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# Plasmid DNA in Strains of *Pediococcus cerevisiae* and *Pediococcus pentosaceus*†

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**Five parental strains of *Pediococcus* were examined for plasmid content. Each strain contained three to six resident plasmids, ranging in size from 4.5 to 39.5 megadaltons. A bacteriocin-like substance produced by *Pediococcus cerevisiae* FBB63 was tentatively linked to a 10.5-megadalton plasmid after being cured with novobiocin.**

The pediococci are saprophytes often found in fermenting vegetable material (10). Many of these organisms are important to the food industry in that they are involved in a variety of food fermentations, i.e., fermentation of pickles, green olives, sauerkraut, soy sauce, sausage products (15), and Cheddar cheese (6). Processes involving the use of pediococci as starter cultures have been developed for some of these products. These pediococcal fermentations not only help to preserve the products but aid in flavor development (6, 9) and inhibit the growth of some pathogenic organisms (4, 5).

Lactic acid starter cultures used in the dairy and meat industries occasionally fail as a result of genetic change or the presence of lytic phage (11, 17). Failures caused by genetic changes may result from gene mutation or loss of plasmid DNA (11). The presence of plasmids in lactic acid bacteria, particularly in the group N streptococci, has been known for some time (3). The first evidence of plasmid DNA in pediococci was reported recently by Gonzales et al. (7) in strains of *Pediococcus pentosaceus* and *P. acidilactici*; however, no information about their metabolic functions was presented.

The purpose of the present investigation was to determine the plasmid profile of several pediococci, to ascertain whether plasmid DNA was responsible for the production of a bacteriocin-like substance (Bac) by *P. cerevisiae* FBB63, and to determine whether mutant strains of *P. cerevisiae* FBB63 showed any other metabolic changes.

Pediococcal strains selected for plasmid isolation (Table 1) were grown for 12 h at 35°C in 10, 40, or 200 ml of APT broth (1% inoculum). Cells were harvested by centrifugation, washed with Tris-EDTA-NaCl buffer (8), and either used immediately or stored at −70°C for later use. The protocol used for plasmid DNA preparations was described by Anderson and McKay (1), except that the renatured lysate was extracted twice with phenol. Agarose gel electrophoresis was carried out as described previously (1).

TABLE 1. Strains of pediococci used

| Strain | Plasmid composition (Mdal) | Relevant phenotype | Comment and source |
|---|---|---|---|
| *P. pentosaceus* E66 | 34, 6.1, 4.6 | $Bac^-$ | Obtained from J. R. Stamer[a] |
| *P. pentosaceus* 991 | 34, 6.1, 4.6 | $Bac^-$ | Obtained from NCDO-NIRD[b] |
| *P. pentosaceus* 996 | 34, 26, 5.9, 5.4, 4.5 | $Bac^-$ | Obtained form NCDO-NIRD[b] |
| *P. cerevisiae* FBB39 | 39.5, 26, 10.2, 5.9, 5.2, 4.5 | $Bac^-$ | Obtained from R. N. Costilow[c] |
| *P. cerevisiae* FBB63 | 39.5, 26, 10.5, 5.2 | $Bac^+$ | Obtained from R. N. Costilow[c] |
| *P. cerevisiae* FBB63 DG-1 | 26, 10.5, 5.2 | $Bac^+$ | Novobiocin-induced mutant; this study |
| *P. cerevisiae* FBB63 DG-2 | 26, 10.5 | $Bac^+$ | Novobiocin-induced mutant; this study |
| *P. cerevisiae* FBB63 DG-3 | 5.2 | $Bac^-$ | Novobiocin-induced mutant; this study |
| *P. cerevisiae* FBB63 DG-4 | None | $Bac^-$ | Novobiocin-induced mutant; this study |
| *P. cerevisiae* FBB63 DG-6 | 10.5 | $Bac^+$ | Novobiocin-induced mutant; this study |

[a] New York State Experiment Station, Geneva, N.Y.
[b] National Collection of Dairy Organisms, National Institute for Research in Dairying, Shinfield, Reading, England.
[c] Department of Microbiology and Public Health, Michigan State University, East Lansing, Mich.

In testing for bacteriocin production ($Bac^+$), approximately 0.05 ml of a 16-h, 35°C pediococcal culture was spotted onto Trypticase soy agar (TSA) (BBL Microbiology Systems) plates. *P. cerevisiae* FBB63 ($Bac^+$) spotted onto TSA served as the control. Plates were incubated at 30°C for 24 h and then overlaid with 5 ml of Trypticase soy soft agar (TSSA; Trypticase soy broth [TSB] plus 0.5% agar) which was seeded with 0.1 ml of a 16-h culture of *P. cerevisiae* FBB39, a Bac-sensitive strain. The plate was incubated at 30°C for 24 h. A colony which produced a clear zone of inhibition extending 0.5 mm or more from the edge of the colony was considered $Bac^+$ (5).

Curing trials were carried out to determine whether Bac production in *P. cerevisiae* FBB63 was plasmid linked. A 1% inoculum of *P. cerevisiae* FBB63 was made into APT broth containing 0.5 to 3.0 μg of acriflavine per ml, 0.5 to 20 μg of

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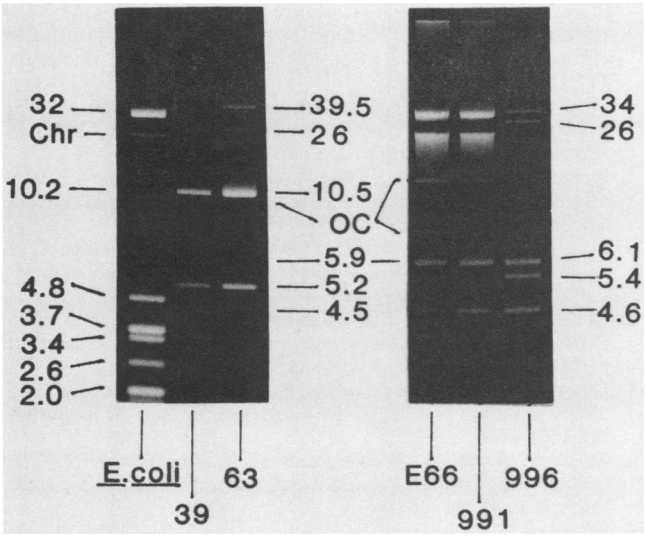


FIG. 1. Agarose gel electrophoresis of plasmid DNA detected in *P. cerevisiae* FBB39, *P. cerevisiae*, FBB63, *P. pentosaceus* E66, *P. pentosaceus* 991, and *P. pentosaceus* 996. *E. coli* V517 (far left) was used as molecular mass standard.

acridine orange per ml, or 5 to 100 μg of novobiocin per ml. Cultures were incubated at 35°C for 24 h, transferred into fresh broth containing the appropriate mutagen, and incubated again. This process was continued through several transfers. Cultures which had passed through two or more transfers were diluted, plated on TSA, and incubated at 30°C for 24 h. Plates containing 30 to 100 colonies were replica plated onto fresh agar, and the original plate was overlaid with TSSA seeded with *P. cerevisiae* FBB39 and incubated for 24 h at 35°C. Colonies without clear zones of inhibition were judged to no longer produce Bac. From the replica plate which was not overlaid, presumptive $Bac^-$ colonies were picked into APT broth, incubated at 35°C, and retested for absence of Bac production. $Bac^-$ mutants were subsequently examined for plasmid DNA (14).

Each of the five parental pediococcal strains contained three to six resident plasmids ranging in size from 4.5 to 39.5 megadaltons (Mdal) (Fig. 1; Table 1). The profiles of *P. pentosaceus* E66 and *P. pentosaceus* 991 were found to be the same, and the profiles of *P. cerevisiae* FBB39 and *P. cerevisiae* FBB63 were similar, except that *P. cerevisiae* FBB39 contains two additional plasmids. The presence of plasmid DNA in pediococci and the relationship between plasmid-directed functions and food fermentation capabilities is just beginning to be assessed. Gonzalez and Kunka (7) found one or two cryptic resident plasmids in three strains of *P. pentosaceus*, two plasmids in one strain of *P. acidilactici*, and no plasmid DNA in a second *P. acidilactici* strain. The plasmid sizes ranged from 4.7 to 30 Mdal. A larger number of resident plasmids were found in each strain of *P. pentosaceus* and *P. cerevisiae* examined, with sizes up to about 40 Mdal. Thus the pediococci, like the streptococci (2, 13), contain a variety of plasmids which may code for functions necessary for their use in fermentation processes. The small plasmids observed in the pediococci examined may prove useful as vector plasmids. Further study is necessary to elucidate the metabolic functions controlled by the many cryptic pediococcal plasmids.

Of the five original strains of pediococci examined, only *P. cerevisiae* FBB63 was found to produce bacteriocin (Table 1). Additionally, the four nonproducing strains would not grow in the presence of this bacteriocin. The production of an inhibitory substance or Bac in pediococci was first reported by Fleming et al. (5). Bac was found to be active against several genera of bacteria, including $Bac^-$ pediococci. Only one of the *Pediococcus* spp. screened in this study was found to be $Bac^+$. Furthermore, it was inhibitory to the other species of pediococci examined, which confirms the findings of Fleming et al. (5). Many genera, including *Bacillus*, *Clostridium*, *Corynebacterium*, *Lactobacillus*, *Mycobacterium*, *Sarcina*, *Staphylococcus*, and *Streptococcus* spp., have been reported to produce Bac. Tagg et al. (16) suggested that the genes coding for bacteriocin production are plasmid linked and that the $Bac^+$ phenotype can be eliminated by using standard techniques for plasmid curing. *P. cerevisiae* FBB63 was cultured at 35°C in broth containing 5 to 50 μg of novobiocin per ml, with consecutive transfers every 24 h. Treatment with novobiocin yielded $Bac^-$ derivatives. Some of these derivatives had lost 1, 2, or 3 plasmids (Table 1); however, Bac production could not be linked to a specific plasmid. $Bac^+$ strain DG-2, which contained two plasmids, of 26 and 10.5 Mdal, was selected for further curing studies. Strain DG-2 was treated with 50, 75, or 100 μg of novobiocin per ml for several transfers. When cultures were plated and analyzed for $Bac^+$-$Bac^-$ colonies, over 60% of the isolates were $Bac^-$. Figure 2 is a photograph of $Bac^+$ colonies (with clear zones) and $Bac^-$ colonies contained in a novobiocin-treated culture overlaid with the Bac-sensitive strain. From cultures treated with 75 μg of novobiocin per ml, 336 $Bac^-$ mutants were isolated that were cured of both plasmids (Table 1) and one $Bac^+$ mutant was isolated that contained only one plasmid, of 10.5 Mdal (Fig. 3). Thus, this physical evidence tentatively links the 10.5-Mdal plasmid in *P. cerevisiae* FBB63 to Bac production. Genetic evidence is now needed to confirm this linkage. This is the first reported evidence that suggests a plasmid-linked trait in *Pediococcus* spp. The value of this information is twofold. First, Bac has been shown to be inhibitory toward a number of microorganisms, including *Staphylococcus aureus*, *Bacillus cereus*, and *Streptococcus faecalis* (5), which are bacteria some-

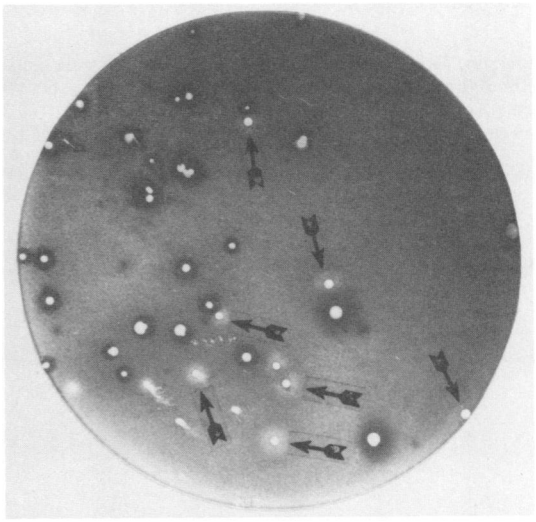

FIG. 2. Curing of *P. cerevisiae* FBB63 DG-2 after two transfers in broth containing 75 μg of novobiocin per ml. The culture was plated on TSA, allowed to grow, and overlaid with a Bac-sensitive strain (FBB39). Colonies surrounded by a clear zone are $Bac^+$ and contain the 10.5-Mdal plasmid; those without clear zones (arrows) are $Bac^-$, having lost the 10.5-Mdal plasmid.

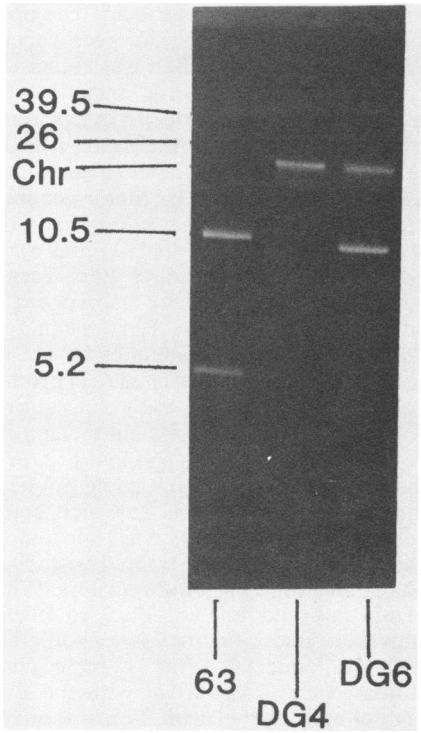


FIG. 3. Agarose gel electrophoresis of plasmid DNA detected in novobiocin-treated mutants DG-4 ($Bac^-$) and DG-6 ($Bac^+$). $Bac^+$ parental strain *P. cerevisiae* FBB63 is on the left.

times found in foods. Second, Bac may be plasmidborne, which means that the characteristics may be transferred to other organisms for strain improvement purposes. Although the results presented here show that pediococci, like the group N streptococci, contain a variety of plasmids (12), much work must be done before the plasmid functions can be elucidated.

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