Regulation of a Mixed Culture of *Streptococcus lactis* and *Saccharomyces fibuliger*

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**Summary.** In a mixed culture of *Saccharomyces fibuliger* Y76 and *Streptococcus lactis* 65.1 on starch the main interactions are commensalism and competition. Oxygen-limited batch and continuous cultures of *S. fibuliger* showed accumulation of sugars. When oxygen was used as an external regulatory parameter in the mixed culture lactic acid, acetic acid and formic acid were formed. Ethanol produced by *S. lactis* was most likely assimilated by *S. fibuliger*. Continuous mixed cultures were stable under conditions of oxygen limitation at the dilution rates tested (0.10 h⁻¹ and 0.15 h⁻¹). Conversion yields of 35 to 40% were obtained but may be improved.

**Introduction**

Interactions between two microbial species in mixed cultures may be of different types such as commensalism, mutualism and competition (Harrison 1978). One difficulty in using mixed cultures industrially is the instability of most systems, especially during continuous steady-state operations. Stability is ensured, in a mixed continuous culture, if the product of the first organism is the growth limiting substrate for the other organism (commensalism) (Lee et al. 1976). Mixed continuous cultures based on pure mutualism are instable but may stabilize when component species are competing for a third substrate (Meyer et al. 1975). A system where the organisms are competing for the same growth-limiting substrate is unstable. Double substrate-limitation in a competing system may lead to the creation of stable steady-state dilution rate regions (Yoon and Blanch 1977).

*Streptococcus lactis* which belongs to the N-streptococci is regarded as homofermentative and is used in the dairy industry where it produces lactic acid from lactose in milk. These organisms are fermentative but aerotolerant. In batch culture *S. lactis* 65.1 ferments glucose to lactic acid whereas it forms acetic acid, ethanol, formic acid and lactic acid from maltose. Glucose and maltose are sequentially metabolized in batch culture but are both utilized simultaneously in continuous carbohydrate-limited cultures (Häggström unpublished data). *Saccharomyces fibuliger* (Endomycopsis fibuliger) is an aerobic organism which can hydrolyze starch.

This report concerns a mixed culture system of *S. lactis* and *S. fibuliger* in which the main interactions are commensalism and competition. The aim of the investigation was to stabilize and regulate the composition of the mixed culture by limiting the supply of oxygen and to study the product formation.

**Materials and Methods**

**Organisms**

*Streptococcus lactis* 65.1 from the Swedish Dairies Association, Malmö, Sweden, was stored in litmus milk at −20°C. *Saccharomyces fibuliger* NRRL Y76 was kept on yeast extract peptone agar at +4°C and was transferred every third week.

**Media**

The medium used for fermenter cultivations of *S. fibuliger* in monoculture contained in g l⁻¹: yeast extract, 5.0; K₂HPO₄, 2.5; KH₂PO₄, 2.5; MgSO₄·7H₂O, 0.5; (NH₄)₂SO₄, 5.0 and soluble starch, 15.0. For growing inocula the same medium was used but with 10 g l⁻¹ glucose instead of starch. In mixed culture fermenter cultivations the monoculture medium described above was used but tryptone (5.0 g l⁻¹) and casamino acid (1.0 g l⁻¹) were added. Inocula of *S. lactis* were grown on the mixed culture medium without (NH₄)₂SO₄ and starch bath with the addition of maltose (20.0 g l⁻¹).
Growth Conditions and Measurements

Fermenters with working volumes of 2.5 and 0.7 litres (Chemoferm, Hagersten, Sweden) were used for the batch and continuous cultures. The pH was kept constant at 6.5 with sodium hydroxide and the temperature was maintained at 30 °C. The volume in the continuous culture experiments was kept constant with a conductivity type level controller.

Inocula for the batch mixed cultures were prepared by growing the two component strains separately and then mixing them on inoculation. The continuous mixed cultures were started as a continuous monoculture of *S. fibuliger*. When steady-state conditions had been reached 20% of the culture was pumped out and replaced by *S. lactis* from a batch culture grown on maltose.

The rate of cell growth was followed by measuring culture turbidity in a Turner photometer at 620 nm. The dry weight of the cells in the monocultures was determined by centrifuging a cell suspension, washing the pellet and drying it for 12 h at 105 °C.

Analyses of Substrate and Products

The presence of starch was qualitatively tested with Lugol's solution. Cells in the samples from the fermenter were rapidly removed by centrifugation and membrane filtration and the supernatants were analysed. Reducing sugars were determined according to the method described by Miller et al. (1960) and the values were expressed as g glucose equivalents. Glucose was measured with a hexokinase/glucose 6-phosphate kit (Boehringer Mannheim, Germany). Maltose was assayed as glucose following a hydrolytic step using α-glucosidase (Sigma Chemical Co, Mo, USA). A L-lactate dehydrogenase kit was used for measuring L-lactate (Boehringer). Formate was assayed according to the method of Lang and Lang (1972) using citric acid. Acetic acid and ethanol were determined on a Varian 940 gas chromatograph equipped with a 1/16 inch I.D., 8-ft-long teflon column containing Chromosorb 101. The column temperature was 160 °C and nitrogen saturated with formic acid was used as the carrier gas at a flow rate of 30 ml/min⁻¹.

Results

Monoculture of *S. fibuliger*

*S. fibuliger* was grown in batch and continuous culture on starch and the sugar released into the medium under different growth conditions was measured. In a batch culture in which the aeration was terminated after a certain time during the cultivation, accumulation of sugar, measured as glucose, and limited cell growth were obtained (Fig. 1). The air supply was stopped while the starch reaction was still positive. The overall glucose production rate after aeration had been stopped was 0.8 g l⁻¹ h⁻¹.

The accumulation of sugars, after the supply of oxygen to the culture was limited, was also studied in continuous cultures. At a constant oxygen transfer rate the production of sugars increased with the dilution rate while the steady-state cell concentration decreased (Fig. 2). In this experiment the concentrations of glucose, maltose and reducing sugars were measured. Residual starch and maltose appeared in the broth at the higher dilution rate. At the highest dilution rate the productivity and specific production rate of reducing sugars obtained were 1.5 g l⁻¹ h⁻¹ and 1.7 g g cell⁻¹ h⁻¹, respectively.

The results obtained thus show that sugar may accumulate under oxygen-limiting conditions and that the relative amounts of individual sugars vary with the dilution rate.

Batch Cultures of *S. lactis* and *S. fibuliger*

Mixed cultures in which *S. fibuliger* produces fermentable sugars for *S. lactis* under oxygen-limited conditions were