

When 3 branched-chain AA were omitted from the complete CDM, Ile did not affect the growth of *S. thermophilus* T1C2, whereas Val and Leu affected the growth of *S. thermophilus* T1C2 (Figure 3). This feature was in agreement with the observation for *S. thermophilus* ST18 in the complete CDM deprived of the branched-chain AA (Letort and Juillard, 2001). The results indicated that the biosynthesis of branched-chain AA might not meet requirements for growth of *S. thermophilus* T1C2. In addition, growth of *S. thermophilus* T1C2 was characterized by prolonged lag times (up to 12 h) in a simplified growth medium containing essential AA (data not showed). This suggested that nonessential AA were also conducive to the growth of *S. thermophilus* T1C2; similar results were also reported by Law and Kolstad (1983). Results of the present study indicated that essential AA did not have to have high amounts of consumption. The casein hydrolysates containing His, Lys, Glu, and Ser AA residua were beneficial for growth of *S. thermophilus* (Zhang et al., 2011). The soy protein hydrolysate, containing large amounts of Asp, Ile, Leu, His, Met, Glu, and Val, were able to promote the proliferation of *S. thermophilus* (Hongfei et al., 2013). According to the present study, it might be speculated that these protein hydrolysates contained AA that could meet AA consumption and necessary profiles of *S. thermophilus*. As His, Lys, Glu, Ser, Leu, His, Met, and Val were essential AA, Lys, Ser, Leu, Met, and Val were intensively consumed AA; Glu, Asp, and Met had high necessary amounts.