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GROWTH AND PHYSIOLOGICAL ACTIVITY OF *LACTOBACILLUS SANFRANCISCO* AND *LACTOBACILLUS PLANTARUM* IN CONTINUOUS CULTURE

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Key words: *L. sanfrancisco*, *L. plantarum*, bread starter, lactic acid fermentation, biomass production.

It was found that the physiological activity of *L. sanfrancisco* and *L. plantarum* monocultures in conditions of continuous culture, expressed as sugar consumption and lactic acid production per 1 g dry substance, was similar in the culture time (Θ) interval 15-40 h. The maintenance of both strains in associated culture required $\Theta > 10$ h. In such a case there occurred a 20% stimulation of biomass production and a ca. 15% increase of physiological activity as compared to the monopopulation cultures.

Among the many species of lactic acid bacteria active in the fermentation of bread starters, the strain *Lactobacillus sanfrancisco*, first described by Kline and Sugihara [8], displays physiological properties that are especially favourable in this environment. The uniqueness of these heterofermentative bacilli [14] stems from their ability to transform maltose to glucose and beta-glucose-1-phosphate [19]. This property causes that in environments with maltose as the main hydrocarbon substrate, *L. sanfrancisco* bacteria enhance the growth and physiological activity of other microorganisms for which glucose is a more available and rapidly metabolized sugar. Studies of interactions in an associated population of *L. sanfrancisco* and *Saccharomyces cerevisiae* [16, 17] showed that the coexistence of these cultures is mutualistic. Also observations of the growth of an associated population of *L. sanfrancisco* and the strain *Lactobacillus plantarum*, isolated from spontaneously ripening bread starters, in periodic cultures [15, 17] justify the surmise that the coexistence of these strains is beneficial to the partner with higher affinity to glucose than to maltose.

The present research was intended to evaluate the growth and physiological activity of *L. sanfrancisco* and *L. plantarum*, in continuous monocultures and in associated culture, with the aim of determining the character of the coexistence of these cultures.

MATERIAL AND METHODS

BIOLOGICAL MATERIAL

Experiments were performed with the strains *L. plantarum* 26 and *L. sanfrancisco* 119 isolated from spontaneously fermenting bread starters [17]. The cultures are at present kept in the Collection of Pure Cultures of the Institute of Fermentation Technology and Microbiology of the Łódź Technical University.

CULTURE MEDIUM

The composition of the medium was determined experimentally [17, 18] and contained: rennin whey (45 g lactose/l) — 0.460 l, condensed malt wort (432 g sugars/l) — 75 g, technological glucose — 5 g, sodium citrate — 5 g, tap water ad 1 l; pH was 6.2, and sterilization was carried out for 30 min at 0.8 atmospheres. Riboflavine concentration determined after medium sterilization was 3.4×10^{-3} g/l.

CULTURE CONDITIONS

Cultures were maintained according to the flow method in an I.I.CH.1 chemostat manufactured in the Institute of Chemical Engineering of the Łódź Technical University [10]. Working volume — 0.85 l, temperature — 28°C, mixing speed — 400 r.p.m., constant pH reading. Experiments were performed for the range of 3.3-40 h of time spent in the fermenter (Θ).

CELL YIELD DETERMINATION

This was done with the nephelometric method. Biomass level was read from the standard curve E = f (dry substance) and results were given in g dry substance/l medium.

CHEMICAL ASSAYS

Lactic acid was determined by the colorimetric method according to Barker et al. [1] as modified by Jakubowska et al. [7]. Results were given in g/g dry substance of cell yield. Volatile acids were assayed after distil-

lation with water vapour after Hopenius et al. [5]; results are in g CH_3COOH/g dry substance. Concentration of sugars was determined colorimetrically with anthron reagent [11] and results given in g/g dry substance. Riboflavine level was determined spectrophotometrically [4]; results are in $g \cdot 10^{-3}$/g dry substance. All determinations were performed for steady states of continuous cultures.

RESULTS AND DISCUSSION

The strains for experiments in associated culture were selected after an analysis of the qualitative and quantitative state of Polish industrial bread starters. *L. plantarum* was dominant among homofermentative lactobacilli [17]. Its specific growth rate in the culture medium used in the experiments was 0.30 h^{-1}, while that of *L. sanfrancisco* was 0.35 h^{-1}. It was found that a characteristic feature of *L. sanfrancisco* in periodic cultures [15, 17] was the linear course of the exponential growth phase lasting for about 10 h. In identical conditions the growth of *L. plantarum* underwent a second adaptation related to the transition to maltose utilization after 13 h following the exhaustion of glucose in the environment. These observations encouraged the extension of studies to conditions of continuous culture.

We intended to find out whether the ability of *L. sanfrancisco* to phosphorylate maltose enhances the growth and activity of *L. plantarum* in associated culture, and to see how the coexistence of these cultures develops. An amount of medium ensuring the stability of its composition throughout the period of fermentation Θ (3.3-40 h) was prepared for each culture cycle.

The dependence g dry substance/l = f(0) presented in Fig. 1 indicates that for *L. sanfrancisco*, both in monopopulation culture and in associated culture, cell yield in excess of 1 g dry substance/l requires more than 10 h in the fermenter. The highest yields, of 1.20 and 1.34 g dry substance/l,

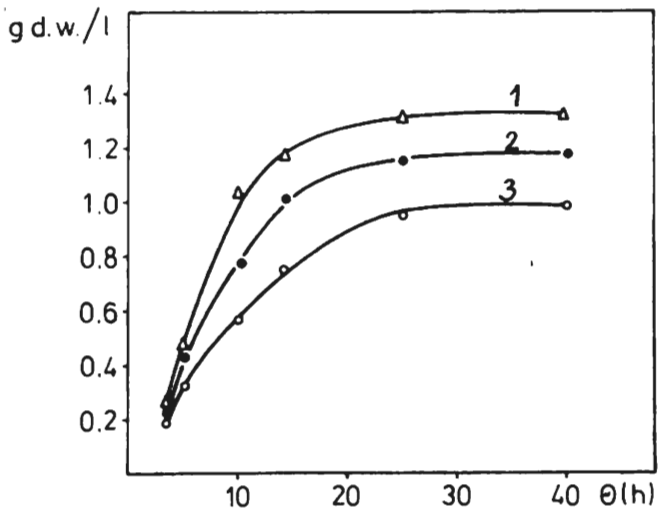
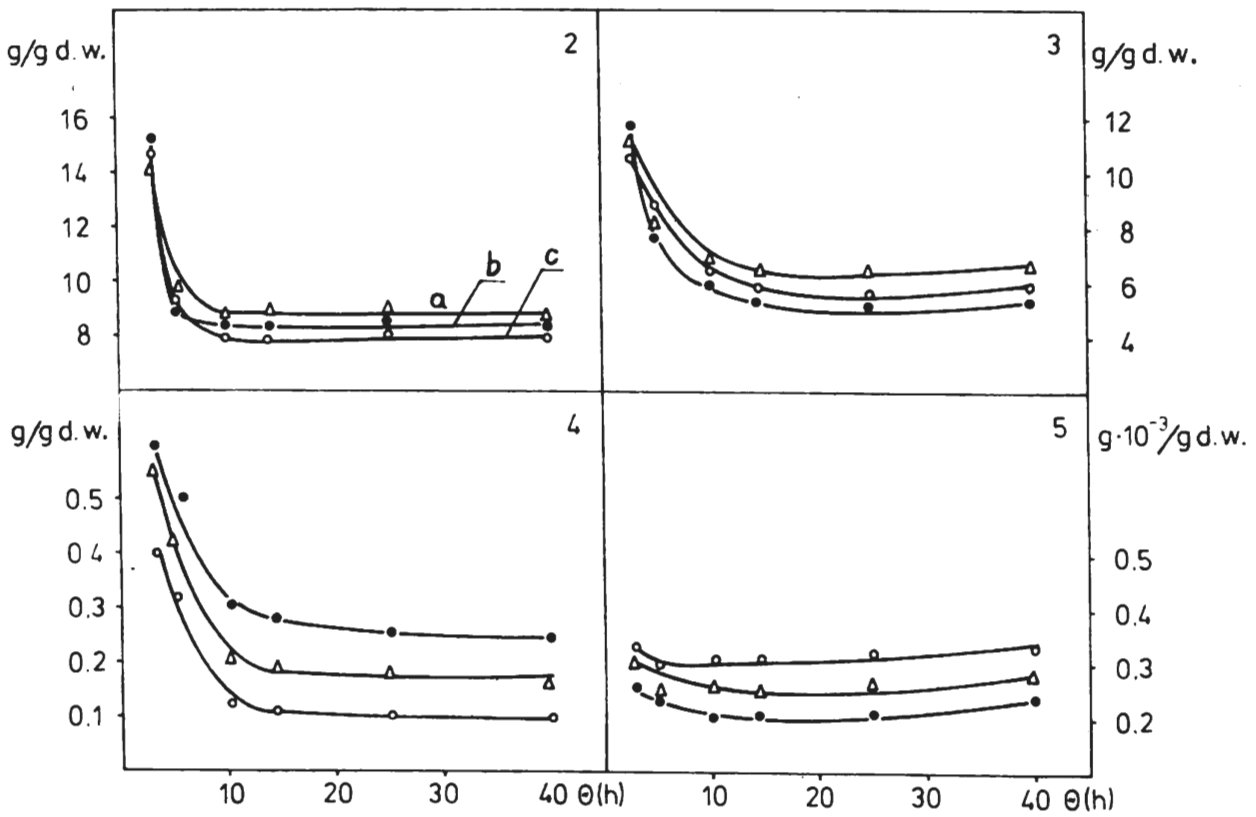


Fig. 1. Cell yield in continuous culture; 1 — associated culture of *L. sanfrancisco* and *L. plantarum*, 2 — *L. sanfrancisco*, 3 — *L. plantarum*

were found in the medium at $\Theta > 20$ h. This was in agreement with reports of Jakubowska et al. [6] and Oberman et al. [12] concerning the growth of *L. casei* in continuous culture and demonstrating the functional dependence of biomass yield on the time spent in the fermenter. The physiological activity of *L. plantarum* and *L. sanfrancisco* regarding the utilization of sugars, the production of lactic acid acetic acid, and riboflavine demand (per 1 g dry substance of cell mass) is illustrated as a function of Θ in Figs 2-5. To ensure stable physiological activity of the strains in the applied medium it was necessary to keep the cells in the chemostat for



Figs 2-5. Physiological activity of *Lactobacillus sanfrancisco* (b), *Lactobacillus plantarum* (c) and of the associated population of these strains (a) in continuous culture

2. Sugars consumption **3. Lactic acid production**
4. Acetic acid production **5. Riboflavine consumption**

more than 10 h. The activity of *L. plantarum* and *L. sanfrancisco* assessed on the basis of sugars utilization and lactic acid production was similar after that time: about 8.0 g/g dry substance (sugars) and about 5.8 g/g dry substance (lactic acid). In associated culture the figures were higher by about 15%.

There were significant differences in the level of acetic acid (Fig. 4); in *L. sanfrancisco* cultures the volatile acidity was over twice higher (0.28 g/g dry substance) than in cultures of *L. plantarum* (0.12 g/g dry substance). The demand for riboflavine also differed: in *L. plantarum* it was twice higher than in *L. sanfrancisco*. In associated cultures the concentration of acetic acid in 1 g of biomass dry substance, as well as the index of riboflavine demand attained values that were exact means of

the values obtained for the monocultures. These observations, evidenced in Figs 4 and 5, allow to assume that the maintaining in a chemostat of an associated population of equally valuable contribution of both partners requires that the culture be kept in the chemostat for more than 10 h.

Attempting to determine the character of the coexistence of the studied strains, we adopted after Pirt [13] the condition of the convergence of the relation μ_{max}/S (where S — concentration of hydrocarbon substrate in steady state of culture) as a condition necessary for the preservation of two-species populations. This allows to rule out the phenomenon of competition for fermentation time longer than 10 h. The interaction of neutralistic type, often characteristic for coexisting populations of lactic acid bacteria [2, 9], also does not seem to reflect the nature of coexistence in the case in hand. The higher yield of the associated population, exceeding that of the monocultures by an average of 25%, as well as the ca. 15% greater physiological activity reflected in the level of sugars consumption and lactic acid production suggest that the coexistence of *L. plantarum* and *L. sanfrancisco* in the applied nutritive environment corresponds to the commensalism class [3, 13].

The ability of *L. sanfrancisco* to phosphorylate maltose should thus be regarded as a unilateral contribution favouring (by making glucose available in the medium) the growth and activity of *L. plantarum*.

CONCLUSIONS

1. A stable level of physiological activity of *L. sanfrancisco* and *L. plantarum* in chemostat cultures on the applied culture medium may be attained only after the cells remain in the chemostat for more than 10 hours. After that time the associated culture gives a ceil mass yield that is on the average about 20% higher than that of monocultures.

2. The consumption of the hydrocarbon substrate and the production of lactic acid per 1 g dry substance are both similar in monocultures of *L. sanfrancisco* and *L. plantarum*; in the associated culture the figures are about 15% higher.

3. The observations indicate that the coexistence of *L. sanfrancisco* and *L. plantarum* for $\Theta > 10$ h is commensal.

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OBSERWACJE WZROSTU I AKTYWNOŚCI FIZJOLOGICZNEJ *LACTOBACILLUS SANFRANCISCO* I *LACTOBACILLUS PLANTARUM* W HODOWLI CIĄGŁEJ

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Streszczenie

W przeprowadzonych w warunkach hodowli ciągłej badaniach wzrostu *Lactobacillus sanfrancisco* i *Lactobacillus plantarum* wykazano, że w przyjętym zakresie czasu przebywania — Θ: 3,3-40 h plon komórek pozostawał w funkcyjnej zależności od tego parametru. Utrzymanie w chemostacie skojarzonej populacji badanych szczepów uwarunkowane było $\Theta > 10$ h. Obserwowano wówczas stabilizację poziomu aktywności fizjologicznej w mono i skojarzonych hodowlach. Dla $\Theta > 10$ h plon komórek skojarzonej populacji był wyższy w porównaniu z monopropagacjami średnio o 20% (rys. 1).

W hodowlach *L. sanfrancisco* i *L. plantarum* wskaźniki wykorzystania substratu węglowodanowego i produkcji kwasu mlekowego dla $\Theta > 10$ h wyrażały się bliskimi wartościami, odpowiednio: ok. 8,0 g cukru/g ss i ok. 5,8 g kwasu mlekowego/g ss (rys.

2, 3). W hodowli skojarzonej notowano wyższą w tym zakresie aktywność populacji o ok. 15%. Zróżnicowanie dla badanych kultur przedstawiały się: zdolność do tworzenia kwasu octowego (dwukrotnie więcej w monohodowlach *L. sanfrancisco*) i zapotrzebowanie na ryboflawinę (dwukrotnie wyższe u *L. plantarum*). W hodowli skojarzonej wartość tych wskaźników stanowiła średnią uzyskanych w monohodowlach (rys. 4, 5). Poczynione obserwacje wskazywały na komensalny charakter współżycia skojarzonej populacji *L. sanfrancisco* i *L. brevis*.