

ISOLATION AND CHARACTERISATION OF PHYTASE-PRODUCING BACTERIA FROM THE RUMEN

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Introduction

Phytate is the major form of phosphorus containing compounds in the plants and pollens. Thus, plant seed meals consumed by farm animals contain a high level of phytate. However, the nutrient availability of this form of phosphorus is poor in monogastrics (such as chickens and pigs) because of the low phytase activity in their digestive tracts. The low availability of phytate poses three problems: i) the need to supplement inorganic phosphorus to the diets, ii) the excretion of indigestible phosphorus in the faeces resulting in environmental pollution, and iii) the reduction of nutritional value of the feedstuff. In contrast, ruminants readily utilise phytate phosphorus because of the abundance of phytase produced by the rumen bacteria. However, very little research has been carried out on these phytase-producing bacteria from the rumen. The objectives of the present study were to isolate, identify and evaluate the phytase activity of some rumen bacteria.

Materials and Methods

Rumen fluid taken from a cattle fed 100% commercial concentrate was inoculated into tubes containing phytase-screening medium (MPSM) (modified from the medium described by Howson and Davis, 1983). After the incubation period, the bacterial colonies with surrounding clear zones were picked and transferred to a MPSM broth containing Na-phytate. The bacterial colonies that liberated large amounts of phosphorus (Pi) from Na-phytate were selected for further studies. For identification of the phytase-producing rumen bacteria, morphological and physiological characteristics as well as the complete sequence of the 16S rRNA gene were studied. For most of the physiological studies, the isolates were incubated in peptone-yeast-glucose (PYG) medium. Characteristics such as acid production from carbohydrates, volatile and non-volatile fatty acids production from the fermentation of the PYG broth, methyl red reaction, acetyl-methylcarbinol production, nitrate reduction, catalase, oxidase, indole and H₂S production, gelatin and Tween 80 hydrolysis, and growth at 20°C and 45°C were studied. Tolerance of the isolates to bile, acid and antibiotics was also determined. Phytase activity of the isolates was determined using the method described by Yanke et al. (1998).

Results and Discussion

A total of 125 bacterial colonies with surrounding clear zones on MPSM agar medium were isolated, but only five colonies produced detectable amounts of Pi from Na-phytate in MPSM broth. All the five isolates possessed similar morphological and physiological characteristics. They were

Gram-negative, non-spore forming, non-motile short rods, occurring singly or in short chains. The physiological characteristics of the isolates showed that they belong to the genus *Mitsuokella*. Although most of the characteristics of the isolates were similar to those of the type (and sole) species, *Mitsuokella multiacida*, some characteristics were distinctly different. The distinct characteristics of the isolates included the ability to grow at 20°C and 45°C; the tolerance to penicillin and kanamycin; the ability to ferment glycerol and sorbitol but not mannitol, rhamnose and melezitose; and the ability to produce trace amounts of butyric, isovaleric and caproic acids as end-products of glucose fermentation. The complete sequence of the 16S rRNA gene of a representative of the isolates revealed that 98.7% of the sequence was similar to *M. multiacida*. A dendrogram of the genetic relatedness of the isolate with other bacteria based on the complete sequence of the 16S rRNA gene showed that although the isolate is most related to *M. multiacida*, it is a distinct species from *M. multiacida*. As the name of the new species has not been established, the isolates are designated as *Mitsuokella* sp. Growth of *Mitsuokella* sp. was enhanced by the presence of glucose (or other fermentable carbohydrates) in the medium, but it was not enhanced by the addition of bile. The growth pattern of *Mitsuokella* sp. in MPSM broth under aerobic or anaerobic condition was similar. Growth was slow during the initial incubation period of 0-4 h, then rapid till 6 h, after which growth was constant till the end of the incubation period. Although the growth patterns were similar, *Mitsuokella* sp. grew better and hydrolysed more phytate in anaerobic than in aerobic condition. Under anaerobic condition, complete (100%) hydrolysis of Na-phytate took place within 6 h of incubation, while under aerobic condition, complete hydrolysis occurred within 8 h of incubation. The highest phytase activity of 1200 nmol Pi min⁻¹ ml⁻¹ was detected after 4 h of incubation. Phytase could be produced without a phytate substrate, but significantly ($P < 0.05$) more phytase was produced in the presence of a phytate substrate. This indicates that the production of phytase by *Mitsuokella* sp. is partially constitutive and partially inducible. *Mitsuokella* sp. was unable to survive at pH 2.0, but it could survive at pH 3.0 for 0.5-1 h. Most of the phytase activity was lost at pH lower than 3.0. The relative activity of phytase at pH 3.0 was 66.5%, but at pH 2.0 it was only 7.7%.

Conclusions

Five rumen bacterial isolates were found to produce high phytase activity and were able to hydrolyse phytate effectively. Their morphological and physiological characteristics, and the complete sequence of the 16S rRNA gene showed that they belong to the genus *Mitsuokella*, but are distinct from the type species, *M. multiacida*. Thus, a new species has to be established to accommodate them.

References

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