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Fermentation of Glucose and Fructose by *Leuconostoc mesenteroides*

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Abstract: The preferred order of glucose and fructose uptake by *Leuconostoc mesenteroides* was investigated in Glucose Fructose Yeast Extract Amino Acid medium with and without the addition of salt under anaerobic conditions. Strains NCIMB 8023 and NCFB 811 preferentially utilised glucose over fructose. A higher biomass was obtained when employing strain NCIMB 8023 compared with strain NCFB 811. The strains studied produced lactate and ethanol as major end-products, with lactate synthesis being greater than ethanol. Small amounts of acetate and mannitol were also formed.

Leuconostoc mesenteroides Tarafından Glikoz ve Fruktozun Fermantasyonu

Özet: *Leuconostoc mesenteroides* tarafından sentetik Glikoz Fruktoz Maya Ekstraktı Amino Asit (GFMA) ve tuz ilave edilmiş GFMA ortamlarında bulunan glikoz ve fruktozdan hangisinin öncelikle metabolize edileceği havasız koşullarda araştırılmıştır. Glikozun fruktozdan daha önce kullanıldığı belirlenmiştir. NCIMB 8023 bakterisi NCFB 811 bakterisine göre daha fazla biokitle oluşturmuştur. Fermantasyon sonucu esas ürün olarak laktik asit ve etanol meydana gelmiştir. Miktar olarak daha fazla laktik asit ve daha az etanol saptanmıştır. Az miktarda asetat ve mannitolun oluştuğu belirlenmiştir.

Introduction

Lactic acid fermentations have been carried out to preserve vegetables and fruits and to develop organoleptic properties of these foods for centuries. Fermentative preservation of plant foods for human consumption plays an important economic role. Pickles, olives and sauerkraut are produced in the largest amounts by fermentation (1). For example, 300,000 tonnes of cucumber and 200,000 tonnes of cabbage are preserved annually by fermentation in the USA (2). The commercial production of fermented foods of plant materials has recently increased in Turkey (3).

Species of lactic acid bacteria mainly responsible for the natural fermentation of plant materials are *Leuconostoc* (*Leuc.) mesenteroides*, *Lactobacillus* (*Lb.*) *brevis*, *Pediococcus* (*P.*) *pentosaceus* (formerly *P. cerevisiae*) and *Lb. plantarum* (4). *Leuc. mesenteroides* is the predominant bacterium in the early stage of most of the fermented vegetables. It initiates fermentation and helps the succession of other lactic acid bacteria (5). The sugar contents of fresh vegetables are between 1 and 4%, depending on the degree of maturation, and glucose, fructose and sucrose are the main fermentable sugars (2, 4).

Heterofermentative lactic acid bacteria, *Leuc. mesenteroides* and *Lb. brevis* ferment sugars to lactate, ethanol, acetate, mannitol, dextran and carbon dioxide. Homofermentative bacteria, *Lb. plantarum* and *P. pentosaceus* primarily produce lactate (6, 7). *Leuconostoc* spp. take up fructose at a faster rate than glucose with the exception of *Leuc. mesenteroides* ssp. *cremoris* (8). Residual glucose was reported in commercial samples of sauerkraut analysed by Hughes and Lindsay (7). Nuraida (9), however, stated that glucose was utilised first in a mixture of glucose/fructose by *Leuc. mesenteroides*.

The aim of the present study was to examine the preferred order of glucose and fructose fermentation by *Leuc. mesenteroides*.

Materials and Methods

Organisms

Leuc. mesenteroides NCIMB 8023 was obtained from the National Collection of Industrial and Marine Bacteria, Aberdeen, UK. *Leuc. mesenteroides* NCFB 811 was obtained from the National Collection of Food Bacteria, Reading, UK. The former was isolated from olive fermentation and the latter was from sauerkraut fermentation. The cultures were maintained in All Purpose Tween-Chalk Semi Solid Preservation medium (10).

Medium

Medium Glucose Fructose Yeast Extract Amino acids (Medium GFYA) was modified from Westby (11). Medium GFYA contained (g/l purified water): K_2HPO_4,

0.52; KH_2PO_4, 1.15; $CaCl_2.6H_2O$, 0.05; $MgSO_4.7H_2O$, 0.2; NaCl, 0.1; yeast extract (Difco-0127-01), 0.1; vitamin free casamino acids (Difco-028801), 0.2; L-histidin, 3.1; glucose, 0.9; fructose, 0.9 and trace metals mixture, 3 ml. The trace metals mixture was prepared according to Owens and Keddie (12). Medium GFYA was autoclaved at 121°C for 2 min and incubated at 30°C for 3 days to check the sterility.

Medium GFYA-NaCl was similar to medium GFYA but contained 22.5 g/l NaCl.

Chemicals were purchased from Sigma Chemical Ltd. or BDH Chemical Ltd. Poole, UK. They were analytical grade or the highest grade avaible.

Deoxygenation of Medium

Deoxygenation of the medium was done by bubbling an oxygen free mixture of 97 % N_2 and 3 % H (British Oxygen Co. Ltd., London) passed through an oxy-purge for 20 min.

Preparation of Inoculum

A 40 ml deoxygenated sample of medium GFYA and medium GFYA-NaCl was inoculated with two drops from a Pasteur pipette of each microorganism from stock culture. The head space was filled with sterile paraffin. The culture was incubated at 25°C for 24 h. The inoculum size was 5 % (v/v) of this culture.

Experimental Procedure

Fermentations were carried out in screw-capped 300 ml bottles containing 250 ml deoxygenated medium under anaerobic conditions. After inoculation, the head space was filled with sterile paraffin and the bottles were incubated at 25°C for 2 days. All experiments were carried out in duplicate.

Determination of Biomass

The optical density of the culture was measured at 550 nm against a distilled water blank, using a SP-350 Spectrophometer with a 1.0 cm light path. Optical density was converted to biomass from a standard curve.

Determination of Substrates and Metabolic End-products

High Performance Liquid Chromatography was used to determine glucose, fructose, lactate, ethanol, acetate and mannitol as described by Erten (13).

Results and Discussion

Fermentations were done in medium GFYA and medium GFYA-NaCl with equal concentrations of a mixture of glucose and fructose under anaerobic conditions at 25°C.

Sugar Utilisation

Figures 1 and 2 illustrate the utilisation of sugars in GFYA medium by *Leuc. mesenteroides* strains NCIMB 8023 and NCFB 811, respectively. Glucose and fructose were metabolised simultaneously, but the former was fermented at a faster rate than the latter. Negligible concentrations (less than 0.04 mmol/l) of sugars were found within 48 h.

The metabolism of a mixture of glucose and fructose by *Leuc. mesenteroides* was also investigated in the presence of salt (22.5 g/l NaCl) as shown in Fig. 3 for strain NCIMB 8023 and Fig. 4 for strain NCFB 811. Sugars were fermented simultaneously, however glucose was preferentially consumed over fructose. A negligible amount (less than 0.04 mmol/l) of glucose was determined at the end. Unfermented concentrations of fructose were also detected after 48 h fermentation. The residual amounts of fructose were 0.25 mmol/l and 0.60 mmol/l for *Leuc. mesenteroides* NCIMB 8023 and *Leuc. mesenteroides* NCFB 811, respectively when samples were taken at 48 h.

Malolactic bacterium, *Leuc. oenos*, fermented very little fructose, slightly more glucose and metabolised all L-malic acid (14). *Leuc. mesenteroides* NCDO 518, *Leuconostoc* sp. Pz. 45 and *Leuconostoc* sp. Pz. 10 metabolised glucose at a faster rate than fructose (9).

Added salt helps drawing out water and nutrients, favouring lactic acid bacteria and inhibition of undesirable microorganisms (15). Shredded cabbage is mixed with 2-3% of salt for sauerkraut fermentation. Cucumber and olives are usually fermented in 4-8% salt brines (6). In low salt cucumber juice brine, *P. pentosaceus*, *Lb. plantarum* and *Leuc. mesenteroides* fermented glucose fast, while *Leuc. oenos* preferred fructose over glucose (16). Notable concentrations of glucose were detected at the end of sauerkraut fermentations and accumulation of mannitol indicated that fructose was removed rapidly from brine (7, 17). Chen et al. (18) studied *Leuc. mesenteroides* strains LC 33, 42 and 43 in bean juice with and without the addition of 2.5% salt. The strains tested preferred fructose over glucose and residual concentrations of sugars were also found.

The results in this study for *Leuc. mesenteroides* support the previous observations of Nuraida (9) and Chavasit et al. (16) but not those of Garvie (8), Fleming et al. (17) or Chen et al. (18). The reason why fructose is a preferred substrate over glucose in sauerkraut and green bean fermentations (7, 17, 18) or vice versa in

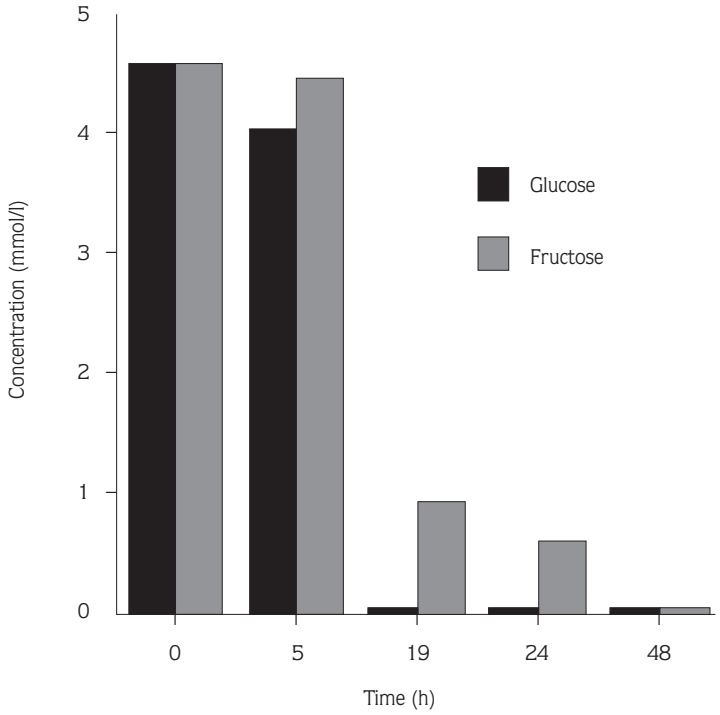

Figure 1. Utilisation of glucose and fructose by *Leuc. mesenteroides* NCIMB 8023 in medium GFYA under anaerobic conditions.

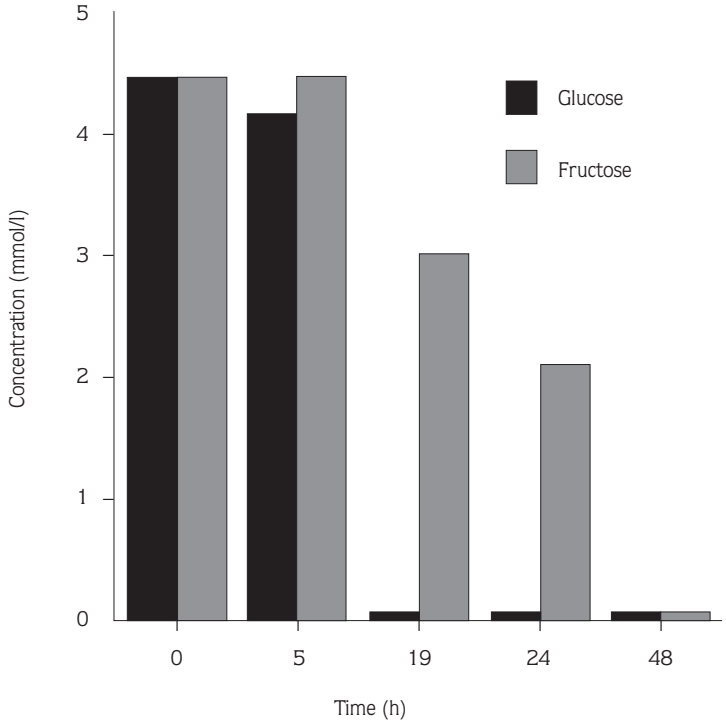

Figure 2. Utilisation of glucose and fructose by *Leuc. mesenteroides* NCFB 811 in medium GFYA under anaerobic conditions.

other previous studies (9, 16) is not known. Vegetable fermentations such as cucumber, olives, sauerkraut involve a mixed microbial population, therefore it would be interesting to study lactic acid bacteria isolated from these vegetable fermentations.

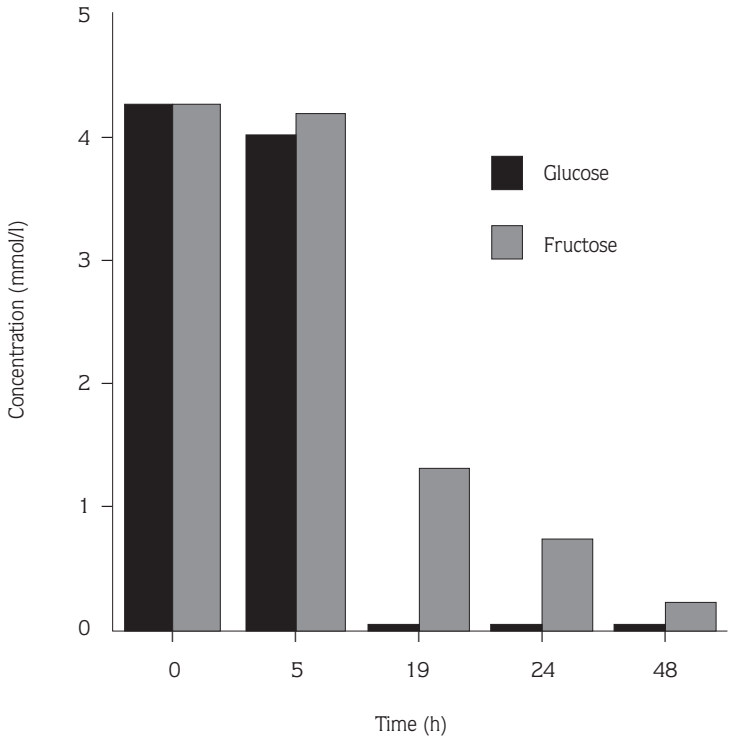

Figure 3. Utilisation of glucose and fructose by *Leuc. mesenteroides* NCIMB 8023 in medium GFYA-NaCl under anaerobic conditions.

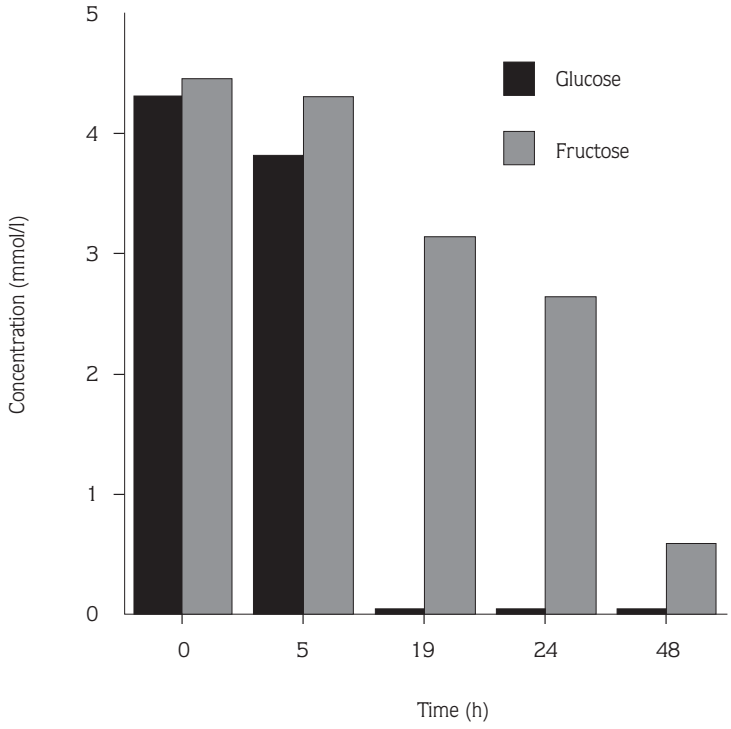

Figure 4. Utilisation of glucose and fructose by *Leuc. mesenteroides* NCFB 811 in medium GFYA-NaCl under anaerobic conditions.

Formation of Biomass and Metabolic End-products

Biomass formation is given in Fig. 5. *Leuc. mesenteroides* NCIMB 8023 grew faster than strain NCFB 811 with and without the addition of salt under anaerobic conditions. Plihon et al. (19) and Nuraida et al. (20) showed that growth occurred in aerated and unaerated cultures, but there was an increase in biomass formation under aerobic conditions. Condon (21) also

stated that biomass production was higher in the presence of oxygen than in its absence.

The metabolic end-products of medium GFYA fermented by *Leuconostoc* spp. were summarised elsewhere by Erten (13). *Leuc. mesenteroides* strains NCIMB 8023 and NCFB 811 also produced the same metabolic end-products in medium GFYA-NaCl. The results are given in Table 1. Lactate and ethanol were the

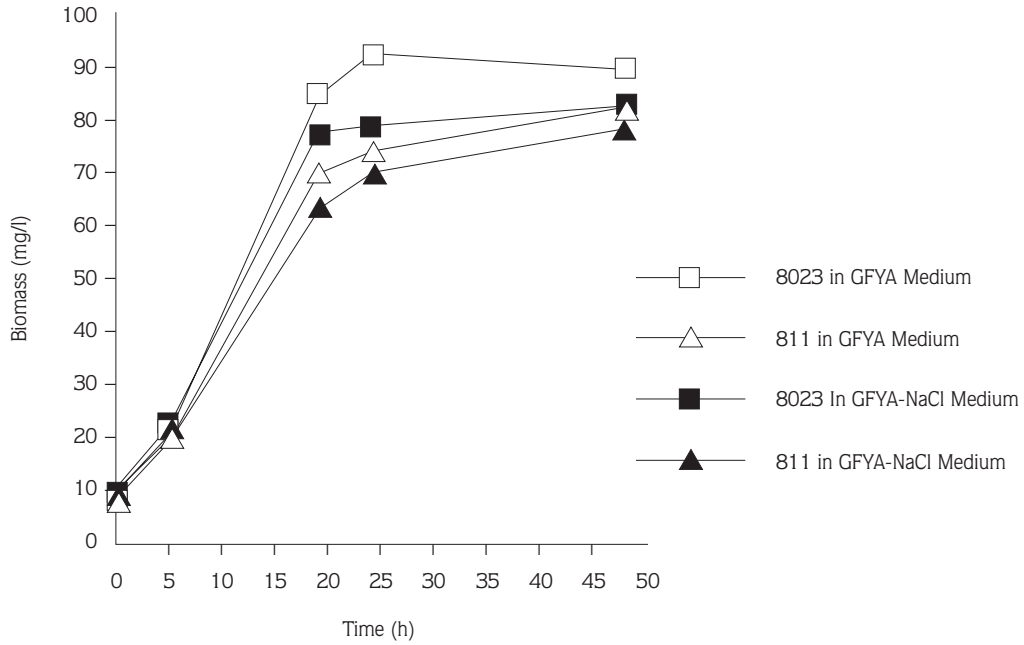

Figure 5. Changes in bacterial biomass during the fermentation of glucose and fructose in cultures of *Leuc. mesenteroides* in media GFYA and GFYA-NaCL under anaerobic conditions.

Table 1. Metabolic end-products from glucose plus fructose by *Leuc. mesenteroides* in medium GFYA and medium GFYA-NaCl.

Strain	Medium	Compounds	Concentrations (mmol/l)* Initial	Final
NCIMB 8023	GFYA	Lactate	0.40	6.75
		Ethanol	<0.20	5.45
		Acetate	<0.02	1.55
		Mannitol	<0.04	2.40
NCFB 811	GFYA	Lactate	0.40	5.85
		Ethanol	<0.20	4.95
		Acetate	<0.02	1.75
		Mannitol	<0.04	2.55
NCIMB 8023	GFYA-NaCl	Lactate	0.40	7.00
		Ethanol	<0.20	6.00
		Acetate	<0.02	1.30
		Mannitol	<0.04	1.10
NCFB 811	GFYA-NaCl	Lactate	0.40	6.15
		Ethanol	<0.20	5.63
		Acetate	<0.02	1.40
		Mannitol	<0.04	1.55

* Results are means of determinations of duplicate experiments. For initial values, samples were taken in 1-2 min after inoculation; final values were analysed after 48 h.

main end-products from a mixture of glucose and fructose under anaerobic conditions. It is important to note that ethanol was formed less than lactate. Small concentrations of acetate and mannitol compared with the main compounds were also produced. Strain NCIMB 8023 formed higher amounts of mannitol than strain NCFB 811.

Leuc. mesenteroides fermented a mixture of glucose and fructose to lactate, ethanol, acetate and mannitol (9). The same end-products were detected in sauerkraut, cucumber and green bean juice fermentations (7, 16-18). *Leuc. mesenteroides* utilised fructose as an electron acceptor and some amount of fructose was reduced to mannitol (9, 13, 22).

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