Phenotypic Features and Enzymatic Profile of a *Lactococcus lactis* Strain Isolated from Wara, an Indigenous Cheese Product from Nigeria

NURUDEEN AYOADE OLASUPO

Department of Botany and Microbiology, Lagos State University Ojo, Apapa, Lagos, Nigeria

Abstract

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A lactic strain coded NA 1300, isolated from wara, a Nigerian cheese product, was identified as *Lactoccus lactis,* on the basis of sugar fermentation pattern, and other physiological and biochemical characteristics and by DNA-DNA hybridization. The enzytmatic profile of strain NA 1300 indicated the presence of a wide spectrum of enzymes. The organism exhibited relatively weak esterase and lipase activities as compared with peptidases. The strain showed no proteinase activity. The results are discussed in relation to the role of enzymes in starter selection for cheese production.

Key word: phenotypic features; enzymatic profile; *Lactococcus lactis*; wara

The species *Lactococcus lactis* contributes substantially to human nutrition and well-being. Economically and technically, the species is of utmost importance as starter culture for the production of fermented milk and its derivative products (TEUBER 1995). The early role of *L. lactis* in milk fermentation is a sufficiently fast and quantitatively defined conversion of lactose into lactic acid. Later functions include formation of diacetyl, CO_2, and other aroma compounds, some possibly generated by controlled proteolysis during cheese ripening (PRITCHARD & COOLBEAR 1993).

Wara is a traditionally prepared white soft cheese that is commonly consumed in Nigeria and some African countries notably in the Republic of Benin. It is manufactured by coagulating milk with a crude extract from the leaves of sodom apple plant (*Calotropis procera*) which have been known to contain calotropain, a rennet-like enzyme. Following the coagulation process, the mixture is heated and the resulting loose curds are drained, moulded into conical shape in small raffia baskets and then submerged in whey or water without any further processing (OGUNDINWIN & OKE 1982).

The association of *L. lactis* with cheese products is well documented. So is the enzymology of the strain. However, most of this information has been limited to strains of *L. lactis* originating from cheese products obtained from the developed countries. No such knowledge is known to be in existence about *L. lactis* strain isolated from cheese products indigenous to the African environment. In the light of this, the present study describes the identification and enzymology of a *L. lactis* strain isolated from wara, a traditional cheese food from Nigeria in Africa.

MATERIAL AND METHODS

Isolation method: Coccus-shaped lactic acid bacteria (LAB) were isolated from home-made and wara products obtained at the markets in many parts in Nigeria. Samples (10 g) of wara were homogenised with 90 ml of 0.85% (w/v) sterile physiological saline and serially diluted in the same diluent. The LAB were selectively isolated on M17 agar plates incubated both aerobically and anaerobically at 30°C for 3 days. The predominant LAB were obtained from M17 plates with the highest dilutions, colonies were either selected randomly or all colonies were sampled if the plate contained less than 10 colonies, according to LEISNER *et al.* (1997). The purity of the isolates was checked by repeated streaking on fresh M17 agar plates, followed by a microscopic examination.

Phenotypic characterization: Initial characterization of isolates included colony and cell morphology and Gram, catalase and oxidase reactions, growth at 45°C and in pH 9.6. Gram-positive cocci, catalase-negative, oxidase-negative non-motile cells which were unable to grow at 45°C, 6.5% NaCl and in pH 9.6 were presumptively identified as *lactococci*. The ability of the isolates to ferment carbohydrates was studied using the API 50 CHL system. Isolates were further identified phenotypically using gas production from glucose, growth at different temperatures, pH and in varying concentrations of NaCl. Arginine

hydrolysis, presence of diaminopimelic acid in the cell wall and lactic acid configuration were determined as described by SCHILLINGER and LUCKE (1987).

DNA base composition and DNA-DNA hybridization: Only one strain was suspected to be *L. lactis* based on the above phenotypic features. In order to confirm the identity of the strain, the homology of its DNA with that of reference strains (including *L. lactis* DSM 20729, *Enterococcus faecalis* DSM 20477 and *Ent. faecium* DSM 20478) was determined. Chromosomal DNA was isolated and purified according to a modification of the method of MAMUR (1961) as described by STACKEBRANDT and KANDLER (1979). The DNA base composition was estimated from the thermal melting point (Tm) as described by MAMUR and DOTY (1962), using a Gilford response spectrophotometer. The spectrophotometric determination of DNA-DNA hybridization from renaturation rates was performed using the modified (HUSS *et al.* 1983) optical method of DE LEY *et al.* (1970).

Enzymatic profile assay: The enzymatic profile of the predominant LAB was assayed following the method of ARORA *et al.* (1990), in API-zym (bioMerieux) galleries by testing for the activity of the following 19 enzymes; alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine, valine and cystine arylamidase, trypsin, chymotrypsin, acid phosphatase, naphtho-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase.

RESULTS

The phenotypic features of strain NA 1300 isolate from wara are presented in Table 1. Morphologically, the cells of strain NA 1300 were coccoid in shape and existed in short chains. The strain was Gram-positive, catalase and oxidase-negative, non-motile and did not produce any gas from glucose. It hydrolysed arginine, grew at 10, 15 and 40°C and at pH 3.9 and 9.2 and in 4.0% NaCl but did not grow at 45°C, pH 9.6 or in 6.5% NaCl. It produced L(+) lactic acid and had a mol %G + C of 33.7. In the API test, it fermented L-arabinose, ribose, D-xylose, galactose, D-glucose, D-fructose, D-mannose, N-acetyl glucosamine, amygdaline, arbutine, esculine, salicine, cellobiose, maltose, lactose, melibiose, saccharose, trehalose, D-raffinose, amidon, β-gentiobiose and gluconate. The other carbohydrates included in the API system were not fermented. Based on these characteristics, the isolate was classified as *Lactococcus lactis*. The identity of the strain was confirmed by DNA-DNA hybridization. The DNA of strain NA 1300

Table 1. Phenotypic features of strain NA 1300 isolated from *wara*

Characteristics/Tests	Reaction	Characteristics/Tests	Reaction	Characteristics/Tests	Reaction
Morphology	*coccus*	4 L-arabinose	+	28 maltose	+
Catalase test	–	5 ribose	+	29 lactose	w
Gram reaction	+	6 D-xylose	+	30 melibiose	+
Growth at [°C]		7 L-xylose	–	31 saccharose	+
10	+	8 adonitol	–	32 trehalose	+
40	+	9 β-methyl-xylose	–	33 inuline	–
45	–	10 galactose	w	34 melesitose	–
Growth at pH		11 D-glucose	+	35 D-raffinose	+
9.2	+	12 D-fructose	+	36 amidon	w
9.6	–	13 D-mannose	+	37 glycogene	–
Growth in NaCl [%]		14 L-sorbose	–	38 xylitol	–
4	+	15 rhamnose	–	39 β-gentiobiose	+
6.5	–	16 dulcitol	–	40 D-turanose	–
Arginine hydrolysis	+	17 inositol	–	41 D-lyxose	–
Presence of meso-2,6-diaminopimelic acid	–	18 mannitol	–	42 D-tagatose	–
Lactic acid configuration	L	19 sorbitol	–	43 D-fucose	–
Gas production from glucose	–	20 α-methyl-D-mannoside	–	44 L-fucose	–
Mol %G + C in DNA	33.7%	21 α-methyl-D-glucoside	–	45 D-arabitol	–
Sugar fermentation (API)		22 N-acetyl glucosamine	–	46 L-arabitol	–
0 control	–	23 amygdaline	w	47 gluconate	w
1 glycerol	–	25 esculine	w	48 2-ceto-gluconate	–
2 erythritol	–	26 salicine	+	49 5-ceto-gluconate	–
3 D-arabinose	–	27 cellobiose	+	Identity	*Lactococcus lactis*

+ = positive; – = negative; w = weak reaction

showed 85% homology with the DNA of the reference strain *Lactococcus lactis* DSM 20729, but only 9 and 29% homology with that of negative controls *Enterococcus faecium* DSM 20478 and *Enterococcus faecalis* DSM 2047, respectively. As the organisms showing homology values higher than 65% are considered members of one species (SCHLEIFER & STACKEBRANDT 1983), the results further confirmed the identity of strain NA 1300 as *Lactococcus lactis*.

The enzymatic profile of strain NA 1300 is presented in Table 2. The lactic strain produced a wide spectrum of enzymes. The organism showed relatively weak esterase and lipase activities as compared with peptidases. It showed no proteinase activity.

Table 2. Enzymatic activity of *Lactococcus lactis* NA 1300 isolated from wara

No.	Enzyme assayed for	Reaction	nm/4 hr
1	control		
2	alkaline phosphatase	–	0
3	esterase (C4)	+	5
4	esterase lipase (C8)	+	10
5	lipase (C14)	–	0
6	leucine arylamidase	+	≥40
7	valine arylamidase	+	≥40
8	cystine arylamidase	+	30
9	trypsin	–	0
10	chymotrypsin	–	0
11	acid phosphatase	+	≥40
12	naphthol-AS-BI phosphohydrolase	+	5
13	α-galactosidase	–	0
14	β-galactosidase	+	≥40
15	β-glucuronidase	–	0
16	α-glucosidase	+	30
17	β-glucosidase	+	10
18	N-acetyl-β-glucosaminidase	+	5
19	α-mannosidase	–	0
20	α-fucosidase	–	0

DISCUSSION

The isolation of *L. lactis* in the present study from *wara* further confirms the close association of the strain with cheese and dairy products. However, despite the extensiveness of information about the identification of the strain from dairy products. This report describes for the first time, the identification of the strain in a typical traditional cheese product (wara) from Nigeria.

The use of the API-zym technique was reported (ARORA *et al.* 1990) as a rapid and simple means of evaluating and localizing 19 different hydrolases of organisms. This method is also of relevance to select strains with superior enzyme profiles, especially in their peptidases and esterases, for accelerated maturation and enzyme modification of cheese. The process of cheese maturation involves a sequential breakdown of milk components, such as fat, protein and lactose by the enzymes of starter bacteria (DAVIES & LAW 1984). Therefore fundamental understanding of starter enzymes is of prime importance in evaluating their suitability and in predicting their influence on the final cheese quality.

The absence of proteinases (trypsin and chymotrypsin), presence of high peptidases (leucine-, valine- and cystine-arylamidase) and esterase-lipases (C4 and C8) produced by strain NA 1300 isolated from wara (Table 2) are traits of desirable quality for their use in accelerated ripening of cheese and production of typical cheese flavours (ARORA *et al.* 1990). Cheese starters with low proteinases and high peptidases are also useful for reducing bitterness and improving body and textural defects, which are often caused by most of the microbial preparations when used as rennet substitutes (DAVIES & LAW 1984). In addition, soapiness defect caused by the accumulation of long-chained fatty acids in many ripened cheese varieties can be removed by using strains with high esterase and lipase activities.

The information obtained from the API-zym enzymatic profiles will help starter companies to assess pertinent enzyme activities of starter strains they provide for industry. Further research would be needed in order to select strains that will provide optimum enzyme activities to catalyse desirable reactions in food systems.

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Souhrn

OLASUPO N. A. (2000): **Fenotypové vlastnosti a enzymatický profil kmene *Lactococcus lactis* izolovaného z nigerijského místního sýrového produktu wara**. Czech J. Food Sci., **18**: 91–94.

Mléčný kmen označený NA 1300, který byl izolován z nigerijského sýrového produktu wara, byl na základě cukerné fermentace cukru a dalších fyziologických a biochemických vlastností a pomocí DNA-DNA hybridizace identifikováni jako *Lactoccus lactis*. Bylo zjištěno, že kmen NA 1300 se skládá z širokého spektra enzymů. Ve srovnání s peptidasami vykazoval tento organismus relativně slabou esterasovou a lipasovou aktivitu. Proteinasovou aktivitu kmen nevykazoval žádnou. Získané výsledky potvrzují význam enzymů v startovací fázi výroby sýrů.

Klíčová slova: fenotypické vlastnosti; enzymatický profil; *Lactococcus lactis*; wara

Corresponding author:

Dr. NURUDEEN AYOADE OLASUPO, Department of Botany and Microbiology, Lagos State University Ojo, P.M.B. 1087 Apapa, Lagos, Nigeria, tel./fax: 00234-1-49 21 423, 00234-1-49 21 478, e-mail: fiiro@rcl.nig.com