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# Influence of Growth Conditions on the Production of a Bacteriocin, Pediocin AcH, by *Pediococcus acidilactici* H†

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**The influence of growth parameters on the production of pediocin AcH by *Pediococcus acidilactici* H was studied. This strain produced large quantities of pediocin AcH in TGE broth (Trypticase [1%], glucose [1%], yeast extract [1%], Tween 80 [0.2%], $Mn^{2+}$ [0.033 mM], $Mg^{2+}$ [0.02 mM] [pH 6.5]) within 16 to 18 h at 30 to 37°C (final pH, 3.6 to 3.7). Pediocin AcH production was negligible when the pH of the medium was maintained at 5.0 or above, even in the presence of high cell mass.**

Antibacterial peptides or bacteriocins from food-grade lactic acid bacteria are bactericidal to many gram-positive bacteria associated with food spoilage and food-borne illnesses (2, 6, 13, 14, 19) and retain this property after heat treatment and in food (1, 2, 4, 13, 16). They are degraded by the proteolytic enzymes of the gastrointestinal tract and seem to be nontoxic and nonantigenic to animals; thus, they can be used to enhance the safety and shelf life of many foods (2, 3, 5, 8, 10, 13, 14). Nisin and pediocins, which are bacteriocins, have wide spectra of bactericidal activity against gram-positive bacteria and are suitable candidates for use as food biopreservatives (2, 6, 9, 12, 13, 17). For effective commercial application, optimization of their production by modification of both genetic regulation and environmental growth parameters will be important. Genetic determinants of nisin and pediocin production are currently being studied (6, 7, 9, 12, 18). The effects of environmental factors on nisin production have been well studied (11, 13). We report here the influence of certain growth factors on the production of pediocin AcH by *Pediococcus acidilactici* H.

Bacterial strains used were *P. acidilactici* H (for pediocin AcH production) and *Lactobacillus plantarum* NCDO 955 (for assay of pediocin AcH) (2–4). The pediocin AcH concentration in a culture broth was determined by a bioassay method described previously with some modifications and is expressed here in activity units per milliliter (4). An aliquot of a test culture broth was heated in boiling water for 15 min and serially diluted (1:10 to 1:200), and 5 μl from each dilution was spotted onto a lawn of *L. plantarum* NCDO 955 in an assay plate. The assay plate had a bottom layer of TGE agar (TGE broth plus 1.5% agar) and a top layer of TGE soft agar (TGE broth plus 0.8% agar) and was seeded with about $10^6$ *L. plantarum* NCDO 955 cells. The TGE broth consisted of Trypticase (1%), glucose (1%), yeast extract (1%), Tween 80 (0.2%), $Mn^{2+}$ (0.033 mM), and $Mg^{2+}$ (0.02 mM) (pH 6.5). The plates were incubated at 30°C for 16 h, and the highest dilution that produced a distinct inhibition zone was multiplied by 200 (1 ml/5 μl) to obtain the activity units per milliliter. Initially, Lactobacillus MRS broth (Difco) and several modifications of it, including TGE broth, were compared for pediocin AcH production. TGE broth was found to yield maximum amounts of pediocin AcH and was used in subsequent experiments. The influence of supplementation with and/or replacement of nutrients on acid production (pH), cell density (optical density at 600 nm), and pediocin AcH production (activity units per milliliter) after growth of *P. acidilactici* H in TGE broth (10-ml portions, in duplicate) for 16 h at 37°C was studied. The nutrients tested were as follows: sucrose, galactose, arabinose, xylose, trehalose, and raffinose (each at 1%); glucose (0 to 2%); Trypticase (0 to 2%); yeast extract (0 to 2%); Tween 80 (0 to 1%); $Mn^{2+}$ (0 to 0.33 mM); $Mg^{2+}$ (0.02 mM); sodium citrate (9 mM); potassium acetate (20 mM); and sodium phosphate (7.5 mM). The influence of incubation time (4 to 24 h), temperature (30 to 40°C), initial culture pH (4.0 to 7.0), and final culture pH (4.0 to 6.5) on pH, cell mass, and pediocin AcH production during growth of *P. acidilactici* H was studied with 800 ml of TGE broth in a fermentor (Biostat M; B. Braun Ltd.). In all studies, a 1% inoculum of *P. acidilactici* H which had been grown for 16 h at 37°C was used.

*P. acidilactici* H incubated for 16 h at 37°C produced 15% less pediocin AcH in Lactobacillus MRS broth than it did in TGE broth, although the cell mass was greater in Lactobacillus MRS broth (data not shown). Trypticase, glucose, and yeast extract at the 1% level in TGE broth produced large amounts of pediocin AcH, cell mass, and acid (Table 1); at 2% concentrations of these nutrients, cell mass and/or pediocin AcH production increased only slightly (data not shown). Both cell mass and pediocin AcH production were optimum at 0.2% Tween 80 and 0.033 mM $Mn^{2+}$ concentrations. $Mg^{2+}$ at 0.02 mM in TGE broth had a stabilizing effect on cell mass and optimized pediocin AcH production. Of the carbohydrates, glucose and sucrose yielded the highest levels of pediocin AcH and cell mass (Table 1). Incorporation into TGE broth of acetate, citrate, and phosphate together increased the cell mass but reduced acid and pediocin AcH production. Cell mass, acid, and pediocin AcH production were maximum in TGE broth with an initial pH of 6.5 after 16 h of incubation at 30 or 37°C. At 40°C, there was a reduction in both cell mass and pediocin AcH production (Table 1).

*P. acidilactici* H grew and produced acid at maximum rates at between 4 and 8 h at 37°C, as indicated by an increase in optical density and a decrease in pH, respectively (Fig. 1). About 60% of the pediocin AcH was produced by 8 h, and the final 40% was produced during the next 8 h

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TABLE 1. Influence of medium composition, incubation temperature, and culture broth initial pH on optical density at 600 nm ($OD_{600}$), final pH, and activity units (AU) of pediocin AcH after 16 h of growth of *P. acidilactici* H at 37°C[a]

| Parameter | $OD_{600}$ | Final pH | $10^4$ AU/ml |
|---|---|---|---|
| Medium components[b] | | | |
| Trypticase (1%) | 3.5 | 3.7 | 3.6 |
| Glucose (1%) | 3.5 | 3.7 | 3.6 |
| Yeast extract (1%) | 3.3 | 3.7 | 3.6 |
| Tween 80 (0.2%) | 3.5 | 3.7 | 4.0 |
| $Mn^{2+}$ (0.033 mM) | 3.5 | 3.7 | 4.0 |
| Sucrose (1%) | 4.0 | 3.6 | 2.0 |
| Xylose (1%) | 2.0 | 4.4 | 1.0 |
| Galactose (1%) | 2.3 | 3.9 | 0.6 |
| Arabinose (1%) | 0.6 | 5.3 | <0.01 |
| Raffinose (1%) | 0.4 | 4.9 | <0.01 |
| Trehalose (1%) | 1.4 | 4.6 | <0.01 |
| Acetate (20 mM) + citrate (9 mM) + phosphate (7.5 mM) | 3.9 | 4.5 | 3.0 |
| Incubation temp[c] | | | |
| 30°C | 3.6 | 3.7 | 4.0 |
| 37°C | 3.8 | 3.6 | 4.0 |
| 40°C | 2.7 | 3.9 | 2.6 |
| Initial broth pH[d] | | | |
| 7.0 | 3.3 | 3.6 | 2.4 |
| 6.5 | 3.5 | 3.6 | 3.6 |
| 6.0 | 3.4 | 3.6 | 3.2 |
| 5.0 | 3.2 | 3.6 | 3.0 |
| 4.0 | 2.8 | 3.6 | 2.0 |

[a] TGE broth containing the following components: Trypticase, glucose, and yeast extract, each at 1%; Tween 80, 0.2%; $Mn^{2+}$, 0.033 mM; and $Mg^{2+}$, 0.02 mM, pH 6.5, used as a basal broth. Tryptone (Difco) could be substituted for Trypticase (BBL Microbiology systems). The optical density at 600 nm and pH were used to determine cell density (or mass) and acid production, respectively. The concentration of the initial inoculum was 1% in all studies.

[b] Numbers in parentheses indicate the concentrations of the components used. Variable concentrations were used for Trypticase (0 to 2%), yeast extract (0 to 2%), glucose (0 to 2%), Tween 80 (0 to 1%), and $Mn^{2+}$ (0 to 0.33 mM), but a relatively higher level of pediocin AcH was produced by using the concentrations of components listed in the table.

[c] Cells were grown in duplicate 10-ml portions of TGE broth. Negligible amounts of pediocin AcH were produced in 8 h at 30 and 40°C. After 24 h of incubation, the pediocin AcH level was slightly reduced at all temperatures studied.

[d] Cells were grown in TGE broth in a fermentor at 37°C for 16 h, with the initial pH adjusted with sterile HCl or NaOH.

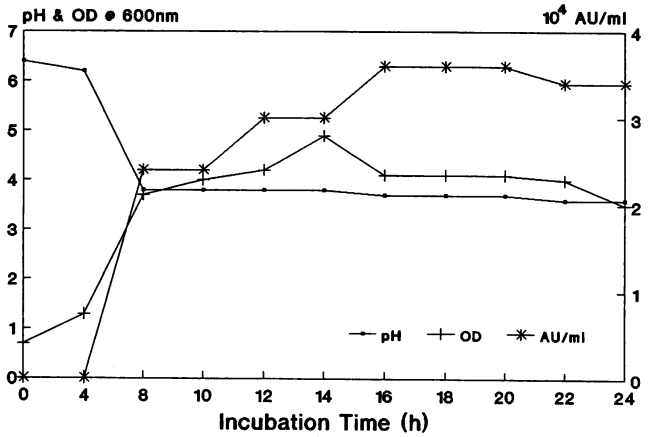


FIG. 1. Changes in pH, cell density (optical density at 600 nm), and pediocin AcH activity (activity units per milliliter) during growth of *P. acidilactici* H in TGE broth at 37°C for 24 h.

TABLE 2. Effect of terminal pH control on cell mass (optical density at 600 nm [$OD_{600}$]) and pediocin AcH production by *P. acidilactici* H after growth at 37°C for 16 h

| pH of the broth | | $OD_{600}$ | $10^4$ AU/ml |
|---|---|---|---|
| Initial | Terminal[a] | | |
| 6.5 | 6 | 3.0 | 0 |
| 6.5 | 5.5 | 3.1 | 0 |
| 6.5 | 5.0 | 4.0 | 1.0 |
| 6.5 | 4.5 | 4.0 | 3.0 |
| 6.5 | 4.0 | 4.0 | 3.2 |

[a] As soon as the pH was reduced from the initial pH (pH 6.5) to the desired terminal pH, the desired pH was maintained by the addition of sterile NaOH automatically by the fermentor.

(stationary phase). Thus, pediocin AcH appeared to be a secondary metabolite, as is nisin (13). *P. acidilactici* H produced negligible quantities of pediocin AcH when the final pH of TGE broth was maintained at 5.0 or above, even in the presence of a substantial increase in cell density (Table 2). A lower terminal pH (below 4.0) along with a large cell mass seemed to facilitate a high level of pediocin AcH production. A low level of pediocin AcH production in the presence of acetate, citrate, and phosphate in TGE broth could be the result of a high terminal pH. This situation is different from nisin production, which is greatest when the terminal pH is maintained at about 6.0 along with a large cell mass (11, 13). *P. acidilactici* H growing at a terminal pH above 5.0 did not lose the ability to produce pediocin AcH, because pediocin AcH production resumed when the pH was allowed to drop below 5.0 (data not shown). A reaction(s) necessary for active pediocin AcH synthesis probably occurs at low pH. Posttranslational modification for the production of active nisin has been reported (7). This aspect of pediocin AcH production is now being studied.

The results indicated that pediocin AcH can be produced by *P. acidilactici* H at a high level in a simple medium (TGE broth) which contains relatively inexpensive, food-grade ingredients. A low final pH and a large cell mass are necessary for a high level of pediocin AcH production. Other workers have reported pediocin production in media which contained costly, non-food-grade ingredients and components which prevented the terminal pH from dropping below 4.0 (6, 9, 12, 15, 16). These aspects should be considered in the commercial production of pediocin AcH for use as a food biopreservative.

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