Ribosomal efficiency and growth rates of freshly isolated *Escherichia coli* strains originating from the gastrointestinal tract

Camilla U. Rang[a,*], Riitta Mikkola[b], Sören Molin[c], Patricia L. Conway[d]

[a]*Department of General and Marine Microbiology, Göteborg University, Medicinaregatan 9C, 413 90 Göteborg, Sweden*
[b]*Department of Molecular Biology, Uppsala University, Biomedical Center, Box 590, 751 24 Uppsala, Sweden*
[c]*Department of Microbiology, The Technical University of Denmark, Building 301, 2800 Lyngby, Denmark*
[d]*CRC for Food Industry Innovation, School of Microbiology and Immunology, University of New South Wales, Sydney, NSW 2052, Australia*

Received 1 October 1997

Abstract It has been previously reported that for natural *Escherichia coli* isolates from the ECOR collection, there were differences in the ribosomal efficiencies and there was a direct correlation between growth rate and the ribosome efficiency (R-factor). The aim of this study was to determine whether strains freshly isolated (i.e. subcultured <5 times) from the gastrointestinal tract ecosystem also exhibited this correlation. Eleven *E. coli* and two *Enterobacter* spp. isolates from either humans, pigs, rats or a mammoth were investigated. Considerable variability in the R-factor was noted using an in vitro translation assay, however no consistent correlation between the R-factor and growth rate was noted.

Key words: Enteric isolate; *Escherichia coli*; Ribosomal efficiency; Growth rate

1. Introduction

In normal laboratory conditions, an enterobacterium invests more than 50% of its total biomass in the protein synthesis system [1] and the translation system is the dominant metabolic pathway of bacteria growing in media normally used in laboratories. Although most studies on bacterial ribosomes have used *Escherichia coli*, information from other species reveals a remarkable similarity among all prokaryotic ribosomes. Both the rRNAs and ribosomal proteins have been highly conserved throughout evolution, since proteins from the *E. coli* 30S subunit bind specifically to the 16S rRNA of several other genera, including other enteric bacteria, *Bacillus* as well as some cyanobacteria, strict anaerobes and even some archaea [2].

It is known that during balanced growth of bacterial cultures, the number of ribosomes per cell is observed to vary in a regular fashion with the growth rate of the culture [3] as was originally observed by Schaechter et al. [4]. The correlation between cellular ribosome (rRNA) concentration and growth rate has recently also been successfully used to study growth rate in vivo directly from fixed cells [5].

Recently it has been suggested that natural strains can contain ribosomes with different efficiencies and that a linear correlation exists between the efficiency of the ribosomes and the specific growth rate of the cell [6]. These authors applied a kinetic model to analyze results from in vitro assays in which the active concentration of the ribosomes from the different strains was the same. It has since been shown [5] that two variants of *E. coli* BJ4 which was isolated from the rodent digestive tract grew at different rates but had almost identical ribosomal efficiencies. It was therefore interesting to investigate further these parameters for gastrointestinal isolates.

In this study the in vitro translation method [7] was used to study the ribosomal efficiencies of 11 freshly isolated *E. coli* strains, of gastrointestinal origin from pigs, humans and rats, and also of two *Enterobacter* strains isolated from a 11 600 year old mammoth. The ribosomal efficiencies of the strains were compared to their growth rate.

2. Materials and methods

2.1. Bacterial strains

In preliminary studies the growth rates of 81 isolates from the gastrointestinal tract were studied. These included pig isolates obtained from O. Söderlind [8] plus human, rat and mammoth strains from the sources outlined below. None of these isolates was subcultured more than five times. A small number of these strains were selected for further study.

Strain G1108E (obtained from Carl Denecke, Tufts University, Boston, MA) was a porcine enteropathogenic *E. coli* strain bearing K88 fimbriae. It has been extensively studied and subcultured in the laboratory over a 10 year period and hence was used here as a reference strain included in each experiment. The human *E. coli* strains (Ucb2 and Ucb6) (obtained from M. Katouli, Karolinska Institute, Stockholm) were isolated from patients with ulcerative colitis. The two rat *E. coli* isolates (RU5 and RX7) (obtained from M. Katouli, Karolinska Institute, Stockholm) were isolated from mesenteric lymph nodes and most probably had originated from the gut. An *Enterobacter cloacae* and an *Enterobacter* sp. from the gastrointestinal system of a mammoth (obtained from J. Tiedje, Michigan State University, East Lansing, MI) were also included.

2.2. Growth rates

The growth rates of the strains were measured at least in duplicate by following their optical density at 540 nm and are reported as mean values. The growth rates were measured using Luria broth (LB) containing 1% NaCl, minimal medium (M9) [9] which contained 0.2% glucose and thiamine, and minimal medium (AB) [10] which contained 1% casamino acids and 0.2% glucose.

2.3. In vitro ribosome assays

The ribosomes were prepared as described by Jelenc [11] and the ribosome concentration was determined as described by Ehrenberg et. al. [7]. The ribosomes were stored at −80°C until use. All other translation components were prepared from *E. coli* MRE 600 as previously described [7]. Phenylalanine, phosphoenolpyruvate (PEP), GTP, putrescine, spermidine, myokinase (EC 2.7.4.3) and pyruvate kinase (EC 2.7.4.6) were purchased from Sigma (St. Louis, MO). Poly-U was obtained from Pharmacia (Sweden). Radioactive amino acids, ^{14}C-Phe and ^{3}H-NAc-Phe, were obtained from Amersham International (Bucks, UK).

The in vitro translation method was performed as described by Ehrenberg et al. [7]. Briefly it is a method where all components are added in very precise and controlled concentrations and the concen-

*Corresponding author. Fax: (46) (31) 773 25 99.
E-mail: Camilla.Rang@gmm.gu.se

PII S0014-5793(97)01341-0

Table 1
Growth rates and kinetic parameters from in vitro translation of ribosomes from freshly isolated pig *E. coli* strains and a reference pig *E. coli* strain, G1108E, subcultured extensively

Strain	Growth rate[a]		R-factor[b]		k_{cat}[b]
	LB	M9	($\times 10^7$ M^{-1} s^{-1})	S.D.	(s^{-1})
G1108E	2.25	1.40	1.26	0.07	5.74
1452	2.47	1.30	1.17	0.10	5.62
604	2.63	1.29	1.14	0.07	6.01
2069	2.40	1.33	1.11	0.15	5.09
1491	2.26	1.17	0.92	0.08	4.86
562	2.47	1.29	0.77[c]		3.70[c]
27	2.65	1.39	0.69	0.01	4.03

[a]Growth rate in doublings per hour when growing in Luria broth (LB) and minimal medium (M9).
[b]Mean of duplicate assays.
[c]$n = 1$ assay.

trations of active ribosomes from the different strains are added in equal and known amounts. The amount of ribosomes in every assay was calculated to contain 10 pmol active ribosomes. The catalytic rate constant (k_{cat}) and the k_{cat}/K_M (the R-factor, which represents the ribosomal efficiency) were calculated from Eadie-Hofstee plots as described by Bohman et al. [12]. The buffer used for the in vitro assay was poly-mix buffer [7]. The in vitro assays were run in duplicate and the mean is reported.

3. Results

3.1. Growth rates

The growth rates for the porcine strains growing in LB and M9 are presented in Table 1 while growth rates for some of the porcine strains and all other strains grown in the AB minimal medium are presented in Table 2.

It is interesting to note that while the reference strain, G1108E, had a slightly faster growth rate than other pig strains when grown in M9, this was not the case for growth rates in LB (Table 1) or AB broth (Table 2).

3.2. In vitro ribosomal kinetics

Two kinetic parameters are described: the R-factor (k_{cat}/K_M) and the k_{cat} (V_{max}). The k_{cat} describes the maximum turnover rate of the ribosomes obtained at excess ternary complex concentrations. Since efficient translation requires that elongation factors associate rapidly with the ribosome and that they spend a short time in complex with the 70S particle, it is the R-factor that determines the efficiency of the ribosomes which describes the amount of amino acids captured per ribosome per molar elongation factor Tu.

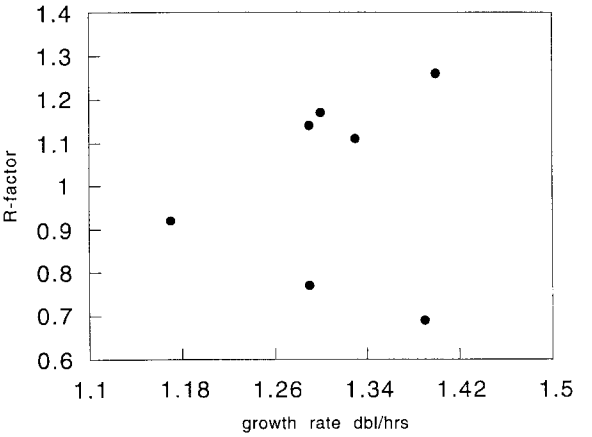

Fig. 1. The ribosomal efficiencies (R-factor$\times 10^7$ M^{-1} s^{-1}) and growth rates in M9 of the *E. coli* strains of porcine origin listed in Table 1.

The percentage active ribosomes in the ribosomal preparations varied between 15 and 26%, with no correlation to R-factor.

The results for the porcine strains grown in M9 are presented in Table 1. The k_{cat} varied between the different strains, with 3.7 being the lowest and 6.0 the highest. The laboratory reference strain (G1108E) had one of the highest values (5.74). The R-factor, which represents the ribosomal efficiency, varied between 0.69 to 1.26 with the reference strain having the highest R-factor and hence having the most efficient ribosomes.

There was no consistent correlation between the R-factor or k_{cat} values and growth rates (Fig. 1, Table 1). It is possible that there is a trend for the five strains containing the most efficient ribosomes (R-factor > 0.90) since the R-factor and the growth rate may exhibit a linear relation (Fig. 1). However, there is definitely no correlation for strains with an R-factor below 0.8.

The kinetic parameters from the second series of in vitro translation assays using strains from different hosts and grown in AB media are presented in Table 2. The reference laboratory strain (G1108E) had the highest value for both R-factor and k_{cat} and hence the values for R-factor and k_{cat} are expressed relative to those of the reference strain for ease of

Table 2
Growth rate and kinetic parameters from in vitro translation of ribosomes from strains freshly isolated from the digestive tract of different hosts, and a laboratory reference strain, G1108E, after growth in minimal medium containing casamino acids (AB)

Strain[a]	Origin	Growth rate	Relative R-factor	Relative S.D.[b]	Relative k_{cat}
G1108E	pig	1.96	1.00	0.09	1.00
Ucb6	human	2.02	0.88	0.11	1.06
1452	pig	2.41	0.67	0.02	1.20
3	mammoth	2.28	0.57	0.07	1.00
Ucb2	human	1.84	0.57	0.09	0.98
10	mammoth	2.17	0.48	0.0	1.00
RX7	rat	2.17	0.39	0.05	1.14
RU5	rat	1.95	0.36	0.01	1.27
27	pig	2.14	0.20[c]		0.97[b]

Growth rate is expressed as the number of doublings per hour and the R-factor and k_{cat} are presented relative to the values obtained for the reference strain (G1108E) which is expressed as 1.00.
[a]*E. coli* strains except those from mammoth which were *Enterobacter* spp.
[b]The observed S.D. as compared to % of the relative R-factor of the strain.
[c]$n = 1$ assay.

comparison. Even though G1108E had the highest R-factor, the growth rate was slower than almost all of the other strains, when minimal medium containing casamino acids (AB) was used. From the data presented in Table 2, there appears to be no correlation between the growth rates of the strains and the kinetic parameters (k_{cat} and R-factor). Furthermore, the origin of the strain had no demonstrable impact on the relation between R-factor and growth rate (Table 2).

4. Discussion

In this study, the ribosomal efficiencies and growth rates of freshly isolated enteric bacteria from various hosts were studied. Of all the natural isolates that were screened in the preliminary studies ($n = 81$), there were no strains that had markedly different growth rates although they were freshly isolated from very different origins. Consequently, 12 isolates were randomly selected for further study such that there were some isolates from all hosts.

It has been shown that all the strains studied showed a wide variation in R-factor values, as can be seen in Tables 1 and 2. This finding that natural isolates can have different ribosomal efficiencies is consistent with the findings of Mikkola and Kurland [6], who found that strains in the ECOR collection [13] had very different ribosomal efficiencies.

Interestingly the control strain, G1108E, had the highest ribosomal efficiency (Tables 1 and 2). G1108E is a wild-type strain which was isolated over 10 years ago and has been used extensively in the laboratory ever since and therefore has been subcultured many times. This is consistent with the findings of Mikkola and Kurland [14] that strains with a high ribosomal efficiency are selected for in laboratory conditions. They found that the wild-type control strain they used also showed the highest R-factor. When they grew the natural strains from the ECOR collection in continuous culture for 300 generations, those strains which used to have a low ribosomal efficiency now had increased their efficiency and were now indistinguishable from the control strain [14].

There were no major differences in growth rates for the strains when grown in a minimal medium even though different ribosome efficiencies were noted. Consequently, there was no demonstrable correlation between growth rate and ribosomal efficiency for many of the strains studied (Table 2 and Fig. 1). This is not consistent with the study of Mikkola and Kurland [6] where they reported both very different growth rates in vitro in minimal medium among the strains in the ECOR collection, and also a correlation between the specific growth rates and the corresponding R-factor. The faster the growth the better ribosomal efficiency.

The study of Poulsen et al. [5] also failed to show a correlation between R-factor and growth rate since ribosomal efficiencies were almost identical between two different variants of the *E. coli* BJ4 strain isolated from the rodent digestive tract, even though growth rates of 1.2 dbl/h and 2.2 dbl/h were noted.

It is interesting to note that all intestinal isolates that were subcultured < 5 times had a slightly slower growth rate than the reference laboratory strain in minimal medium, however the reference strain grew more slowly than the fresh isolates in richer broth. One can speculate that the fresh isolates have not adapted to the minimal medium conditions.

We can only speculate that the concentration of ribosomes is the most important contribution to the growth rate of bacteria, as previously described [4,5], and that ribosomal efficiency could be a more minor complement which may not always be detectable in some strains.

In summary, while the strains studied did contain ribosomes which differed in efficiency, there was no direct correlation between growth rate and ribosomal efficiency, although for some, but not all, porcine strains there was a trend towards a linear relationship between growth rate and the R-factor.

Acknowledgements: The study was financially supported by the Swedish Environmental Protection Agency. The authors thank Prof. Staffan Kjelleberg for valuable discussions and Ioannis Tubulekas and Farhad Abdulkarim at Uppsala University for their guidance with the translation assay. The authors also thank Dr. Söderlind, Dr. Katouli and Prof. Tiedje for providing the various bacterial isolates.

References

[1] Maaloe, O. (1979) in: Biological Regulation and Development (Goldberger, R.F., Ed.), Vol. 1, pp. 487–542, Plenum Press, New York.
[2] Neidhardt, F.C., Ingraham, J.L. and Schaechter, M. (1990) pp. 506, Sinauer Associates, Sunderland, MA.
[3] Kjeldgaard, N.O. and Gausing, K. (1974) in: Ribosomes, (Nomura, M., Tissieres, A. and Lengyel, P., Eds.), pp. 369–416, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
[4] Schaechter, M., Maaloe, O. and Kjeldgard, N.O. (1958) J. Gen. Microbiol. 19, 592–606.
[5] Poulsen Kongsbak, L., Rask Licht, T., Rang, C., Krogfelt, K.A. and Molin, S. (1995) J. Bacteriol. 177, 5840–5845.
[6] Mikkola, R. and Kurland, C.G. (1991) Biochimie 73, 1061–1066.
[7] Ehrenberg, M., Bilgin, N. and Kurland, C.G. (1990) in: Ribosomes and Protein Synthesis: A Practical Approach (Spedding, G., Ed.), pp. 101–129, Oxford University Press, Oxford.
[8] Söderlind, O., Thafvelin, B. and Möllby, R. (1988) J. Clin. Microbiol. 26, 879–884.
[9] Reeve, C.A., Bockman, A.T. and Matin, A. (1974) J. Bacteriol. 157, 758–763.
[10] Clark, D.J. and Maaloe, O. (1967) J. Mol. Biol. 23, 99–112.
[11] Jelenc, P.C. (1980) Anal. Biochem. 105, 369–374.
[12] Bohman, K., Ruusala, T., Jelenc, P.C. and Kurland, C.G. (1984) Mol. Gen. Genet. 198, 90–99.
[13] Ochman, H. and Selander, R.K. (1984) J. Bacteriol. 157, 690–693.
[14] Mikkola, R. and Kurland, C.G. (1992) Mol. Biol. Evol. 9, 394–402.