Growth of Candida ingens on Supernatant from Anaerobically Fermented Pig Waste: Effects of Temperature and pH

D. P. HENRY* AND RUTH H. THOMSON
Central Animal Breeding House, University of Queensland, St. Lucia, Brisbane, Australia 4067

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Candida ingens, a pellicle-forming yeast utilizing volatile fatty acids, grew over a pH range of 4.1 to 6.0 on nonsterile supernatants derived from anaerobically fermented pig wastes; growth was inconsistent between pH 4.1 and 4.6. When ambient temperature above the pellicle was 21°C and the temperature of the medium was 29 to 32°C, a pH range of 4.8 to 5.0 gave yields of 1.90 to 3.31 g of dry matter per liter, and 0.059 to 0.065 mol of volatile fatty acids was utilized per liter. There was no advantage in utilization of volatile fatty acids and yield of dry matter in keeping the pH constant during a 24-h growth period. C. ingens grew at pH 4.8 and 5.0 when both ambient and medium temperatures were 30°C. When ambient temperature was 10°C, maximum yield and utilization of volatile fatty acids occurred at a medium temperature of 28 to 30°C.

Candida ingens grows as a thick, wrinkled pellicle on supernatants derived from the fermented wastes of monogastric animals and simultaneously reduces the concentration of dissolved organic compounds in the medium. Volatile fatty acid (VFA) is used as a source of carbon (7). Feeding trials, using rats, suggest that this organism has a potential as a fodder protein (4). The organism also grows in submerged culture using liquor derived from in vitro anaerobically fermented improved tropical pasture (6). This paper reports some of the factors influencing the growth of C. ingens and provides data that may help in the design and operation of an on-farm plant for effluent treatment and protein production.

Experiments were designed to investigate the effects of pH and temperature on the utilization of VFA and on the yield of yeast. pH was considered to be important because the medium on which C. ingens was grown was not sterile. An optimum pH would not only contribute to the growth requirements of the yeast, but would also inhibit the growth of possible competitors. Since the organism was being grown as a surface pellicle, the temperature of both the ambient air and the medium might modify the growth pattern.

MATERIALS AND METHODS

Microorganism. C. ingens was maintained in pure culture on Wickerham agar. For the experiments described here it was grown on the surface of a liquid medium prepared from anaerobically fermented pig-gery wastes, and it was subcultured every 24 h. The strain used was originally isolated from a pellicle growing in liquid taken from a piggery septic tank (4).

Medium. The liquid medium consisted of the supernatant from pig wastes fermented without aeration in a 45-liter vessel. The vessel contained pig feces, pig urine, and tap water, with a total solids concentration of approximately 7%. The vessel was stirred manually for 5 min/day until the total VFA reached a concentration of >0.04 mol/liter. The supernatant was then decanted, centrifuged, and frozen until required for use. The urine in the wastes provided nitrogen and inorganic salts used by the anaerobic bacteria and by C. ingens. The concentration of ammonia nitrogen (N) in all liquid media used exceeded 700 mg/liter.

Assays. Ammonia N was estimated according to Standard Methods for Examination of Water and Wastewater (1). Total VFA was estimated after steam distillation of 2 ml of medium with pH controlled at 2.7 by 2 ml of an aqueous solution made up of equal parts of 10% potassium oxalate and 5% oxalic acid. A titrimetric finish was used for both N and VFA estimations, using 0.007 M H₂SO₄, and 0.01 M NaOH, respectively. Measurement of pH was made with a Pye Unicam model 291 pH meter.

Statistical analyses. The method of least squares was used to estimate if there was a relationship between pH of the medium and (i) moles of VFA utilized per liter or (ii) yield of dry matter (DM) in grams per liter and between moles of VFA utilized per liter, yield of DM in grams per liter, and (i) the temperature or (ii) the pH of the medium.

The techniques used in the experiments were as follows.

For each experiment, VFA was estimated before the inoculation of C. ingens and after 24 h of growth, when the yeast had been harvested. Unless otherwise stated, the same batch of medium was used through-
out the experiment. Before inoculation, the pH of the medium was adjusted using concentrated H₂SO₄. A peristaltic pump recirculated the medium at a rate of 300 ml/h without causing surface turbulence, except that for one series of experiments magnetic stirrers were used in the medium. The medium was dispensed in 100-ml volumes into open glass vessels (65-mm diameter) of known weight. Inoculation onto the medium was made by transferring loops of pellicle from a 24-h culture so that a thin unwrinkled film of C. ingens covered approximately 80% of the surface area. The mean weight of the inoculum was 0.08 ± 0.02 g of DM per liter.

At the conclusion of an experiment, the tubes of the peristaltic pumps were removed from the medium. The liquid beneath the pellicle was withdrawn with a hypodermic syringe and needle, and the volume removed was measured. Yeast adhering to the needle and the pump tubes was washed back into the dish, which with its contents was dried in an oven at 60°C for 24 h and then weighed.

(i) Effect of pH of the medium at time of inoculation on VFA utilized and yield of C. ingens when ambient and medium temperature were constant at 21°C. A minimum of three estimates was made at each pH over the range 4.1 to 6.0. The initial concentration of VFA in the media ranged from 0.05 to 0.097 mol/liter. A total of 78 experiments were performed.

(ii) Effect of pH and temperature of the medium on VFA utilized and on yield of C. ingens when ambient temperature was constant at 21°C. Sixty experiments were performed over four levels for pH at time of inoculation and over five levels for temperature of the medium. The initial concentration of VFA in the media ranged from 0.06 to 0.09 mol/liter.

(iii) Effect of maintaining a constant pH of the medium throughout the experiment. In each of the previous experiments pH could be expected to change during the course of the experiment as the VFA was utilized (7). This time, during growth of the yeast, a constant pH was maintained throughout, and this was compared with a control without pH adjustment. Two magnetically stirred vessels were used, each containing 5 liters of medium adjusted to pH 4.5. Ten inocula of unwrinkled C. ingens pellicle, each 2.5 cm in diameter, were sown onto the surface of the medium in each container. Every hour, the pH in one vessel was estimated and maintained at 4.5 with concentrated H₂SO₄. The pH in the other vessel was not controlled, and it increased as the growing yeast utilized the VFA. Similar experiments were done using pH 5.0 and 5.5. The pellicles were harvested after 24 h by skimming and were oven dried at 60°C.

(iv) Effect of maintaining the temperature of both air and medium at 30°C. C. ingens was grown for 24 h as a pellicle in two vessels, one at pH 4.8 and the other at pH 5.0, in an air-conditioned room maintained at 30°C.

(v) Effect of varying the temperature of the medium when the ambient temperature was 10°C. C. ingens was grown as a pellicle in two vessels, one at pH 4.8 and the other at pH 5.0. The temperature of the medium for each 24-h growth period was maintained at 18, 20, 23, 25, 28, or 30°C while ambient temperature was 10°C.

RESULTS

During a 24-h incubation period, C. ingens grew over a pH range from 4.1 to 6.0 at an ambient temperature of 21°C (Fig. 1). However, at pH 4.1 to 4.6, during 31 growth trials, there was little or no growth on 13 (42%) occasions. These unsuccessful events were not included in the statistical analysis, nor are they shown in Fig. 1. Growth of C. ingens was achieved at every attempt at pH 4.7 to 6.0, and a dense pellicle grew on each occasion at pH 4.7 to 5.2. Above pH 5.2, yield of DM tended to decline. Another pellicle, which was seen to be bacterial on microscopic examination, frequently appeared at the higher pH levels.

There was no significant relationship between pH and the amount of VFA utilized, but there was a significant relationship between pH and yield of DM (P < 0.001). A regression equation shows this relationship (Fig. 1). Yields were generally higher at the lower end of the pH range. At the 5% level of probability, changes in temperature of the medium resulted in significant differences between the means of both the amounts of VFA utilized and the yield of DM. The optimum medium temperature was around 30°C. There were no significant differences between the means resulting from the levels of pH used (Fig. 2).

C. ingens grew over a range of medium temperatures from 23 to 35°C when ambient temperature was approximately 21°C and the pH of the medium at the time of inoculation was 4.6.
4.8, 5.0, or 5.2. At pH 4.6, C. ingens sometimes failed to grow. Growth occurred in each case at pH 4.8, 5.0, and 5.2. The experiments showed that, at pH 4.8, 5.0 at 29 to 32°C, C. ingens utilized 0.059 to 0.065 mol of VFA per liter and yielded 1.90 to 3.31 g of DM per liter (Fig. 2). This is equivalent to a range of yield of 0.54 to 0.85 g of DM per g of VFA expressed as acetic acid.

![Graph showing mean yield of C. ingens in media with varying pH and temperature](image)

**Fig. 2.** Effect of pH, at time of inoculation, and temperature of the medium on VFA utilized and yield of DM of C. ingens during a 24-h period when ambient temperature was constant at 21°C. (—-) pH 4.6; (—-) pH 4.8; (—-) pH 5.0; (—-) pH 5.2.

<table>
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<th>Initial pH</th>
<th>VFA utilized (mol/liter)</th>
<th>Yield of DM (g/liter)</th>
<th>Final pH</th>
<th>VFA utilized (mol/liter)</th>
<th>Yield of DM (g/liter)</th>
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**Table 1.** VFA utilized and yield of C. ingens in media with constant or uncontrolled pH

![Graph showing yield of DM and VFA utilized during varying temperature](image)

**Fig. 3.** Effect of varying the temperature of the medium on yield of C. ingens DM and utilization of VFA when ambient temperature above the pellicle was 10°C.

There was no advantage in terms of amount of VFA utilized or yield of DM when the pH was kept constant with H₂SO₄ during a 24-h growth period (Table 1).

C. ingens grew when ambient and medium temperatures were coincident at 30°C and the pH was 4.8 or 5.0 (Table 2).

At an ambient temperature of 10°C, C. ingens grew most successfully at pH 4.8 or 5.0 and at a medium temperature of 28 or 30°C and progressively less successfully down to 18°C (Fig. 3). This applied to the yield of DM, the percentage of total VFA utilized, and the quantity of VFA utilized.

**DISCUSSION**

In previous studies, Henry (4, 5) and Henry et al. (7) suggested that C. ingens could reduce the VFA and N concentration of waste organic effluents from intensive animal production units and produce a yield of potentially useful feed yeast. Before such a proposal could be implemented, basic data on the growth characteristics of the organism would be needed. The experiments described here attempt to define the parameters of temperature and pH on the yield of the organism and its utilization of VFA when using a liquid medium from anaerobically fermented pig wastes.

Whereas Henry et al. (7) showed that C. ingens could utilize almost all of the VFA up to
concentrations of 0.09 mol/liter, the present work shows that, in a 24-h growth period, concentrations of the order of 0.05 mol/liter could be utilized consistently. The yield of \textit{C. ingens} DM per gram of VFA is of the same order as was obtained by other yeasts using substrates of lactic acid, lactose, methanol, and ethanol. A substrate of \textit{n}-paraffin gave increased yields of a \textit{Candida} sp. when compared to the other substrates (Table 3). Within limits, the concentration of VFA produced during fermentation should be proportional to the concentration of substrate within the medium; thus in an anaerobic fermentor receiving pig wastes there would need to be sufficient substrate and fluids to maintain a daily concentration of acid of at least 0.05 mol/liter. This would provide nutrients for an optimal daily yield of \textit{C. ingens}. Under these circumstances the oxidation rate of VFA would approximate 0.002 mol/liter per h. If the fermentation produced a VFA yield significantly greater than 0.05 mol/liter, dilution of this concentrate for yeast growth should be a simple matter.

Though the experiments showed that \textit{C. ingens} could grow through a range of acid pH, growth did not always occur at the lower levels. At the higher levels of pH a bacterial pellicle appeared, and this seemed to compete with \textit{C. ingens} for the available surface area. Although significant statistical differences in yield or utilization of VFA were not evident between pH levels 4.6 and 5.2, the difficulties of initiating growth and the presence of bacteria at the lower and higher levels, respectively, indicated that the optimum pH was 4.8 to 5.0. In this pH range, it was shown that the optimum temperature of the medium was between 29 and 32°C.

Yield was maintained whether or not the pH was controlled during growth. This could be of economic importance to the operation of an on-farm effluent utilization process. Whereas \textit{C. ingens} will grow as a pellicle in ambient temperature of the order of 30°C, heating of the medium produces a positive growth response when ambient temperature is 21°C or lower.

After the fermentation of the wastes and the utilization of various components by the anaerobes, some of the nutrients may have reached limiting concentrations. This may have adversely affected the growth of \textit{C. ingens}. More work needs to be carried out to investigate this aspect of the process. It may be possible to optimize the quality of the medium by management of the wastes when conveying them from the pigs to the fermentor and by management of the fermentation so as to maximize VFA production and minimize loss to gas.

This work was carried out on the wastes of pigs in the 30- to 80-kg weight range. Significant variations might be expected in the concentration of the different VFA end products of the fermentation which result from the various classes of animals in a herd, and also from the feeding of different batches of diet. Hungate (8) tabulated the changes in concentration of the specific VFA that can occur in the rumen following various dietary regimes. Since \textit{C. ingens} has different rates of utilization for the various straight-chain VFA (7), a wide range could be expected in yield of DM per liter and moles of VFA utilized per liter.

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**LITERATURE CITED**


