Arch Microbiol (1984) 140:218–224

Archives of
Microbiology

Cloning of the ribokinase gene of *Staphylococcus hyicus* subsp. *hyicus* in Staphylococcus carnosus

Günter Keller, Karl Heinz Schleifer, and Friedrich Götz

Lehrstuhl für Mikrobiologie, Technische Universität München, Arcisstraße 21, D-8000 München 2, Federal Republic of Germany

Summary. A gene library with DNA of *Staphylococcus hyicus* subsp. *hyicus* was established in *S. carnosus* by using the plasmid vector pCT20. Two clones of *S. carnosus* were isolated which were able to ferment D-ribose. The two hybrid plasmids (pRib 1) and (pRib 2) were isolated and characterized. They contained inserted DNA fragments of *S. hyicus* subsp. *hyicus* with sizes of 10.2 and 8.2 kb, respectively. D-Ribose uptake and enzyme activities were studied. All strains tested [*S. hyicus* subsp. *hyicus*, *S. carnosus* (wild type) and the two *S. carnosus* clones] possessed an inducible uptake system for D-ribose. *S. hyicus* subsp. *hyicus* possessed in addition enzyme activities of D-ribokinase and D-ribose-5-P isomerase. None of these enzyme activities could be detected in *S. carnosus* (wildtype). Only in the *S. carnosus* clones containing (pRib 1) or (pRib 2) could a D-ribokinase activity be demonstrated, indicating that the gene for D-ribokinase of *S. hyicus* subsp. *hyicus* was cloned in *S. carnosus*.

Key words: Recombinant DNA – Cloning – *Staphylococcus carnosus* and *S. hyicus* subsp. *hyicus* – Ribose degradation – Ribokinase

Pentose metabolism has been extensively studied in the *Lactobacillaceae* and *Enterobacteriaceae*. The lactic acid bacteria convert pentoses to the common intermediate, D-xylulose-5-P, by means of a series of inducible isomerases, kinases and epimerases (Burma and Horecker 1958a, b; De Moss et al. 1951). D-Xylulose-5-P is split by the key enzyme, phosphoketolase, into acetyl phosphate and glyceraldehyde-3-P (Heath et al. 1958). In the *Enterobacteriaceae*, pentose catabolism follows a similar route as in *Lactobacillaceae* and involves isomerization of the aldopentose to its respective ketopentose, followed by phosphorylation of the ketose, and epimerization of the product to D-xylulose-5-P (Martlock and Wood 1964). The latter compound is further degraded via transketolase and the pentose phosphate pathway (Bagatell et al. 1959).

The only known exception to the sequential isomerization, phosphorylation, and epimerization of aldopentoses is found in ribose catabolism, where phosphorylation precedes isomerization. Ribose metabolism in *Escherichia coli* appears to be more complex than arabinose or xylose metabolism. Ribose metabolism in *E. coli* involves two ribose permeases (1), an inducible ribokinase (2) and two distinct ribose-5-phosphate isomerases (3) (David and Wiesmeyer 1970a, b).

(1) D-ribose$_{(external)}$ $\xrightarrow{\text{Permease}}$ D-ribose$_{(internal)}$;

(2) D-ribose + ATP $\xrightarrow{\text{Ribokinase}}$ D-ribose-5-P + ADP;

(3) D-ribose-5-P $\xrightarrow[\text{isomerase}]{\text{P-ribose}}$ D-ribulose-5-P.

Ribose metabolism in staphylococci has been much less extensively studied than in lactobacilli or enterobacteria. A single gene mutant (car$^-$) was isolated from *Staphylococcus aureus* and analysed. The mutant had lost the ability to utilize sucrose, maltose, galactose, fructose, lactose, mannitol, ribose and trehalose (Egan and Morse 1966). It was proposed that carbohydrate transport was mediated by specific permeases and a common membrane carrier and that the car locus was concerned with the formation of a functional carrier. Recently, the metabolism of pentoses and pentitols was studied in *Staphylococcus xylosus* and *S. saprophyticus* (Lehmer and Schleifer 1980). Pentoses were taken up in an unsubstituted state and the uptake was inducible. A phosphoenolpyruvate phosphotransferase system (PTS) was not involved in the uptake of pentoses and xylitol. However, there are still several unsolved problems regarding ribose metabolism in staphylococci.

In this study, we describe the cloning of a gene in *S. carnosus* which is able to complement D-ribose fermentation in this host. By comparative analysis of enzyme activities of *S. carnosus* wild-type and *S. carnosus* clones, it was established that the ribokinase gene was cloned.

Materials and methods

Materials

T4 DNA ligase and alkaline phosphatase, enzymes and some of the restriction enzymes were obtained from Boehringer, Mannheim, other restriction enzymes were obtained from Bethesda Research Laboratory or Amersham. All enzymes were used according to manufacturers' instructions, unless otherwise indicated. Pentoses and derivatives were obtained from Sigma; chemicals and solutions for thin layer chromatography were obtained from Merck. 35S-dATP αS

Offprint requests to: F. Götz

Abbreviations: bp, base pairs; C-TLC, cellulose-thin layer chromatography; kb, kilo base pairs; pR$_i$b 1 and 2, ribokinase activity conferring hybridplasmids; MBq, megabequerel; wt, wild type

and D-(1-^{14}C) ribose were obtained from New England Nuclear Corp.

Bacterial strains, medium and optical density of cell suspension

Transformations were carried out with *Staphylococcus carnosus* TM 300 (Götz et al. 1983a). Gene library was performed with DNA from *S. hyicus* subsp. *hyicus* DSM 20459 (De Vriese et al. 1978). The strains were cultivated in PYS (10 g peptone, 5 g yeast extract and 5 g NaCl per liter of distilled water, pH 7.2). Growth studies with D-ribose or other sugars were carried out in a protein deficient medium of the following composition (g/l H_2O dist.): Casamino acids, 0.2 g; yeast extract, 0.2 g; Na_2HPO_4, 0.2 g; $MgSO_4$, 0.1 g; KCl, 0.1 g; sugar, 10 g (separately sterile filtered); pH of the medium was adjusted to 7.2. Indicator agar contained in addition 0.8 g yeast extract, 10 g agar and 20 mg bromocresol purple as an indicator for acid production. For preparation of crude cell-free extract, the liquid medium contained a higher concentration of yeast extract (1 g/l).

Optical densities of bacterial suspensions were routinely measured at a wavelength of 578 nm. From a calibration curve it was found that suspensions at an absorbancy = 1.0 contain ca. 0.3 mg cell dry weight/ml.

Preparation of plasmid and chromosomal DNA

Plasmid DNA isolation was described previously (Götz et al. 1983b). Chromosomal DNA of *S. hyicus* subsp. *hyicus* (ca. 100 µg) was partially digested for 20 min with *Sau*96 (0.3 U/µg DNA). After phenolyzation, the DNA was separated by sucrose gradient (10–40%) centrifugation and DNA fragments in a size range of 4–20 kb were pooled and used for cloning.

Cloning of DNA

As a cloning vector the recently described pCT20 was used (Keller et al. 1983). pCT20 was linearized with *Sau*96, treated with alkaline phosphatase, and was ligated with the partially digested (*Sau*96) chromosomal DNA of *S. hyicus* subsp. *hyicus*. The ratio of vector and chromosomal DNA was 1:5 (µg/µg). The ligation mixture was analysed by agarose gel electrophoresis and then transformed by protoplast transformation (Götz et al. 1983a) into *S. carnosus* TM 300. Transformants were initially selected on DM 3 agar containing 10 µg/ml chloramphenicol. Chloramphenicol resistant transformants were picked out onto PYS-agar plates containing 10 µg of chloramphenicol per ml. From these plates the transformants were replicated onto tetracycline (25 µg/ml) PYS-agar plates. Chloramphenicol resistant but tetracycline sensitive clones were selected for the gene library.

Nick translation of plasmid DNA and Southern hybridization

DNA restriction fragments were transferred from agarose gels to a nitrocellulose filter (Schleicher & Schuell) by the method of Southern (1975). The hybridization was performed in a buffer containing 6 × SSC (standard saline citrate) at 65°C for 16 h, as described previously (Götz et al. 1983b). Plasmid DNA was used as a probe and was labelled in vitro with ^{35}S-dATP αS by nick translation (Rigby et al. 1977).

Uptake studies with D-(1-^{14}C)ribose

To 3 ml cell suspension in 0.1 M K-phosphate buffer, pH 7.0 (optimal density at 578 nm was 40), 1 ml of D-ribose (10 mM) and 0.025 ml of D-(1-^{14}C)ribose (3.7 MBq/ml) were added. This cell suspension was incubated in a rotary shaker at 37° C. At the indicated times (0, 1, 30, 60, 100, 150, 240, and 360 min) 200-µl samples were rapidly filtered using cellulose acetate membrane filters of 0.2 µm pore size. The filters with the retained bacteria were washed with 5 ml K-phosphate buffer, dried at 80° C under vacuum and the radioactivity was determined by liquid scintillation counting, using a scintillation solution for water-free samples (1 l toluene, 3 g PPO and 0.25 g POPOP).

Immediately after addition of D-(1-^{14}C)ribose, 200 µl of the cell suspension was removed and its radioactivity was determined in a scintillation solution for aqueous samples (1 l toluene, 0.5 l Triton X100, 4 g PPO and 0.1 g POPOP). The obtained radioactivity was taken as 100%.

Enzyme assays

Cells were grown to end-log phase in D-ribose medium. Preparation of crude cell-free extract from ca. 1 g cell wet weight was carried out as described previously (Götz et al. 1979). Crude cell-free extract was dialysed against 50 mM Tris-HCl, pH 7.4, and 0.1 mM dithiothreitol.

Isomerase activity was determined by measuring the formation of D-ribulose from D-ribose or of D-ribulose-5-P from D-ribose-5-P by the aid of cellulose thin layer plates. The reaction mixture contained in a total volume of 1.07 ml 40 mM K-Na-phosphate buffer, pH 7.0; 45 mM D-ribose or D-ribose-5-P; 2 mM $MnCl_2$, and 0.2 ml cell-free extract. The reaction was incubated for 1 h at 37° C and was then stopped by incubation at 100° C for 2 min. The precipitated proteins were centrifuged in an Eppendorf centrifuge for 15 min and 10 µl of the supernatant were applied to a cellulose thin layer plate.

D-Ribokinase activity was determined by measuring the D-ribose and ATP dependent formation of D-ribose-5-P and ADP. The reaction mixture contained in a total volume of 1.22 ml 42 mM K-Na-phosphate buffer, pH 7.0; 14 mM ATP; 40 mM $MgCl_2$; 37 mM D-ribose; and 0.2 ml cell-free extract. The reaction was incubated for 1 h at 37° C and was then stopped by incubation at 100° C for 2 min. After centrifugation, 10 µl of the supernatant were applied to a cellulose thin layer plate. In both, the isomerase and kinase assays, controls were always performed in the absence of either substrates or cell-free extracts.

Cellulose thin layer chromatography (C-TLC)

A. A good separation of D-ribose and D-ribulose was achieved on C-TLC with the solvent system described by Lehmer and Schleifer (1980). The solvent system is composed of pyridine–ethylacetate–glacial acetic acid-water in the ratio 36:36:7:21. Chromatography was normally terminated when the solvent front had ascended ca. 15 cm. After drying C-TLC plates, the pentoses were identified by spraying the chromatogram with phloroglucinol reagent (Clotten and Clotten 1962).

B. AMP, ADP and ATP were separated on C-TLC plates by the solvent system as described by Stahl, 1967: By using C-TLC plates with fluorescence indicator, the nucleotide spots were visualized at 254 nm.

C. A separation of pentoses from pentose phosphates was achieved by the following solvent system (Stahl 1967): isobutyric acid – NH_4OH – water in the ratio 66:1:33. The staining was also carried out with the phloroglucinol staining reagent.

D-Ribose-5-P isomerase activity was assayed by using D-ribose-5-P as a substrate. After incubation in the above described assay system at 37° C, the reaction was stopped by incubation at 100° C for 2 min and the phosphate groups of D-ribose-5-P and D-ribulose-5-P were removed by treatment with alkaline phosphatase (Maniatis et al. 1982). The produced pentoses were separated and stained with the solvent and staining solution as described in A.

Results

Construction of a gene library of S. hyicus subsp. hyicus

Chromosomal DNA of *S. hyicus* subsp. *hyicus* DSM 20459 was partially digested with the restriction endonuclease *Sau*96. DNA fragments in the size range of 4 to 20 kb were pooled and ligated with the linearized (*Sau*96) plasmid vector pCT20 (Keller et al. 1983) as described in Materials and methods. The restriction site of *Sau*96 in pCT20 is located within the tetracycline gene so that DNA insertions can be detected in clones by inactivation of tetracycline resistance.

The ligase treated DNA was transformed into *S. carnosus* TM 300 by protoplast transformation. Transformants were first selected for chloramphenicol resistance. The chloramphenicol resistant transformants were then checked with regard to their tetracycline resistance. 87% of the chloramphenicol resistant transformants were tetracycline sensitive due to insertional inactivation. 2,300 of the chloramphenicol resistant and tetracycline sensitive clones were collected for the gene library. The size of the inserted DNA fragments calculated from 63 clones was 5.6 kb on average. However, among the analyzed hybrid plasmids, DNA inserts in a size of more than 20 kb were found. Since *S. carnosus* TM 300 can only poorly degrade sugar and possesses only a few extracellular enzymes, it is possible to screen the gene library with respect to expression of several phenotypic markers. We have so far cloned the gene(s) for arsenate resistance, lipase and ribose degradation.

Restriction map of pRib 1 and pRib 2

The gene library contained two clones which were positive in ribose fermentation. From these clones the hybrid plasmids (pRib 1 and pRib 2) were isolated and purified by CsCl centrifugation. In order to rule out the possibility that these clones represent spontaneous revertants of *S. carnosus*, both hybrid plasmids were transformed again into *S. carnosus* TM 300. All chloramphenicol resistant transformants were able to ferment ribose, indicating that the cloned fragments in both hybrid plasmids carried genetic information which enables *S. carnosus* to degrade D-ribose.

Figure 1 A and B show a restriction map of pRib 1 and pRib 2. The hybrid plasmid pRib 1 is 15.5 kb in size (5.3 kb comprises the plasmid vector pCT20 and 10.2 kb comprises the inserted chromosomal DNA). With pRib 1, two *Sau*96 fragments of 8.2 and 2.0 kb in size were cloned; with pRib 2 only the 8.2 kb fragment was cloned.

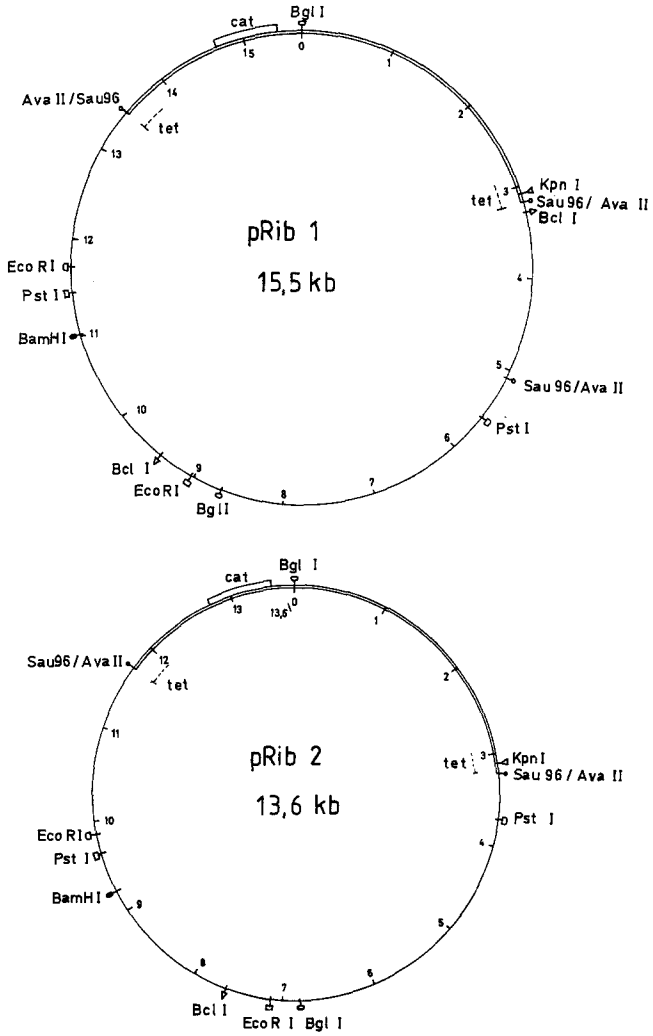

Fig. 1. Restriction maps of pRib 1 (A) and pRib 2 (B). Double lines represent the vector plasmid pCT20

Hybridization studies with pRib 1 as a probe

The chromosomal DNA of *S. hyicus* subsp. *hyicus* DSM 20459 was cut with several restriction endonucleases such as *Bam*HI, *Eco*RI, *Pst*I and *Sau*96. The DNA fragments were separated by agarose-gel-electrophoresis, blotted onto nitrocellulose filter, and hybridized to pRib 1 probe (Fig. 2). The *Sau*96 digested chromosomal DNA revealed two DNA fragments in the autoradiogram of exactly the same size as the cloned DNA of pRib 1. EcoRI cleavage of the chromosomal DNA was incomplete, so that only one weak hybridization band could be seen. With PstI and BamHI, two and one hybridization bands, respectively, were visible at the autoradiogram as was expected from the restriction map of pRib 1. The 8.2 kb insert of pRib 2 also shows strong hybridization with pRib 1. No DNA homology was detectable between the chromosomal DNA and the plasmid vector pCT20.

Growth studies

The two ribose-positive clones of the gene library were detected by acid production on D-ribose indicator agar. In order to obtain more information about the new genetic

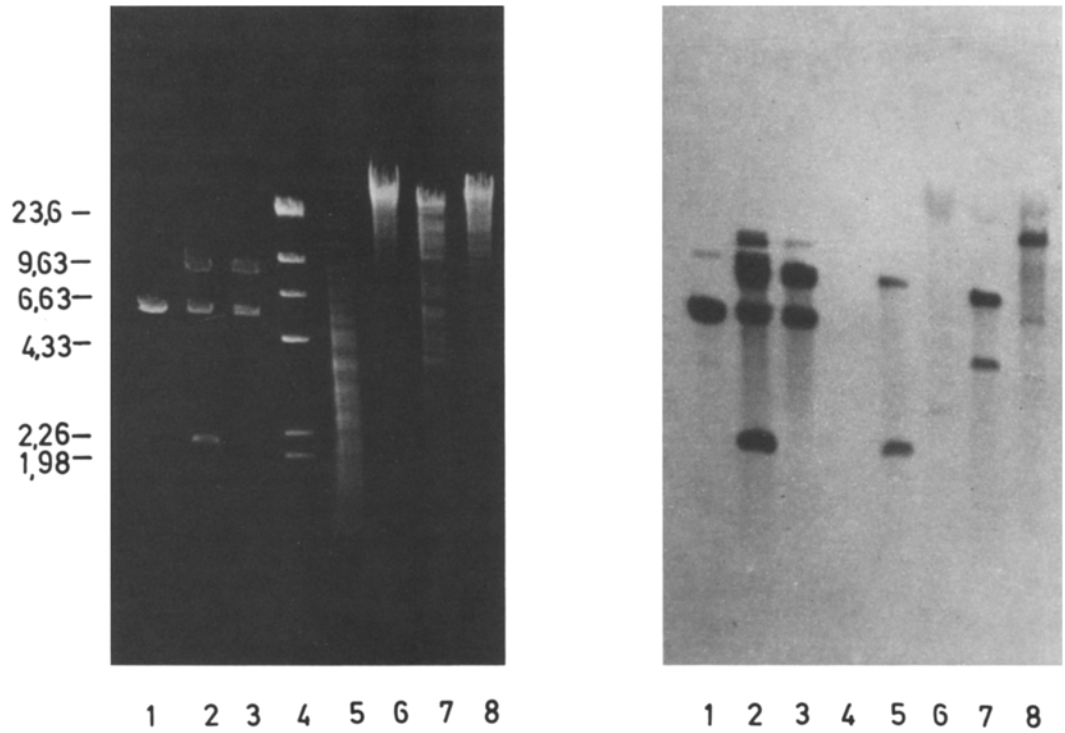

Fig. 2. The various plasmids (lanes *1*–*4*) and the chromosomal DNA of *S. hyicus* subsp. *hyicus* (lanes *5*–*8*) were cleaved with the following restriction endonucleases and separated by agarose gel electrophoresis (left): Lane *1*: pCT20 (*Sau*96); lane *2*: pRib 1 (*Sau*96); lane *3*: pRib 2 (*Sau*96); lane *4*: λ-DNA (Hind III, as a marker); lane *5*: DNA (*Sau*96); lane *6*: DNA (EcoRI); lane *7*: DNA (PstI); lane *8*: DNA (Bam HI). Right: Autoradiograph after Southern blot hybridization of ^{35}S-labelled pRib 1 to DNA fragments of lanes *1*–*8*

and physiological properties of *S. carnosus,* growth, ribose uptake and enzymatic studies were carried out.

The ability to metabolize D-ribose should enable the D-ribose-positive clones to grow better in the presence of this sugar than *S. carnosus* wild type (wt). By using a protein deficient medium which allows only limited growth in the absence of a fermentable sugar, growth studies with glucose (control) and D-ribose were carried out with *S. hyicus* subsp. *hyicus*, *S. carnosus* (wt), *S. carnosus* (pRib 1) and (pRib 2) respectively (Fig. 3). The growth curves show that the *S. carnosus* clones grow better in the presence of D-ribose than *S. carnosus* (wt), while growth in the presence of glucose is comparable for all three *S. carnosus* strains.

If one compares *S. hyicus* subsp. *hyicus* with *S. carnosus* (pRib 1) or (pRib 2) it is noticeable that *S. hyicus* subsp. *hyicus* is much more active in the fermentation of both glucose and D-ribose; with D-ribose also a much higher density was reached as compared to *S. carnosus* clones. These data show that the new genetic information only enables *S. carnosus* clones pRib 1 and 2 to limited growth on D-ribose. The hybrid plasmids pRib 1 and 2 were stably maintained in *S. carnosus*. Even without selective pressure, loss of hybrid plasmids was less than 2% after ca. 60 cell generations.

Uptake studies with D-*(1-*^{14}*C)ribose*

With washed cell suspensions of *S. hyicus* subsp. *hyicus*, *S. carnosus* (wt) and *S. carnosus* clones, the uptake of D-ribose, by using D-(1-^{14}C)ribose as an indicator, was studied (Fig. 4). The cells were precultured either with glucose or with ribose. The time course of the uptake of D-ribose shows that with cells which were precultivated with glucose, D-ribose uptake was either very low or totally repressed in the case of *S. carnosus* (wt). If however, the strains were precultured with D-ribose, then they all were able to take up D-ribose, although the time courses revealed some differences. With *S. hyicus* subsp. *hyicus*, the percent uptake of labelled D-ribose increases during the first 60 min and decreases after this time. *S. carnosus* (wt) is also able to take up D-ribose, however, unlike *S. hyicus* subsp. *hyicus*, there is no decrease in the observed radioactivity. With *S. carnosus* clones, the percentage uptake of labelled D-ribose appears to be markedly reduced. A further difference between *S. carnosus* (pRib 1), (pRib 2) and *S. carnosus* (wt) is that the D-ribose uptake of cells which precultivated with glucose, was only to some extent repressed with both clones.

Isomerases and kinases

Crude cell-free extracts were prepared from D-ribose-grown cells of *S. hyicus* subsp. *hyicus*, *S. carnosus* (wt) and the clones *S. carnosus* (pRib 1) and (pRib 2). By using cellulose-thin layer chromatography (C-TLC), the presence or absence of enzymes which are normally found with D-ribose metabolism were investigated.

Ribose isomerase catalyses the isomerization of D-ribose and D-ribulose and was found in some staphylococcal species (Lehmer and Schleifer 1980). For the measurement of this enzyme activity, a good separation of D-ribose and D-ribulose on C-TLC was necessary. This was achieved by solvent A and staining the chromatogram with the phloroglucinol staining solution. However, none of the crude cell-free extracts contained D-ribose isomerase activity,

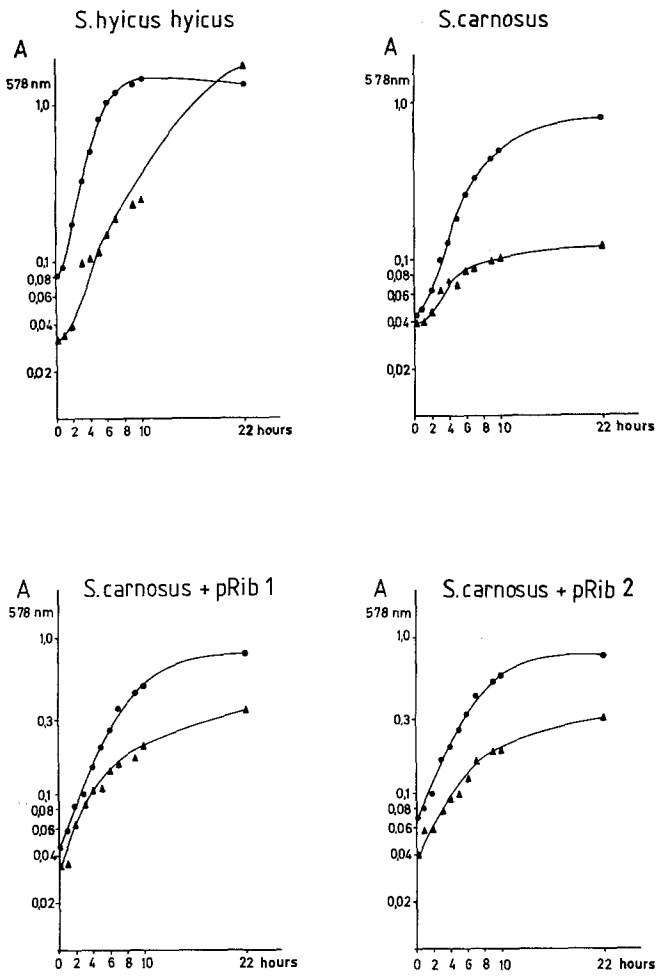

Fig. 3. Growth curves of *S. hyicus* subsp. *hyicus*, *S. carnosus* (wt) and the *S. carnosus* clones with pRib 1 and pRib 2 respectively. Cell growth is represented by the absorption (A) at 578 nm. For growth studies, a protein deficient medium with glucose (●) or D-ribose (▲) as the sole carbohydrate source was used

either with D-ribose or D-ribulose as the substrate. This negative result indicates that D-ribose might be first phosphorylated.

D-Ribokinase catalyses the ATP dependent phosphorylation of D-ribose to form D-ribose-5-P. With all crude cell-free extracts, except that of *S. carnosus* (wt), D-ribose-5-P was produced from D-ribose in the presence of ATP, indicating that the ribose-positive clones of *S. carnosus* have obtained the gene for D-ribokinase from *S. hyicus* subsp. *hyicus*. This result was supported by the finding that only with extracts of *S. hyicus* subsp. *hyicus* and the two *S. carnosus* clones, was ADP formed from ATP and not with the extract of *S. carnosus* (wt). The nucleotides ATP, ADP and AMP were separated on C-TLC with fluorescence indicator and with solvent system B. With D-ribulose as a substrate, no phosphorylating activity was detectable.

D-Ribose-phosphate-isomerase catalyses the isomerization of D-ribose-5-P and D-ribulose-5-P. Since a good separation of these pentose phosphates was not achieved using the solvent systems tested, after the enzyme reaction was stopped, the pentose phosphates were treated with alkaline phosphatase thus forming D-ribose or D-ribulose which could then be well separated. Only *S. hyicus* subsp. *hyicus* possessed a D-ribose-5-P isomerase activity which was absent in both the *S. carnosus* clones (pRib 1) and (pRib 2) and in *S. carnosus* (wt).

Discussion

The species *S. carnosus* is characterized by its inability to ferment D-ribose and other pentoses (Schleifer and Fischer 1982). By cloning DNA fragments of the ribose-positive *S. hyicus* subsp. *hyicus* strain into *S. carnosus*, clones were detected which were capable of fermenting D-ribose. A comparison of the growth curves on protein deficient D-ribose or glucose medium revealed that *S. carnosus* (wt) was unable to grow at the expense of D-ribose (the limited growth observed was due to a low amount of amino acids and yeast extract). The *S. carnosus* clones containing the

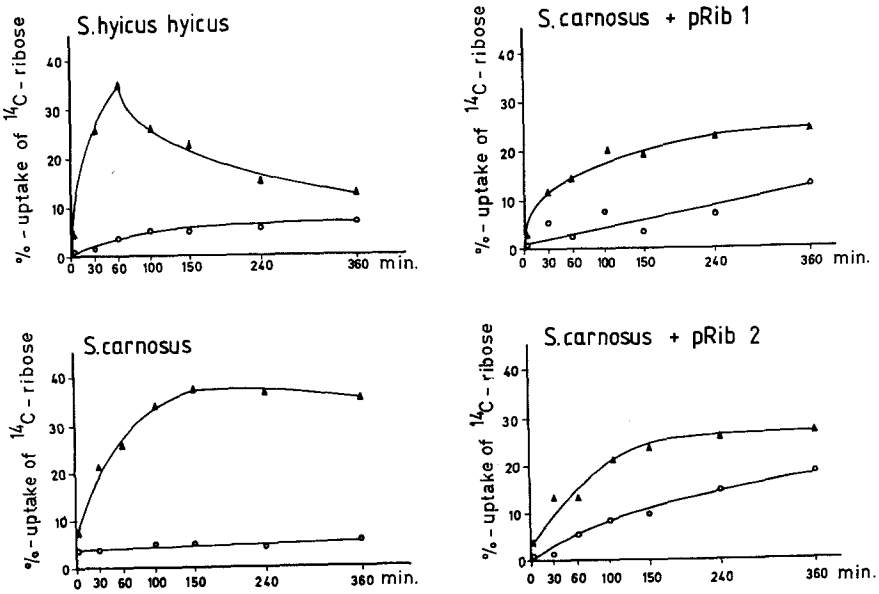

Fig. 4
Time course of the uptake of D-ribose [with D-(1-^{14}C)ribose as indicator] by washed cell suspensions of *S. hyicus* subsp. *hyicus*, *S. carnosus* (wt) and the *S. carnosus* clones with pRib 1 and pRib 2 respectively. Cells were precultivated with glucose (○) or D-ribose (▲) respectively

hybrid plasmids (pRib 1) and (pRib 2) respectively, were able to ferment D-ribose, but fermentation ability was low when compared to *S. hyicus* subsp. *hyicus*. *S. carnosus* (wt) and *S. carnosus* clones showed similar growth with glucose as the only source of carbohydrate.

Uptake studies with D-(1-^{14}C)ribose revealed that *S. carnosus* (wt) was able to take up D-ribose; however, this uptake system is subject to a stringent glucose repression, which is less pronounced with *S. hyicus* subsp. *hyicus* or the *S. carnosus* clones. This difference in glucose repression with *S. carnosus* (wt) and *S. carnosus* clones could indicate that in addition to the ribokinase-gene, an additional ribose uptake system (or part of it) was cloned, which is less sensitive to glucose repression. The D-ribose uptake is not coupled to a phosphotransferase system like that described by Hengstenberg et al. (1969) for hexoses in *S. aureus* (data not shown). This result is in agreement with earlier findings (Lehmer and Schleifer 1980).

In cell-free extracts of *S. hyicus* subsp. *hyicus*, we detected a D-ribokinase- and a P-ribose isomerase activity by C-TLC, indicating that the basic pathway of D-ribose metabolism in *S. hyicus* subsp. *hyicus* is the same as that described in other microorganisms (David and Wiesmeyer 1970b) and includes permeation of D-ribose, its phosphorylation to D-ribose-5-P and isomerization of the product to ribulose-5-P. D-Ribokinase activity was also determined by the use of a coupled enzyme system involving phosphoriboisomerase (EC 5.3.1.6), D-ribulose-5-phosphate 3-epimerase (EC 5.1.3.1), transketolase (EC 2.2.1.1), α-glycerophosphate dehydrogenase (EC 1.1.1.8), and triosephosphate isomerase (EC 5.3.1.1). However, monitoring of NADH disappearance was complicated by the presence of high NADH oxidase activity. If, however, the assay system for D-ribokinase – composed of cell-free extract, D-ribose and ATP – was boiled for 2 min after incubation at 37° C, then the produced D-ribose-5-P could be assayed by the coupled enzyme system; the NADH oxidase activity was destroyed by the heat step.

The situation is different with *S. carnosus* (wt). We were unable to demonstrate D-ribokinase-, D-ribose isomerase- or D-ribose-5-P isomerase activity. Only in strains containing pRib 1 or pRib 2 was a D-ribokinase activity detectable. However, even with these clones, no D-ribose-5-P isomerase activity was detectable. The absence of a D-ribose-5-P isomerase activity in cell-free extracts of *S. carnosus* raises the question, in which way D-ribose-5-P or D-ribulose-5-P are converted to D-xylulose-5-P in *S. carnosus*. It could be possible that *S. carnosus* possesses appropriate enzymes for a by-pass, or, what appears more likely, *S. carnosus* contains a weak and therefore, not detectable D-ribose-5-P isomerase activity. In favour of the latter hypotheses, are the results of the growth studies with D-ribose as the sole carbohydrate source in the reduced medium, where the cloned D-ribokinase genes in *S. carnosus* (pRib 1) and (pRib 2) support only a reduced growth on D-ribose in contrast to *S. hyicus* subsp. *hyicus*. In *E. coli* for example two distinct ribose-5-P isomerase activities were found which were distinguishable by temperature sensitivity and substrate affinity (David and Wiesmeyer 1970b). The heat-stable enzyme does not appear to function in the formation of ribulose-5-P from ribose-5-P in vivo. The heat-stable ribose-5-P isomerase, however, is able to catalyze this reaction.

Since we cannot presently entirely rule out that the cloned gene product only leads to a derepression of an endogenous ribokinase in *S. carnosus*, it is planned to characterize the cloned gene product and to do complementation studies with appropriate *E. coli* mutants.

Acknowledgements. We wish to thank B. Schumacher for technical assistance and H. Huber for typing the manuscript. This research was supported by a grant from the Deutsche Forschungsgemeinschaft (SFB 145).

References

Bagatell FK, Wright EM, Sable HZ (1959) Biosynthesis of ribose and deoxyribose in *Escherichia coli*. J Biol Chem 234:1369–1374

Burma DP, Horecker BL (1958a) Pentose fermentation by *Lactobacillus plantarum*. III. Ribulosekinase. J Biol Chem 231:1039–1051

Burma DP, Horecker BL (1958b) Pentose fermentation by *Lactobacillus plantarum*. IV. L-Ribulose-5-phosphate-4-epimerase. J Biol Chem 231:1053–1064

Clotten R, Clotten A (1962) Hochspannungs-Elektrophorese. Thieme Verlag, Stuttgart

David J, Wiesmeyer H (1970a) Regulation of ribose metabolism in *Escherichia coli*. II. Evidence for two ribose-5-phosphate isomerase activities. Biochim Biophys Acta 208:56–67

David J, Wiesmeyer H (1970b) Regulation of ribose metabolism in *Escherichia coli*. III. Regulation of ribose utilization in vivo. Biochim Biophys Acta 208:68–76

DeMoss RD, Bard RC, Gunsalus JC (1951) The mechanism of the heterolactic fermentation: A new route of ethanol formation. J Bacteriol 62:499–511

Devriese LA, Hajek V, Oeding P, Meyer SA, Schleifer KH (1978) *Staphylococcus hyicus* (Sompolinsky 1953) comb nov and *Staphylococcus hyicus* subsp. *chromogenes* subsp nov. Int Syst Bacteriol 28:482–490

Egan JB, Morse ML (1966) Carbohydrate transport in *Staphylococcus aureus*. Biochim Biophys Acta 112:63–73

Götz F, Nürnberger E, Schleifer KH (1979) Distribution of class-I and class-II D-fructose-1,6-bisphosphate aldolases in various staphylococci, peptococci and micrococci. FEMS Microbiol Letters 5:253–257

Götz F, Kreutz B, Schleifer KH (1983a) Protoplast transformation of *Staphylococcus carnosus* by plasmid DNA. Mol gen genet 189:304–342

Götz F, Zabielski J, Philipson L (1983b) DNA homology between the arsenate resistance plasmid pSX267 from *Staphylococcus xylosus* and the penicillinase plasmid pI258 from *Staphylococcus aureus*. Plasmid 9:126–137

Heath EC, Hurwitz J, Horecker BL, Ginsburg J (1958) Pentose fermentation by *Lactobacillus plantarum*. I. The cleavage of xylulose-5-phosphate by phosphoketolase. J Biol Chem 231:1009–1029

Hengstenberg W, Penberthy WK, Hill KL, Morse ML (1969) Phosphotransferase system of *Staphylococcus aureus*. Its requirement for accumulation and metabolism of galactosides. J Bacteriol 99:383–388

Keller G, Schleifer KH, Götz F (1983) Construction and characterization of plasmid vectors for cloning in *Staphylococcus aureus* and *Staphylococcus carnosus*. Plasmid 10:270–278

Lehmer A, Schleifer KH (1980) Metabolism of pentoses and pentitols by *Staphylococcus xylosus* and *Staphylococcus saprophyticus*. Zbl Bakt Hyg C1:109–123

Maniatis T, Fritsch EF, Sambrock J (1982) Molecular cloning. A laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Martlock RP, Wood WA (1964) Metabolism of pentoses and pentitols by *Aerobacter aerogenes*. I. Demonstration of pentose isomerase, pentulokinase, and pentitol dehydrogenase enzyme families. J Bacteriol 88:838–844

Rigby PW, Dieckman M, Rhodes C, Berg P (1977) Labeling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase. I. J Mol Biol 113:237–251

Schleifer KH, Fischer U (1982) Description of a new species of the genus *Staphylococcus: Staphylococcus carnosus*. Int Syst Bacteriol 32:153–156

Southern EM (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98:503–517

Stahl E (1967) Dünnschichtchromatographie. Springer, New York

Received April 21, 1984/Accepted July 10, 1984