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Nutritionally fastidious *Ruminococcus flavefaciens* strains

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The majority of *Ruminococcus flavefaciens* strains isolated in our laboratories failed to grow or grew poorly both in rumen-fluid medium and in a chemically defined medium containing all the nutrients reported to be required for good growth of this species. Attempts were made to develop a defined medium which would support good growth of the *R. flavefaciens* strains studied. Over 70 different medium formulations free of rumen fluid were tried. However, only one supported growth of all strains and generally growth remained poor. Addition or omission of nutrients, either singly or in combination, from this basal medium, did not result in any material improvement in growth rates.

Die meerderheid van *Ruminococcus flavefaciens* stamme wat in ons laboratorium geïsoleer is, het swak gegroei of glad nie gegroei in 'n rumenvloeistof medium en in 'n chemies gedefinieerde medium wat al die voedingstowwe bevat het wat voorheen gerapporteer is as noodsaaklik vir groei van hierdie spesies nie. Pogings is aangewend om 'n gedefinieerde medium te ontwikkel waarin die *R. flavefaciens* stamme wat ondersoek is, gekweek kon word. Meer as 70 verskillende medium samestellings sonder rumenvloeistof is getoets. Daar was egter net een medium waarin al die stamme gekweek kon word en oor die algemeen het die organismes swak gegroei. Byvoeging of weglating van voedingstowwe, afsonderlik of in kombinasie, van die basale medium, het geen noemenswaardige verbetering in groeitempos tot gevolg gehad nie.

Keywords: *Ruminococcus flavefaciens, Ruminococcus albus,* nutritional requirement, defined media, unknown growth factor

Introduction

Our knowledge of the nutritional requirements of *Ruminococcus flavefaciens* is derived from studies on a small number of strains, mainly by Bryant and co-workers (Bryant, 1973). They found that *R. flavefaciens* resembled *R. albus* in requiring, in addition to minerals, suitable sources of carbon, energy and sulphur, certain B-vitamins, ammonia and one or more of the branched chain volatile fatty acids (BVFA) isobutyric, isovaleric and 2-methylbutyric acids. Relatively large amounts of CO_2 or bicarbonate were also required for optimal growth and the addition of organic nitrogen sources such as amino acid mixtures or casein hydrolysate to defined media containing ammonia, stimulated the growth of some strains.

In the early stages of an investigation into the quantitative requirements for BVFA for optimal growth rates of ruminococci, we discovered that the majority of our isolates of *R. flavefaciens* failed to grow or grew poorly in a defined medium (Roché, Albertyn, Van Gylswyk & Kistner, 1973) containing all the nutrients reported to be required for good growth of this species. These isolates were obtained, over a number of years, from sheep on different diets. In contrast, a group of *R. albus* isolates, obtained from the same sheep, behaved as expected. We tried to develop a chemically defined medium which would support good growth of all our *R. flavefaciens* isolates.

Materials and methods

Organisms. In the initial stages, 22 strains of *R. flavefaciens* and, for comparison, an equal number of *R. albus* strains were used. Five of the *R. flavefaciens* strains, 4A, CE 70, CE 77, CXS 36 and CXS 67 (Shane, Gouws & Kistner, 1969) were dropped at an early stage because of the difficulty experienced in culturing them even in a medium containing rumen fluid. On the basis of the different patterns of growth responses found, the *R. flavefaciens* strains 25, 37, 38, 39, 40, 41, 43, 44, 45, 46, 47, 49 and 51 (Van Gylswyk & Roché, 1970) and the *R. albus* strains 21.09.6E, 22.08.6A (Kistner & Gouws, 1964), 27, 28 (Van Gylswyk & Roché, 1970) were selected for further investigation. The *R. flavefaciens* strains B_1C45, C94 and FD1 and *R. albus* strains 7 and 20, which had been used in earlier nutritional studies by American workers, served as reference material. Cultures of these strains were kindly donated by Prof. M.P. Bryant.

Media. Over 70 different medium formulations free of rumen fluid were tried. Of these, five contained complex sources of growth factors, viz. yeast extract or a tryptic digest of casein, and one contained as a complex source of carbon and energy an acid hydrolysate of cellulose. The compositions of the remaining media will refer to the composition of medium BN. This was based on the medium of Roché *et al.* (1973), with the following modifications: cellobiose, 10 g/1; methionine, 0,51 mM; acetic acid, 3 mM; propionic acid, 1 mM; isobutyric acid, 0,4 mM; 2-methylbutyric acid, 0,4 mM; isovaleric acid, 0,4 mM; valeric acid, 0,4 mM; haemin, 3μM; succinic acid, 0,5 mM; diaminopimelic acid, 168 μM; pyridoxal-5-phosphate, 0,4 mM; thiamine pyrophosphate, 0,2 μM. The following

nutrients were omitted singly or in combinations from medium BN: haemin, succinic acid, diaminopimelic acid, thiamine pyrophosphate, and 0,92 of the concentration of methionine shown. Further media differed from BN in that they contained one or more of the following: tetrahydrofolic acid, 2,25 μM; pantethine, 1,8 μM; co-enzyme M, 6,1 μM; putrescine, 56,7 μM; spermine, 24,7 μM; isohexanoic acid, and 3-methyl pentanoic acid, both 40 μM; Tween 80, 0,1% (v/v); butyric acid, 5 mM; D-glutamic acid, D-aspartic acid, D-methionine, D-alanine, all 45 μM; phenylacetic acid, 230 μmol; acetyl glucosamine, acetyl galactosamine, acetyl muramic acid, diaminobutyric acid, ornithine, homoserine, glycerol phosphate, uridine diphosphate, phosphorylcholine and adonitol, all 50 μM; and different concentrations of Se, Wo and Mo in a constant proportion of 100:1:10. In some of the medium formulations the concentrations of minerals, cysteine and Na_2S were varied. The media were prepared, sterilized and distributed as described by Roché *et al.* (1973). A non-defined medium containing 40% (v/v) membrane-filtered rumen fluid was used for comparison.

Measurement of growth rates. The methods described by Therion, Kistner & Kornelius (1982) were used, except that optical densities were monitored until growth ceased.

Results

The six representative strains of *R. albus* grew well in all the defined media, attaining growth rates close to or higher than those obtained in a medium containing rumen fluid. Growth of all strains was remarkably uniform. Where the same inoculum was used, differences in the qualitative composition of the medium usually had little effect on growth.

In contrast, the *R. flavefaciens* strains were much more variable in their growth responses. BN was the only defined medium in which all 16 strains grew and growth rates ranged from very poor for strain 49 to very good for FD1. The latter attained a higher growth rate than any of the *R. albus* strains. Bryant's strains C94 and FD1 and our strains 40 and 43 grew in practically all the media in which they were tested, and Bryant's strain B_1C45 and our strain 41 on most. The American strain C94 and our strains 38, 40, 41, 43, and, on occasions, 46 and 47 attained relatively high maximal optical densities on extended incubation. With the exception of FD1 and 40 on some media, however, growth rates and maximal optical densities were lower than those of *R. albus*. Some of the locally isolated strains grew very poorly and, as in the cases of strains 25, 39, 45, 49 and 51, showed no growth at all on most media. With the exception of Bryant's strain C94 and our strains 38 and

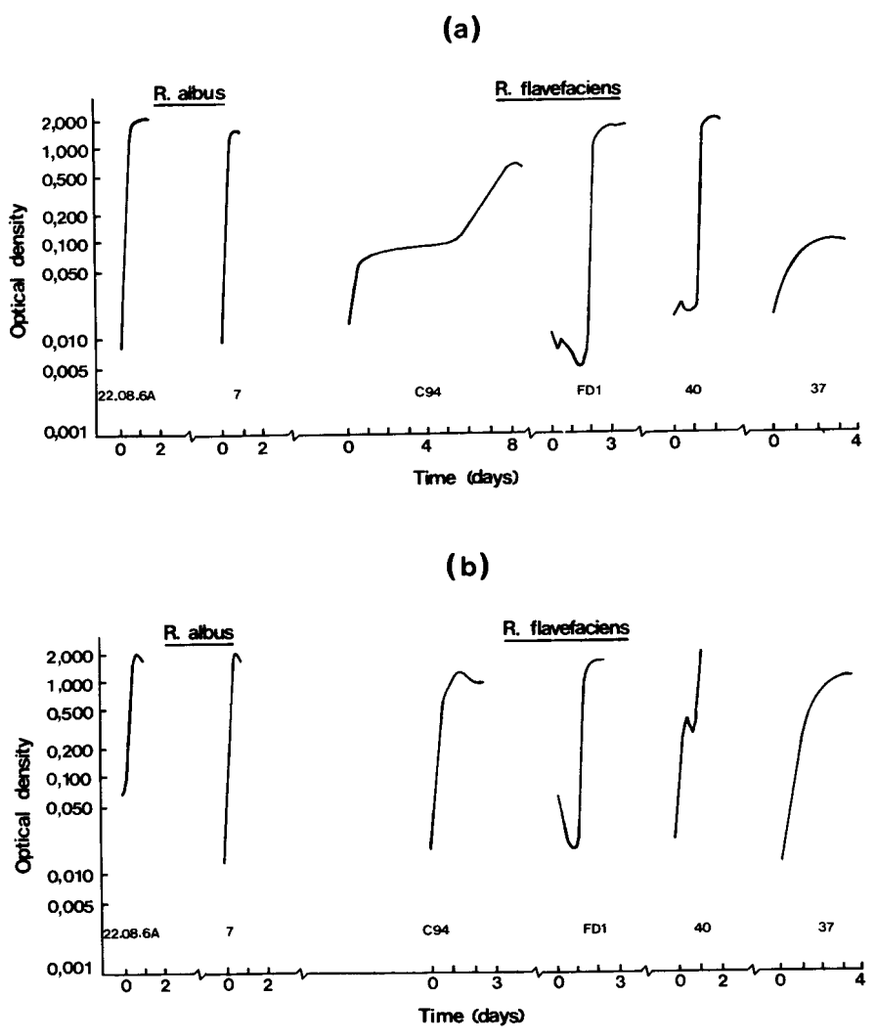

Figure 1 Determination of optical density of strains of *Ruminococcus albus* and *Ruminococcus flavefaciens* as a function of time using the same inocula (a) in defined medium BN (b) in a medium containing rumen fluid.

43, *R. flavefaciens* showed pronounced growth lags on most media. The 48-hour lag of FD1 shown in Figure 1(a) is not unusually long. This again contrasts with the *R. albus* strains, which generally showed little or no lag.

The growth curve of C94 in Figure 1(a) demonstrates diphasic growth. This phenomenon, which was displayed by most *R. flavefaciens* strains, occurred inconsistently on different media.

None of the modifications to medium BN resulted in any material improvement in the growth of the fastidious *R. flavefaciens* strains.

The growth of most of the local *R. flavefaciens* isolates in liquid medium containing 40% (v/v) rume fluid was poor, despite the fact that these isolates were successfully maintained on slants containing rumen fluid. The effect of time of collection and method of processing the rumen fluid was, therefore, examined. Again we found no material improvement in growth.

Using media containing rumen fluid with pH's adjusted over the range pH 5,75 to pH 8,0, it was established that the poor growth of the local *R. flavefaciens* isolates was not a result of these organisms having an abnormal pH optimum.

In conclusion, the majority of locally isolated *R. flavefaciens* strains require an as yet unknown growth factor which may be present as a limiting factor in the rumen.

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