APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Jan. 1986, p. 105–109
0099-2240/86/010105-05$02.00/0

Vol. 51, No. 1

Evidence for Plasmid Linkage of Raffinose Utilization and Associated α-Galactosidase and Sucrose Hydrolase Activity in *Pediococcus pentosaceus*

CARLOS F. GONZALEZ* AND BLAIR S. KUNKA
Microlife Technics, Sarasota, Florida 34243

Received 12 August 1985/Accepted 22 October 1985

The ability to ferment the trisaccharide raffinose was linked with the presence of plasmid DNA in three strains of *Pediococcus pentosaceus*. Parental strains showed associated inducible α-galactosidase and sucrose hydrolase activities when grown in α-galactosides and sucrose, respectively. Derivative strains of PPE1.0, PPE2.0, and PPE5.0, which had lost 30-, 28-, and 23-megadalton plasmids, respectively, had no α-galactosidase or sucrose hydrolase activity.

The pediococci are a group of homofermentative lactic acid bacteria which are used in the food fermentation industry. Recent studies (8) from our laboratory have established the presence of resident plasmids and a conjugal transfer system for the pediococci with the broad-host-range plasmid pIP501. The previous study identified the presence of resident plasmid(s) in *Pediococcus* spp. but did not attribute any phenotypic character to them.

Evidence that plasmid DNA is involved in carbohydrate fermentation (7, 12, 21) and other phenotypic characters (6, 9, 13, 16) expressed by various lactic streptococci is well documented. We present evidence in this study which links the utilization of the trisaccharide raffinose [*O*-α-D-galactopyranosyl(1→6)*O*-α-D-glucopyranosyl(1→2)β-D-fructofuranoside] and associated α-galactosidase and sucrose hydrolase activities to plasmid DNA present in *P. pentosaceus* isolates.

MATERIALS AND METHODS

Bacterial strains and media. The bacterial strains are listed in Table 1. Carbohydrate fermentation was determined by using medium BM (8) containing the desired filter-sterilized carbohydrate at a final concentration of 0.5% and bromocresol purple at a final concentration of 0.008%. Cultures that fermented raffinose or sucrose were propagated on BM-raffinose or BM-sucrose at 32°C. Strains that did not ferment raffinose or sucrose were propagated on BM-glucose or APT (Difco Laboratories, Detroit, Mich.). Stock cultures grown in the appropriate medium were stored in liquid nitrogen with 10% (vol/vol) sterile glycerol added as a cryoprotectant. Cultures for routine use were stored in vials at −60°C.

Plasmid isolation and purification. Plasmid DNA was isolated, and DNA samples were subjected to agarose gel electrophoresis as previously described (8). Reference plasmid DNA was prepared as previously described (8).

Elimination of resident plasmids. Isolates to be used in curing experiments were streaked on BM-sucrose or BM-raffinose agar plates. A single acid-producing colony was then transferred to BM-sucrose or BM-raffinose for use in curing experiments. Stability of plasmid-encoded traits and curing of resident plasmids by temperature or curing agents was accomplished by methods previously described (8). Screening for raffinose- or sucrose-negative isolates was done by plating on BM-raffinose or BM-sucrose. To allow for detection of non-acid-producing isolates in the presence of large numbers of acid-producing colonies, medium BM was supplemented with sodium-β-glycerophosphate (Sigma Chemical Co., St. Louis, Mo.) at a final concentration of 0.475%. The percentage of non-sucrose-fermenting colonies obtained in temperature-curing studies is the average of results obtained in three independent experiments ± the standard deviation. The percentage of non-sucrose-fermenting colonies obtained with the curing agents acriflavin (Sigma) or proflavin (Sigma) is the average of results obtained in two independent experiments ± the standard deviation.

Preparation of cell-free extracts. Cells were grown in medium BM supplemented with the appropriate carbon source (0.5%). After growth at 35°C for 18 h, the cells were harvested by centrifugation and washed twice in 10 mM potassium phosphate buffer (pH 6.8). This procedure and all subsequent operations (except enzyme assay) were carried out at 0 to 5°C. Cells from 1 liter were suspended in 15 ml of buffer and disrupted in a Bead-Beater (Biospec Products, Bartlesville, Okla.) by using the small chamber kit with an ice water jacket and 0.01- to 0.15-mm glass beads. The cell suspension was subjected to 10 30-s intervals of blending separated by 5-min cooling periods. The disrupted cell suspension was subjected to centrifugation at 37,000 × *g* for 30 min to remove cell debris. Total protein concentration in the crude extracts was determined by the microbiuret method of Koch and Putman (10).

Enzyme assays. Sucrose hydrolase activity was assayed by incubation of cell extract in the presence of 0.01 M sucrose and 0.1 M potassium phosphate (pH 6.8). The assay mixture was incubated for 0, 30, and 60 min at 35°C. Enzymatic cleavage of sucrose was stopped by boiling the reaction mixture for 2 min. Controls included reaction mixtures to which boiled extracts had been added. Generation of reducing sugars from sucrose was measured by the method of Nelson (15).

Cells to be assayed for α-galactosidase activity were grown in BM broth containing the appropriate carbohydrate at a final concentration of 0.5%. Cells (100 ml) were harvested by centrifugation at mid-exponential growth (optical density at 600 nm, 0.3), washed twice, and suspended in 10 ml of cold 50 mM potassium phosphate buffer containing 5

* Corresponding author.

TABLE 1. Bacterial strains

P. pentosaceus strain	Resident plasmid(s)	Plasmid size(s) (MDa)	Specific plasmid (phenotype)[a]	Description, source, or reference[a]
PPE1.0	pSRQ1	30	pSRQ1 (Raf^+ Suc^+ Mel^+)	8
PPE1.2	None	None	None	PPE1.0 cured of pSRQ1 (Raf^- Suc^- Mel^-); 8
ATCC 25744	pSRQ12, pSRQ13, pSRQ14, pSRQ15, pSRQ16	28, 19, 8.5, 6, 2.3	pSRQ12 (Raf^+ Suc^+ Mel^+)	Laboratory designation, PPE2.0
PPE2.1	pSRQ13, pSRQ14, pSRQ15, pSRQ16	19, 8.5, 6, 2.3	None	PPE2.0 cured of pSRQ12 (Raf^- Suc^- Mel^-); this study
PPE5.0	pSRQ17, pSRQ18, pSRQ19, pSRQ20	23, 6.8, 6.2, 1.2	pSRQ17 (Raf^+ Suc^+ Mel^+)	Isolated from Kimchi; this study
PPE5.1	pSRQ18, pSRQ19, pSRQ20	6.8, 6.2, 1.2	None	PPE5.0 cured of pSRQ17 (Raf^- Suc^- Mel^-); this study

[a] Symbols: Raf, raffinose; Suc, sucrose; Mel, melibiose; +, ability to ferment designated carbohydrate; −, inability to ferment designated carbohydrate.

mM $MgCl_2$ (pH 6.5) (decryptification buffer). The chilled cell suspension was permeabilized by adding 100 μl of toluene-acetone (1:9) per ml of cell suspension. The mixture was mixed with a Vortex Genie (Scientific Industries, Inc., Bohemia, N.Y.) at maximum for 2 min. α-Galactosidase activity was measured by hydrolysis of the chromogenic substrate *O*-nitrophenyl-α-D-galactopyranoside (ONPG) at 37°C. The reaction mixture (625 μl) contained 125 μl of cell suspension and 500 μl of ONPG (5×10^{-3} M) in decryptification buffer. The mixture was incubated at 37°C in a 1.5-ml microcentrifuge tube for 5 min. The reaction was terminated by adding 625 μl of 0.5 M sodium carbonate. Controls included reaction mixtures to which a cell suspension which had been boiled were added. Cells were removed by centrifugation in an Eppendorf model 5412 centrifuge (Brinkmann Instruments, Inc., Westbury, N.Y.). The A_{420} of the supernatant was measured and converted to molar concentration of *O*-nitrophenol determined from an *O*-nitrophenol standard curve as described by Citti et al. (4). Under the conditions described, the lower limit for detection of *O*-nitrophenol was 0.2 nmol/ml. One unit of activity was defined as the number of micromoles of *O*-nitrophenol liberated from ONPG per min per mg (dry weight) of cells.

Crude extracts were assayed for α-galactosidase activity by a procedure similar to that used for permeabilized cells. The reaction mixture contained 125 μl of crude extract instead of cells. Reaction conditions were the same. Controls included reaction mixtures to which boiled extracts had been added. One unit of activity was defined as the number of micromoles of *O*-nitrophenol liberated from ONPG per min per mg of protein.

α-Glucosidase activity in crude extracts was assayed by the method described above for α-galactosidase activity, except that the substrate was *p*-nitrophenyl-α-D-glucoside. One unit of activity was defined as the number of nanomoles of *p*-nitrophenol liberated from *p*-nitrophenyl-α-D-glucoside per min per mg of protein.

RESULTS

Identification of plasmid and associated phenotype. Survey lysis of *P. pentosaceus* isolates had previously identified the presence of resident plasmids in representative strains (8). Strains PPE1.0 (Fig. 1, lane C) PPE3.0, and PPE4.0 were shown to contain plasmids ranging from 6.7 to 30 megadaltons (MDa) (8). A survey of plasmid DNA in two additional isolates, PPE2.0 and PPE5.0, showed the presence of resident plasmids listed in Table 1 and depicted in Fig. 1 (lanes D and F, respectively).

After determining that plasmid DNA was present in these strains, we initiated studies to determine whether any of the resident plasmids might encode for a detectable phenotype such as carbohydrate utilization or antibiotic or heavy metal resistance. Curing studies were initiated with both elevated growth temperatures and curing agents such as acriflavin and proflavin. Initial studies showed that growth of strain PPE1.0 at elevated temperature resulted in loss of the sucrose-fermenting phenotype. Analysis of plasmid DNA from these isolates showed that plasmid pSRQ1 had been eliminated.

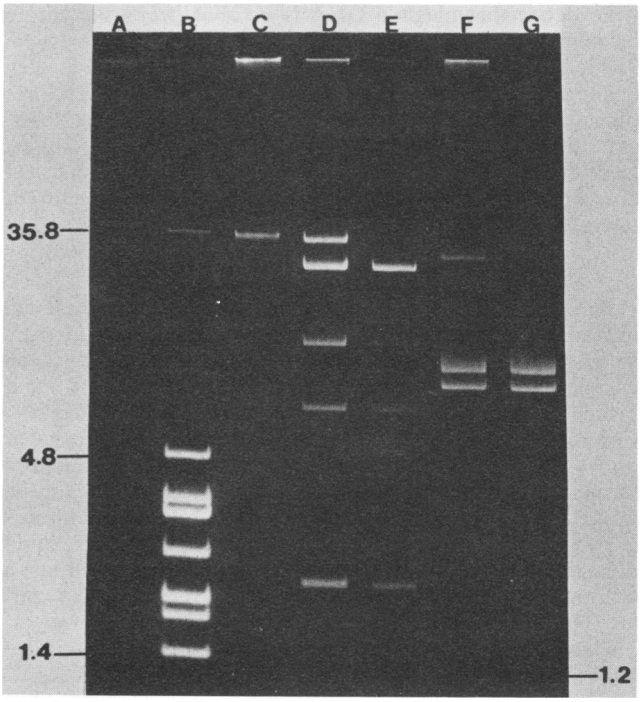

FIG. 1. Agarose gel electrophoresis of CsCl-ethidium bromide-purified plasmid DNA from *P. pentosaceus* strains. Electrophoresis was in 0.7% agarose at 10 V/cm for 2 h. Bands are identified top to bottom. (A) *P. aeruginosa* PAO2 (RP1); 38 MDa. (B) *E. coli* V517; 35.8-, 4.8-, 3.7-, 3.4-, 2.6-, 2.0-, 1.8- and 1.4-MDa covalently closed circular DNAs. (C) PPE1.0; pSRQ1. (D) PPE2.0; pSRQ12, pSRQ13, pSRQ14, pSRQ15, and pSRQ16. (E) PPE2.1; pSRQ13, pSRQ14, pSRQ15, and pSRQ16. (F) PPE5.0; pSRQ17, pSRQ18, pSRQ19, and pSRQ20 (migration indicated to the right, 1.2 MDa). (G) PPE5.1; pSRQ18, pSRQ19, and pSRQ20. The molecular mass of *P. pentosaceus* plasmids is shown in Table 1. The molecular mass of standard plasmid DNA is indicated.

TABLE 2. α-Galactosidase activity[a] of crude extracts from *P. pentosaceus* cells grown in different substrates

Strain	Hydrolysis of ONPG[a] in extracts of cells grown in:					
	Glucose	Fructose	Sucrose	Cellobiose	Melibiose	Raffinose
PPE1.0	0.087	0.062	0.103	0.015	0.526	0.383
PPE1.2	$<10^{-4}$	$<10^{-4}$	ND[b]	$<10^{-4}$	ND	ND
PPE2.0	0.015	0.009	0.016	0.016	0.529	1.129
PPE2.1	$<10^{-4}$	$<10^{-4}$	ND	$<10^{-4}$	ND	ND
PPE5.0	0.153	0.044	0.069	0.059	0.776	3.741
PPE5.1	$<10^{-4}$	$<10^{-4}$	ND	$<10^{-4}$	ND	ND

[a] Specific activity expressed as micromoles of O-nitrophenol liberated per min per mg of protein.
[b] ND, Not determined.

The plasmid-free strain was designated PPE1.2. Growth of strain PPE1.0 at temperatures of 32, 42, 45, or 48°C resulted in the detection of non-sucrose-fermenting (Suc^-) segregants at frequencies of 0.5 ± 0.007, 2.5 ± 1, 1.31 ± 0.6, and 1.5 ± 0.4%, respectively. Growth of strain PPE2.0 at the same temperatures resulted in Suc^- segregants at frequencies of 10.6 ± 1.8, 19.6 ± 6, 26.3 ± 7, and 15.2 ± 2.7%, respectively. Suc^- segregants of strain PPE5.0 were detected at frequencies of 1.75 ± 0.8, 7.3 ± 2, 12.5 ± 1.7, and 11.7 ± 2.7%, respectively, when grown at the same temperatures as PPE1.0 and PPE2.0. The instability of the sucrose-fermenting phenotype (Suc^+) generally increased with growth temperature. The drop in curing frequency observed at the highest temperature (48°C) may be a reflection of cell viability at this temperature. The number of CFU at 48°C (2×10^6) in comparison with the other temperatures (10^9 at 32°C) examined in this study was lower.

Representative isolates of PPE1.0, PPE2.0, and PPE5.0 expressing a sucrose-negative phenotype were lysed and examined for plasmid content. Agarose gel electrophoresis of DNA samples showed that, as previously observed, strain PPE1.0 had lost its 30-MDa plasmid, pSRQ1. Additionally, strains PPE2.0 and PPE5.0 showed losses of 28- (pSRQ12) and 23-MDa (pSRQ17) plasmids, respectively (Fig. 1, lanes E and G, respectively). Agarose gel electrophoresis of DNA samples obtained from survey lysis of representative Suc^+ isolates of strains PPE1.0, PPE2.0, and PPE5.0 grown at the various temperatures showed that these Suc^+ isolates contained plasmids pSRQ1, pSRQ12, and pSRQ17, respectively.

Similar studies were conducted with the curing agents acriflavin and proflavin. Growth of the same isolates in the presence of sublethal concentrations of acriflavin or proflavin resulted in loss of the sucrose-fermenting phenotype. Growth of PPE1.0, PPE2.0, and PPE5.0 in the presence of 15, 12, or 9 μg of acriflavin per ml, respectively, resulted in Suc^- segregants at frequencies of 30.2 (656 of 2,173) ± 4.2, 45.5 (437 of 961) ± 6.7, and 25.2 (188 of 745) ± 7%, respectively. Suc^- segregants of strains PPE1.0 and PPE2.0 were observed at frequencies of 5.2 (28 of 536) ± 1.5 and 68.6 (913 of 1,331) ± 9%, respectively, when the strains were grown in the presence of 20 μg of proflavin per ml. A frequency of 41.4 (375 of 910) ± 2.7% Suc^- segregants was observed for strain PPE5.0 grown in the presence of 12 μg of proflavin per ml. Survey lysis of representative isolates showed results similar to those obtained in the temperature-curing studies with plasmids pSRQ1, pSRQ12, and pSRQ17 being eliminated in Suc^- isolates of PPE1.0, PPE2.0, and PPE5.0, respectively.

Further characterization of the respective Suc^- isolates from both curing studies showed that, along with the sucrose-fermenting phenotype, the isolates had also lost the ability to ferment the trisaccharide raffinose (Raf^-), and the dissaccharide melibiose (Mel^-). The utilization of raffinose has been shown to be plasmid encoded in *Escherichia coli* (17). The characterized *E. coli* raffinose plasmid encodes for a raffinose transport system, invertase, and α-galactosidase (19). The α-galactosidase catalyzes the hydrolysis of raffinose to galactose and sucrose, and the invertase catalyzes the hydrolysis of sucrose to fructose and glucose. The presence of α-galactosidase and sucrose hydrolase activities being associated with plasmids pSRQ1, pSRQ12, and pSRQ17, respectively, was addressed.

α-Galactosidase activity. Curing studies suggested that utilization of raffinose, melibiose, and sucrose in *P. pentosaceus* PPE1.0, PPE2.0, and PPE5.0 was plasmid linked. It was of interest to determine whether quantitative differences in enzyme activity in parent and cured derivatives were correlated with the presence and absence of plasmids pSRQ1, pSRQ12, and pSRQ17. Strains PPE1.0, PPE2.0, and PPE5.0 and their respective Suc^- Raf^- Mel^- isolates, PPE1.2, PPE2.1, and PPE5.1, were assayed for α-galactosidase with permeabilized cells. The parental strains and their respective cured strains were grown in glucose or melibiose. The parental strains PPE1.0, PPE2.0, and PPE5.0 showed activities of 0.011, 0.014, and 0.010 μmol/min per mg of cell dry weight (U), respectively, when grown in the presence of glucose, while plasmid-cured isolates exhibited no detectable activity. Growth of the parental strains in the presence of melibiose appeared to induce the cells. Activities of 0.311, 0.248, and 0.235 U were expressed for PPE1.0, PPE2.0, and PPE5.0, respectively, when grown in the presence of melibiose. The cured isolates were unable to utilize melibiose as a growth substrate. However, it was determined that growth of strain PPE5.0 in the presence of cellobiose, a β-D-glucopyranosyl-D-glucoside, induced α-galactosidase activity to a level of 0.264 U. Activities of 0.025 and 0.014 U were observed in extracts of strains PPE1.0 and PPE2.0, respectively, grown in the presence of cellobiose. Although the cured derivatives were able to utilize cellobiose as a growth substrate, there was no evidence of α-galactosidase activity in extracts of cured cells grown in this substrate.

Since the permeabilized cell assay had confirmed the existence of toluene-resistant α-galactosidase activity, we proceeded to determine if such activity was evident in crude extracts of cells grown on various substrates. Data shown in Table 2 illustrate the presence of α-galactosidase activity in parental strains grown in six different substrates. No detectable activity was observed in the respective cured isolates grown in three of the six substrates. The specific activities of α-galactosidase in crude extracts were 6-, 35-, and 51-fold higher for strains PPE1.0, PPE2.0, and PPE5.0, respectively, when they were grown in the α-galactoside melibiose

TABLE 3. Sucrose-hydrolyzing activity of crude extracts from *P. pentosaceus* cells grown in different substrates

Strain	Sucrose hydrolase activity[a] in extracts of cells grown in:				
	Glucose	Fructose	Sucrose	Melibiose	Raffinose
PPE1.0	0.40	0.04	5.5	0.06	8.1
PPE1.2	<0.01	<0.01	ND[b]	ND	ND
PPE2.0	0.23	0.02	1	0.09	1.8
PPE2.1	<0.01	<0.01	ND	ND	ND
PPE5.0	0.05	0.02	2	0.03	4.1
PPE5.1	<0.01	<0.01	ND	ND	ND

[a] A unit was defined as that amount of activity generating 1 nmol of reducing sugar (expressed as glucose) per min per mg of protein at 35°C. Assay was by the method of Nelson (15).
[b] ND, Not determined.

than when they were grown on glucose. Activities 4-, 75-, and 250-fold higher were observed in extracts of raffinose-grown cells for the same three strains, respectively, compared with those grown in glucose.

Levels of sucrose hydrolase activity in cells grown on various carbon sources. The association between the ability to ferment raffinose and the ability to ferment sucrose was further investigated by determining sucrose hydrolase activity in cell-free extracts. Strains PPE1.0, PPE2.0, and PPE5.0 grown in the presence of glucose, fructose, sucrose, melibiose, or raffinose and respective plasmid-cured derivatives grown in the presence of glucose or fructose were examined for sucrose hydrolase activity. Strains PPE1.0, PPE2.0, and PPE5.0 showed high sucrose hydrolase activity when grown in sucrose or raffinose but not when grown in fructose or melibiose (Table 3). Moderate activity was observed in PPE1.0, and PPE2.0 grown in glucose, with PPE5.0 showing low activity. No activity was observed in extracts of cured isolates grown in either glucose or fructose.

α-Glucosidase activity of extracts was also tested by using *p*-nitrophenyl-α-D-glucopyranoside as a substrate. Extracts of strain PPE1.0 grown in glucose, fructose, sucrose, or raffinose showed low-level activities of 0.2, 0.1, 2.0, and 1.9 units, respectively. No α-glucosidase activity was observed in extracts of strain PPE1.2 grown in either glucose or fructose. Extracts of strain PPE2.0 or PPE5.0 grown in the same substrates as PPE1.0 showed no α-glucosidase activity.

DISCUSSION

Evidence of the presence of plasmid-encoded raffinose utilization and associated α-galactosidase and sucrose hydrolase activities in *P. pentosaceus* has been described. The abilities to ferment the trisaccharide raffinose and the disaccharides sucrose and melibiose were found to be associated with three individual plasmids from three distinct strains of *P. pentosaceus*. Strains PPE1.0, PPE2.0, and PPE5.0 contained plasmids of 30, 28, and 23 MDa, respectively, associated with functions necessary for the metabolism of raffinose. Isolates of *P. pentosaceus* PPE1.0, PPE2.0, and PPE5.0 which had lost the ability to ferment raffinose, sucrose, and melibiose simultaneously were found to be cured of pSRQ1, pSRQ12, and pSRQ17, respectively. In addition to loss of the ability to ferment these carbohydrates by cells, cell-free extracts of the cured derivatives showed no evidence of α-galactosidase or sucrose hydrolase activity when grown in the presence of substrates which induced activity in parental strains (Tables 2 and 3). Also, a permeabilized cell assay for α-galactosidase activity showed the associated enzyme activity in parental strains to be toluene resistant. Characterization of α-galactosidase activity associated with a raffinose plasmid in *E. coli* has shown that, in contrast to chromosomal α-galactosidase specified by the melibiose system of *E. coli* (20), raffinose α-galactosidase which is plasmid encoded can be assayed in toluene-treated cells (19). Cornelis et al. (5) have suggested toluene resistance as a useful test for defining strains which harbor a raffinose plasmid and for distinguishing them from Raf^+ strains which do not.

The raffinose plasmid D1021 enables strains of *E. coli* to utilize raffinose as a carbon source by the action of three inducible plasmid-encoded functions, namely, a transport system (raffinose permease), an α-galactosidase, and an invertase catalyzing the uptake and hydrolysis of the trisaccharide into monsaccharides (19). The three functions are coordinately induced by different α-galactosidase suggesting common regulation (19). Evidence for inducible α-galactosidase and sucrose hydrolase activities was obtained for *P. pentosaceus* strains PPE1.0, PPE2.0, and PPE5.0 containing plasmids pSRQ1, pSRQ12, and pSRQ17, respectively. The presence of an inducible permease can only be inferred from available data. Studies of extracts of *P. pentosaceus* cells showed that growth of these cells in α-galactosides induced high α-galactosidase activity. Extracts of sucrose-grown cells showed α-galactosidase activity equal to that seen with glucose- or fructose-grown cells (Table 2). Extracts of cells grown with sucrose or raffinose showed moderate sucrose-hydrolyzing activity, while those grown in the presence of the α-galactoside melibiose showed only minimal activity. These data suggest that the α-galactosidase and sucrose-hydrolyzing activities are not coordinately induced and may not be under common regulation on plasmids pSRQ1, pSRQ12, and pSRQ17. The α-D-glucosidase activity observed in extracts of strain PPE1.0 suggest that sucrose hydrolase activity in this strain may not be due entirely to invertase (β-D-fructofuranoside fructohydrolase)-like activity but rather to α-glucosidase activity or a combination of the two activities. Lack of such activity in PPE2.0 and PPE5.0 may indicate that such activity was not detected or was not present. Further characterization and purification of enzymes will determine the specificity of the enzymes involved in utilization of raffinose and sucrose in *P. pentosaceus*.

Preliminary results in our laboratory have shown that the *P. pentosaceus* PPE1.2 protoplast can be transformed with plasmid pGB301 (2) at a frequency of 15 transformants per μg of DNA by a procedure similar to that described for *Streptococcus lactis* (11). Attempts to restore PPE1.2 to a Raf^+ phenotype with plasmid pSRQ1 DNA and polyethylene glycol-induced transformation have, to date, been unsuccessful. This inability to restore PPE1.2 to a Raf^+ phenotype is most probably due to lack of optimal conditions for the

transformation and recovery of protoplast or to such parameters as plasmid size. Current efforts to improve protoplast regeneration and transformation frequencies are in progress. Conjugal transfer experiments with the broad-host-range plasmid pIP501 (3) were conducted with a Raf^+ derivative of PPE1.0 designated PPE1.12 (8), which harbors plasmid pIP501. The recipient was an antibiotic-resistant derivative of PPE1.2 designated PPE1.5 (8). Selection in these mating experiments was for transfer of pIP501 and Raf^+ phenotype in transconjugants. It was thought that pIP501 might mobilize plasmid pSRQ1; however, numerous experiments have shown only the transfer of plasmid pIP501 to the recipient at a frequency of 10^{-5} transconjugants per donor with no detectable transfer of the Raf^+ phenotype.

The selective advantage or role of the raffinose plasmid in *P. pentosaceus* is unknown. However, *P. pentosaceus* resides naturally on plants (14) and is associated with fermentation of a variety of vegetables (18). Sucrose is by far the most commonly found disaccharide in nature, its distribution being universal among photosynthetic plants (1). Melibiose is found in many plants and is found in high amounts in soybean roots and stems (1). The trisaccharide raffinose is the most common sugar, next to sucrose, found in higher plants (1). It is, therefore, possible that plasmid-encoded raffinose utilization may give these isolates a selective advantage in being able to ferment sugars which are commonly found in plants.

Knowledge of plasmids which govern the fermentation of carbohydrates in lactic acid bacteria is of importance to the food fermentation industry. In product development it may be necessary to promote or prevent the utilization of certain naturally occurring or added carbohydrates in fermentation systems. By addition or deletion of a naturally occurring plasmid in a microorganism, its fermentation pattern may be tailored either to ferment or not ferment a given carbohydrate. One such example is a recently issued patent (C. F. Gonzalez, U. S. patent 4,508,738, April 1985), in which the naturally occurring raffinose-sucrose plasmid was eliminated so that *P. pentosaceus* could be used to ferment meats and vegetables in the presence of added sucrose without fermenting the sucrose. Further studies will determine the transfer abilities and relatedness of these naturally occurring plasmids.

ACKNOWLEDGMENTS

We gratefully acknowledge the assistance of Loretta S. McMullen in the preparation of the manuscript. We thank Ebenezer R. Vedamuthu for critical reading of the manuscript.

LITERATURE CITED

1. **Axelrod, B.** 1965. Mono- and oligosaccharides, p. 231–254. *In* J. Bonner and J. E. Varner (ed.), Plant biochemistry. Academic Press, Inc., N.Y.
2. **Behnke, D., M. S. Gilmore, and J. J. Ferretti.** 1981. Plasmid pGB301, a new multiple resistance streptococcal cloning vehicle and its use in cloning of the gentamycin/kanamycin resistance determinant. Mol. Gen. Genet. **182**:414–421.
3. **Buu-Hoï, A., G. Bieth, and T. Horaud.** 1984. Broad host range of streptococcal macrolide resistance plasmids. Antimicrob. Agents Chemother. **25**:289–291.
4. **Citti, J. E., W. E. Sandine, and P. R. Elliker.** 1965. β-Galactosidase of *Streptococcus lactis*. J. Bacteriol. **89**:937–942.
5. **Cornelis, G., R. K. J. Luke, and M. H. Richmond.** 1978. Fermentation of raffinose by lactose-fermenting strains of *Yersinia enterocolitica* and by sucrose-fermenting strains of *Escherichia coli*. J. Clin. Microbiol. **7**:180–183.
6. **Davey, G. P., and L. E. Pearce.** 1982. Production of diplococcin by *Streptococcus cremoris* and its transfer to nonproducing group N streptococci, p. 221–224. *In* D. Schlessinger (ed.), Microbiology—1982. American Society for Microbiology, Washington, D.C.
7. **Efstathiou, J. D., and L. L. McKay.** 1976. Plasmids in *Streptococcus lactis*: evidence that lactose metabolism and proteinase activity are plasmid linked. Appl. Environ. Microbiol. **32**:38–44.
8. **Gonzalez, C. F., and B. S. Kunka.** 1983. Plasmid transfer in *Pediococcus* spp.: intergeneric and intrageneric transfer of pIP501. Appl. Environ. Microbiol. **46**:81–89.
9. **Kempler, G. M., and L. L. McKay.** 1979. Characterization of plasmid deoxyribonucleic acid in *Streptococcus lactis* subsp. *diacetylactis*: evidence for plasmid-linked citrate utilization. Appl. Environ. Microbiol. **37**:316–323.
10. **Koch, A., S. Putnam.** 1971. Sensitive biuret method for determination of protein in impure system such as whole bacteria. Anal. Biochem. **44**:237–245.
11. **Kondo, J. K., and L. L. McKay.** 1984. Plasmid transformation of *Streptococcus lactis* protoplasts: optimization and use in molecular cloning. Appl. Environ. Microbiol. **48**:252–259.
12. **LeBlanc, D. J., V. L. Crow, and L. N. Lee.** 1980. Plasmid mediated carbohydrate catabolic enzymes among strains of *Streptococcus lactis*, p. 31–41. *In* C. Struttard and K. R. Rozee (ed.), Plasmids and transposons: environmental effects and maintenance mechanisms. Academic Press, Inc., New York.
13. **McKay, L. L., and K. A. Baldwin.** 1984. Conjugative 40-megadalton plasmid in *Streptococcus lactis* subsp. *diacetylactis* DRC3 is associated with resistance to nisin and bacteriophage. Appl. Environ. Microbiol. **47**:68–74.
14. **Mundt, J. O., W. G. Beattie, and F. R. Weiland.** 1969. Pediococci residing on plants. J. Bacteriol. **98**:938–942.
15. **Nelson, N.** 1944. A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. **153**:375–380.
16. **Neve, H., A. Geis, and M. Teuber.** 1984. Conjugal transfer and characterization of bacteriocin plasmids in group N (lactic acid) streptococci. J. Bacteriol. **157**:833–838.
17. **Ørskov, I., and F. Ørskov.** 1973. Plasmid-determined H_2S character in *Escherichia coli* and its relation to plasmid-carried raffinose fermentation and tetracycline resistance characters. J. Gen. Microbiol. **77**:487–499.
18. **Pederson, C. S.** 1949. The genus *Pediococcus*. Bacteriol. Rev. **13**:225–232.
19. **Schmid, K., and R. Schmitt.** 1976. Raffinose metabolism in *Escherichia coli* K12. Purification and properties of a new α-galactosidase specified by a transmissible plasmid. Eur. J. Biochem. **67**:95–104.
20. **Schmitt, R.** 1968. Analysis of melibiose mutants deficient in α-galactosidase and thiomethylgalactoside permease II in *Escherichia coli* K-12. J. Bacteriol. **96**:462–471.
21. **Snook, R. J., and L. L. Mckay.** 1981. Conjugal transfer of lactose-fermenting ability among *Streptococcus cremoris* and *Streptococcus lactis* strains. Appl. Environ. Microbiol. **42**: 904–911.