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Production of a *Lactobacillus plantarum* Starter with Linamarase and Amylase Activities for Cassava Fermentation

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Abstract: *Lactobacillus plantarum* strain A6 isolated from cassava, cultured on cellobiose MRS medium showed a growth rate of 0·41 h^{-1}, a biomass yield of 0·22 g g^{-1}, and produced simultaneously an intracellular linamarase (76·4 U g^{-1} of biomass) and an extracellular amylase (36 U ml^{-1}). The synthesis of both enzymes was repressed by glucose. The use of such a strain as a cassava fermentation starter for gari production had the following influences: a change from a heterofermentative pattern observed in natural fermentation to a homofermentation, a lower final pH, a faster pH decline rate and a greater production of lactic acid (50 g kg^{-1} DM). However, this starter did not appear to play a significant role in cassava detoxification, since it was observed that the level of endogenous linamarase released during the grating of the roots was sufficient to permit the complete and rapid breakdown of linamarin.

Key words: fermented cassava, lactic acid bacteria, starter, *Lactobacillus plantarum*, amylase activity, linamarase activity, cyanide, *Manihot esculenta* Crantz, gari.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is an important staple food for 500 million people (Cock 1982). However, its use as a food is influenced by its toxicity. Cassava or mincing of the roots permits, through the cell structure damage, the releasing of endogenous linamarase (EC 3.2.1.21, linamarin β-D glucoside glucohydrolase) able to hydrolyse linamarin into glucose and cyanohydrin (Conn 1969); and (ii) the roasting allows

this in mind, the authors have recently reported that various lactic acid bacteria have the ability to hydrolyse linamarin (Giraud *et al* 1992). *Lactobacillus plantarum* strain A6, isolated from retted cassava for its amylolytic activity (Giraud *et al* 1991a), appeared to be the more suitable. Indeed, after culture on cellobiose MRS medium this strain showed a strong linamarase activity

iodine solution (KI, 1·2 g; I_2, 0·12 g; distilled water 1 litre). One enzyme unit is defined as the amount of enzyme that permits the hydrolysis of 10 mg of starch in 30 min under the conditions given above.

Linamarase activity was assayed on whole cells by the method described by Giraud *et al* (1992).

the assay. Linamarin was determined as the difference between free and total cyanides, and cyanohydrin as the difference between free cyanides and HCN.

RESULTS AND DISCUSSION

Production of an *L plantarum* starter

Figures 1–3 represent growth kinetics, sugar consumption, and linamarase and amylase production of *L plantarum* A6 cultured on different media as indicated in the experimental section. The main fermentation parameters are shown in Table 1. In all three tested media, biomass productivity and growth rates were high and practically identical. However, linamarase and amylase amounts differed with the medium used.

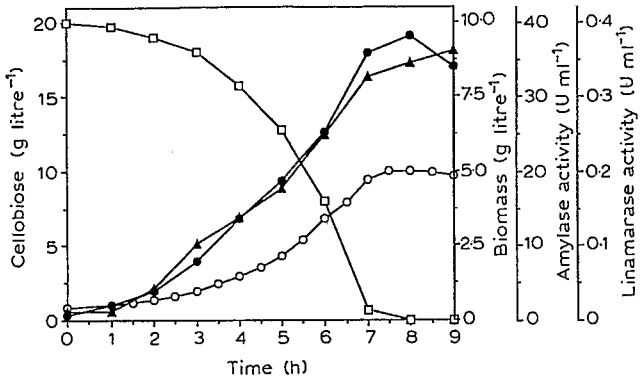


Fig 1. Fermentation of *L plantarum* A6 on cellobiose MRS medium at 30°C and pH 6·0: □, cellobiose; ○, biomass; ▲, amylase activity; ●, linamarase activity.

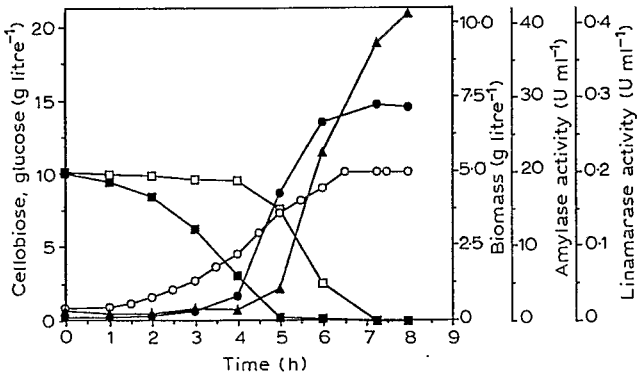


Fig 2. Fermentation of *L plantarum* A6 on cellobiose–glucose MRS medium at 30°C and pH 6·0: ■, glucose; □, cellobiose; ○, biomass; ▲, amylase activity; ●, linamarase activity.

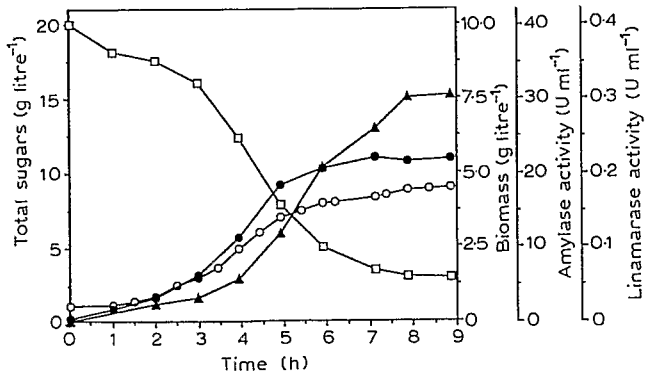


Fig 3. Fermentation of *L plantarum* A6 on cellobiose–starch MRS medium at 30°C and pH 6·0: □, total sugars; ○, biomass; ▲, amylase activity; ●, linamarase activity.

On cellobiose MRS medium

Linamarase and amylase synthesis occurred at the start of fermentation and seemed to be related to biomass formation. Linamarase concentration at the end of the fermentation was 76·4 U g^{-1} of biomass. A recent study (Giraud *et al* 1992), carried out in flasks on the same medium, demonstrated that the amount of linamarase produced was 29 U g^{-1} of biomass. In the bioreactor, under controlled conditions, it increased 2·6 times. It was noticed that the strain produced an amylase while there was no starch in the medium, moreover, the amount was higher than that obtained on cellobiose–starch MRS medium.

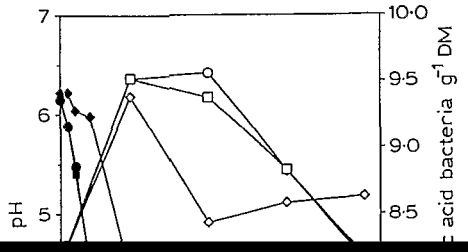
On cellobiose–glucose MRS medium

The kinetics indicated that glucose was rapidly consumed during the first 4 h of fermentation, while cellobiose content remained constant. It appeared that linamarase was not formed as long as the glucose concentration remained at about 3 g $litre^{-1}$. Extremely rapid uptake of

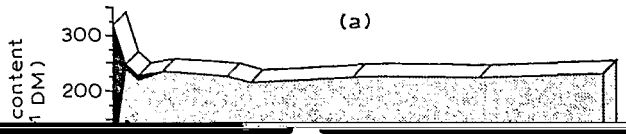
TABLE 1
Fermentation parameters of *L plantarum* A6 cultured on various media at pH 6·0 and 30°C

Medium	*Growth rate* (h^{-1})	*Biomass*		*Enzyme activity*	
		Concn. ($g\ litre^{-1}$)	*Yield* ($g\ g^{-1}$)	*Linamarase* ($U\ g^{-1}$)	*Amylase* ($U\ ml^{-1}$)
MRS cellobiose	0·41	5	0·22	76	36
MRS cellobiose–glucose	0·46	5	0·23	59	42
MRS cellobiose–starch	0·44	4·5	0·23	49	30

cellobiose was then observed and it correlated well with considerable synthesis of linamarase (β-glucosidase). Amylase synthesis was slightly retarded and occurred when the glucose concentration was approximately zero; about 80–90% of the enzymes was synthesised during this stage. It is interesting to note that the production of amylase reached a level higher than that observed on the



and Odunfa (1990) who reported a predominant development of *Leuconostoc mesenteroides*—in the study of characterisation and distribution of the lactic acid microflora during the preparation of fufu—replaced



significant role in the development of sensory qualities, and in the standardisation and preservation of the final product by the large amounts of lactic acid produced and

1991a Isolation and physiological study of an amylolytic strain of *Lactobacillus plantarum*. *Appl Microbiol Biotechnol* **36** 370–383.