Note

An Artificial Medium for Deodorant Microorganisms

Yoshiyuki OHTA and Hideki SATO

Laboratory for Microbial Biochemistry,
Faculty of Applied Biological Science,
Hiroshima University,
Fukuyama, Hiroshima 720, Japan

Received July 13, 1984

Malodorous wastes such as livestock excreta, garbage and waste-activated sludge constitute sources of environmental pollution that are difficult to use due to their offensive odor. We have found microorganisms which are able to deodorize these malodorous wastes simply and rapidly.¹⁻⁴⁾ These deodorant microorganisms grow rapidly on solid malodorous wastes and utilize malodorous components such as volatile fatty acids (V.F.A.), H₂S, mercaptans and so on. They convert these malodorants into odorless substances.¹⁻⁴⁾ It has been necessary to design a synthetic liquid medium for the growth of pure cultures for studies on the physiological and biochemical properties of these deodorant microorganisms. These deodorant microorganisms grow abundantly on a medium containing extracts of the malodorous wastes mentioned above, although their growth is delayed on ordinary nutrient medium (e.g. peptone, 1.0%; meat extract, 0.5%; NaCl, 0.5%; pH 8.5), as shown in Fig. 1. Furthermore, cells grown on the nutrient medium lose the ability to grow on malodorous wastes and the ability to deodorize malodorants.

One deodorant bacterium strain, which was previously isolated⁵⁾ from a seed culture^{2,3)} and identified as *Corynebacterium* sp. No. 922, was selected to test the growth on the new medium. This strain readily utilizes

V.F.A., which are major malodorous components of pig feces.⁶⁾ Feces collected from pigs fed with a concentrated feed was used the test material in this experiment. The following mixture was employed as the basal medium: *n*-Valeric acid, 0.5%; peptone, 1.0%; KH₂PO₄, 0.1%; CaCl₂, 0.05%; MgSO₄·7H₂O, 0.05%; pH, 8.5. The strain was cultured at 38°C on a rotary shaker for 18 hr in a 100 ml Erlenmeyer flask containing 20 ml of the medium.

(1) Carbon sources: V.F.A. other than formic acid were substituted for *n*-valeric acid in the basal medium. Especially the fatty acids with an offensive odor, for example, *n*-caprylic acid and *n*-butyric acid, were excellent carbon sources. On the other hand, this strain did not grow with sugars (i.e. xylose, arabinose, glucose, fructose, mannose, galactose, maltose, sucrose, dextrin and starch) as the carbon source. (2) Nitrogen sources: The strain utilized peptone and soybean extract as nitrogen sources, but not NH₄⁺, NO₃⁻, NO₂⁻, urea, glutamic acid, casamino acid or milk casein. (3) Metal ions: Besides the metal ions contained in the basal medium, the chloride forms of Ba²⁺, Co²⁺, Fe³⁺, Mn²⁺, Pb²⁺ and Zn²⁺ (each 0.01 mM) were tested for stimulation of the growth. Only the Co²⁺ ion was found to stimulate growth at concentrations from

FIG. 1. Growth of Deodorant *Corynebacterium* sp. No. 922.

See the text for the culture conditions. Symbols: ○, 10% pig feces extract; ●, ordinary nutrient broth.

TABLE I. VOLATILE FATTY ACID UTILIZING ACTIVITY OF *Corynebacterium* sp. No. 922

Cells grown on	V.F.A. ^a utilizing activity (nmol O ₂ /min/mg cells) ^b						
	Formic	Acetic	Propionic	<i>n</i> -Butyric	<i>iso</i> -Butyric	<i>n</i> -Valeric	<i>iso</i> -Valeric
Artificial medium	12.5	89.0	68.8	100	68.0	82.5	50.2
P.F.E. ^c	13.5	102	68.2	102	42.3	88.5	47.0

^a Volatile fatty acids.

^b The utilizing activity is expressed as the amount of oxygen consumed by the cells according to the results described previously.⁵⁾ The cells after 16-hr incubation at 30°C were collected and washed with Tris-HCl buffer (20 mM, pH 8.5). The washed cells were used for a respirometric experiment with a Warburg's respirometer.⁵⁾

^c 10% pig feces extract, which was prepared by the same procedure as described previously.¹⁾

1.25 to 320 μM ; a higher concentration inhibited the growth. The other metal ions had no effect on growth at the concentrations tested. (4) Growth stimulation: Trace substances such as yeast extract, soybean extract, wheat bran extract, meat extract, corn steep liquor, molasses and malt extract were tested at the concentration of 0.02% for growth stimulation. Only yeast extract enhanced the growth. Vitamin B₁₂ (0.2 μg /20 ml medium) was also found to stimulate the growth.

The selected deodorant bacterium strain was found to utilize V.F.A. and peptides as sources of carbon and nitrogen, respectively. Consequently, the composition of the artificial medium for the strain was determined to be as follows: Either *n*-valeric acid, acetic acid or *n*-butyric acid, 0.25%; peptone, 1.0%; KH₂PO₄, 0.1%; CaCl₂, 0.05%; MgSO₄·7H₂O, 0.05%; CoCl₂, 10 μM ; pH 8.5.

The strain grew on the artificial medium at almost the same rate as on the medium containing the pig feces extract. Cells grown on this medium showed almost the same V.F.A. utilizing activity⁵⁾ as that of the cells grown on the extract of pig feces, as shown in Table I. Furthermore, the cells grew on the solid malodorous

wastes mentioned above, and deodorized them at similar rates as did the cells grown on extracts of the wastes, and no malodorous sulfur compounds were detected in the solid wastes incubated with cells grown either on the artificial medium or on the extracts of the wastes.

REFERENCES

- 1) Y. Ohta and M. Ikeda, *Appl. Environ. Microbiol.*, **36**, 478 (1978).
- 2) Y. Ohta and M. Ikeda, *Nippon Nôgeikagaku Kaishi*, **53**, 277 (1979).
- 3) Y. Ohta, M. Ikeda and Y. Hemmi, *Hakkokogaku Kaishi*, **57**, 372 (1979).
- 4) Y. Ohta and Y. Kuwada, *Hakkokogaku Kaishi*, **61**, 195 (1983).
- 5) H. Sato and Y. Ohta, Abstract of Papers, Annual Meeting of Hakkokogakukai, Osaka, November, 1983, p. 107.
- 6) T. Hamano, Y. Oka, O. Takada and T. Asano, *Bull. Hyogo Prefect. Exp. Stan. Anim. Husbandry*, **9**, 140 (1972).