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The Nucleic Acid Fractions of a Strain of *Streptococcus faecalis*

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SUMMARY: The Schmidt & Thannhauser (1945) procedure was applied to the nucleic acid fractions of *Streptococcus faecalis*. A part of the deoxypentose nucleic acid was differentiated as being insoluble in *N*-NaOH at 37°, and appeared to be firmly bound to polysaccharide material. Base analyses of the nucleic acid fractions are reported, and qualitative determinations of the amino-acid and sugar composition of the residue described.

The Schmidt & Thannhauser (1945) procedure has been widely used to estimate the nucleic acids in biological materials. It consists, essentially, of incubating the tissue with *N*-NaOH so that the nucleic acids dissolve and the pentose nucleic acid (PNA) is degraded to nucleotides while the deoxypentose nucleic acid (DNA) is still precipitable by acid. The nucleic acid fractions are then resolved by precipitating the DNA, the PNA nucleotides remaining in the supernatant. We have noted, however, in applying this method to a strain of *Streptococcus faecalis*, that a large proportion of the DNA, in association with much cell material, remains undissolved in the *N*-NaOH. Thus the DNA portion appears to exist in two easily separable fractions.

METHODS

Preparation of material. *Streptococcus faecalis* NCIB 8123 was grown in a medium comprising 0.2 g. yeast extract (Oxoid), 0.5 g. peptone (Oxoid), 0.5 g. glucose, dissolved in 100 ml. 0.05 M-phosphate buffer and adjusted to pH 7. The organism was grown in 10 l. batches, harvested on a Sharples supercentrifuge, washed twice with water and acetone-dried.

Schmidt & Thannhauser procedure. A modification of this technique (Davidson, Leslie & Waymouth, 1949) was used to prepare the nucleic acid fractions. The dried cells were extracted twice with ice-cold 10 % (w/v) trichloroacetic acid (TCA) in 50 ml. centrifuge tubes, then, in succession, with 80 % (v/v) ethanol, absolute ethanol, chloroform + ethanol (1:3) twice at 80°, and finally with ether.

The remaining dry powder was incubated overnight with 10 ml. *N*-NaOH at 37°. The insoluble residue was centrifuged off and washed with water (15 ml.) which was added to the supernatant. To this combined aqueous solution, 5 ml. of 2.5 *N*-HCl and 6 ml. 30 % (w/v) TCA were added. The DNA was precipitated and spun down by centrifuging for 20 min. at 5000 r.p.m. The precipitate was washed twice with small volumes of 5 % TCA, the washings being added to the supernatant solution which contained the PNA. This

solution of PNA was concentrated *in vacuo*, transferred to a combustion tube and evaporated to dryness in a vacuum desiccator over P_2O_5 and solid NaOH; this was the PNA fraction.

The precipitated DNA was dissolved in a small volume of 0.1 N-NaOH and reprecipitated by acidification with acetic acid and addition of 1 vol. ethanol. The DNA was redissolved in 0.1 N-NaOH and deproteinized by shaking with chloroform + butanol (8:1, v/v). The protein gel was centrifuged off and the DNA precipitated from the aqueous fraction by the addition of acetic acid and ethanol and finally dried with acetone and ether. This was the 'free' DNA fraction.

The insoluble residue from the N-NaOH hydrolysis was washed twice with N-NaOH and then twice with water. It was finally dried with acetone and ether; this was the 'bound' DNA fraction.

The nucleic acids in the three fractions were assayed by direct phosphorus determinations.

Phosphorus determinations. After combustion with 60 % (w/v) perchloric acid, inorganic phosphorus was measured colorimetrically by the method of Fiske & Subbarow (1925).

Nitrogen determinations. Total nitrogen was assayed by a micro-Kjeldahl method.

The Dische reaction for deoxypentoses was carried out qualitatively by heating the sample with two volumes of the Dische reagent (twice recrystallized diphenylamine, 1 g.; sulphuric acid, 1.5 ml.; glacial acetic acid, 100 ml.) for 20 min. at 100°. A blue colour indicates deoxypentose. The specificity of this test was examined by Deriaz, Stacey, Teece & Wiggins (1949).

Chromatographic methods

For nucleic acids the technique of Wyatt (1951) was used. The nucleic acid fractions were hydrolysed by heating with 72 % (w/v) perchloric acid on a boiling water-bath for 90 min., sufficient perchloric acid being used to give an 8 % solution of nucleic acid, calculated from the P content. The hydrolysate was then diluted with 2 vol. water and centrifuged to remove the carbon. Samples (15–25 μ l.) of the supernatant liquid were taken for chromatography and for further P determinations. The chromatograms were run for 36 hr. in isopropanol + HCl (65 : 35, v/v, with 2 N-HCl) on Whatman no. 1 paper and dried in air.

Purines and pyrimidines were detected by photographing the paper in ultraviolet light on reflex document paper. The source of ultraviolet light used was an Osram UV lamp with the outer glass envelope removed. This was in series with a 3000-ohm resistance (mains voltage 230 V. a.c.) and the light was filtered through an Ilford filter no. 828. This is a method which has been used in this laboratory by Mr R. A. Evans. The positions of the spots were traced out on the chromatogram and the areas cut out and eluted, together with the appropriate blank areas, with 0.1 N-HCl. The bases were estimated by measuring their ultraviolet extinction at their maxima against the extinction of the appropriate blank, in the Unicam spectrophotometer. The identity of

all the spots was confirmed by determining the ultraviolet absorption curves of the eluates.

Amino-acids. The sample was hydrolysed with 6 N-HCl for 16 hr., the hydrolysate evaporated to dryness 3 times *in vacuo* and taken up in a small volume of water. The hydrolysates were run on Whatman no. 1 paper with *n*-butanol+acetic acid+water mixture (4 : 1 : 5, v/v) in one dimension, and in two dimensions with *n*-butanol+acetic acid+water followed by phenol+NH₃ (3 : 1 v/v), the NH₃ solution being 3 ml. sp.gr 0.880 ammonia in 1 l. water, using ascending chromatography. The amino-acids were detected by spraying with ninhydrin.

Sugars. The sample was hydrolysed with 2 N-H₂SO₄ in a sealed tube at 100° for 2 hr., the hydrolysate adjusted to pH 4.5 with Ba(OH)₂ solution, and the mixture centrifuged. The precipitated BaSO₄ was washed, and the hydrolysate+washings evaporated to a small volume *in vacuo*. The hydrolysate was run with *n*-butanol+acetic acid+water mixture in one dimension, followed by phenol+NH₃ in the second. The various sugars were detected by aniline hydrogen phthalate (Partridge, 1949). The presence of rhamnose was confirmed by a specific colour test for methylpentoses (Edward & Waldron, 1952).

Growth experiment

One litre of the glucose peptone medium was inoculated with 1 ml. of a 24 hr. culture of *Strep. faecalis* and incubated aerobically at 37°. At hourly intervals 10 ml. samples were withdrawn, centrifuged and the cells washed. The cells were resuspended in 10 ml. water and 1 ml. of this taken and appropriately diluted for turbidity measurements. To the remaining 9 ml., 1 ml. of N-NaOH was added and the suspension incubated at 37° overnight. The residue was then spun down and the ultraviolet absorption of the supernatant read directly in the Unicam spectrophotometer, at its maximum absorption at 260 mμ. This represents the PNA + 'free' DNA. The residue ('bound' DNA) was hydrolysed with 72 % perchloric acid at 100° for 1 hr., diluted to 10 ml., the carbon fragments centrifuged down and the ultraviolet absorption of the resulting clear solution measured at its absorption peak of 260 mμ. The absorption curves of the two components were measured and conformed to the normal curve for a nucleic acid, thus showing them to be free from appreciable quantities of other ultraviolet absorbing substances.

RESULTS

The quantitative results are expressed as the means of triplicate determinations on three independent preparations.

'Bound' DNA fraction. This is insoluble in N-NaOH at 37°. The cell outlines were still visible and were Gram-negative. The material had P=1.05 % and N=3.85 % and represented 45 % of the cell dry weight. Adenine, guanine, cytosine and thymine were detected and accounted for 78 % of the P in the fraction, assuming that the bases in nucleic acids have a 9 : 10 molar ratio to P (Wyatt, 1951).

The following compounds were also detected in hydrolysates:

(a) Amino-acids: alanine, aspartic acid, glutamic acid, glycine, leucine, lysine, phenylalanine, threonine, tyrosine, valine.

(b) Sugars: galactose, glucose and rhamnose. The fraction gave a strong Dische reaction for deoxypentose.

Extraction of the fraction by boiling N-NaOH gradually removed P from the material, although after four successive 15 min. extractions some 10 % of the P still remained in the residue. The P in the supernatants from each successive extraction was found to be almost equally distributed between the acid-soluble and acid-insoluble material. At the same time much of the carbohydrate became soluble in the N-NaOH, at a rate corresponding to the extraction of the P. Chromatography showed that purine and pyrimidine bases were also distributed between the acid-soluble and acid-insoluble material.

'Free' DNA fraction. The bases adenine, guanine, cytosine and thymine were detected; they accounted for 95 % of the P of the fraction.

PNA fraction. The bases adenine, guanine, cytosine and uracil were detected; they accounted for 85 % of the P of the fraction. No inorganic P was detected.

Whole cells. Values for the P content of the different nucleic acid fractions were: 'bound' DNA 4.75 $\mu\text{g.}/\text{mg.}$; 'free' DNA 1.03 $\mu\text{g.}/\text{mg.}$; PNA 11.6 $\mu\text{g.}/\text{mg.}$ The purine and pyrimidine composition of the fractions is given in Table 1. The following compounds were detected in hydrolysates of the whole organism:

(a) Bases: adenine, guanine, cytosine, uracil, thymine.

(b) Amino-acids: alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, leucine, lysine, phenylalanine, proline, serine, threonine, tyrosine, valine.

(c) Sugars: galactose, glucose, glucosamine, ribose, rhamnose.

Table 1. *The purine and pyrimidine composition of the nucleic acid fractions of washed Streptococcus faecalis*

Base	Molar ratios, calculated to total 4.00		
	'Bound' DNA	'Free' DNA	PNA
Adenine	1.17	1.20	1.00
Guanine	0.73	0.66	1.25
Cytosine	0.81	0.84	0.61
Uracil	—	—	1.14
Thymine	1.29	1.30	—

Results of the growth experiment

The results of the growth experiment are shown in Fig. 1. The amount of bacterial growth was measured by a photo-electric turbidimeter. The relation of this to weight of cell material and to cell numbers once the log phase is reached has been tested by a number of workers (Monod, 1942). The nucleic acid curves were obtained by dividing the ultraviolet absorption by the

turbidity, and thus give a measure of the variation of the amount of nucleic acid/cell during the lag and early log phases, when the mean cell size remains approximately constant. The insoluble fraction persists throughout the growth cycle after the initiation of the log phase and remains proportional to the amount of soluble nucleic acid in the cell. The 'bound' DNA fraction also persists under differing physiological and nutritional conditions (Thomas, unpublished).

Fig. 1. The variation of the nucleic acid fractions of *Streptococcus faecalis* during growth.

●—● = bacterial density, measured turbidimetrically, $\log I/I_0$; ▲—▲ = 'bound' DNA/cell = ultraviolet absorption at 260 $m\mu$. divided by turbidity; ■—■ = PNA + 'free' DNA/cell = ultraviolet absorption at 260 $m\mu$. divided by turbidity.

DISCUSSION

The drastic conditions necessary for extraction of 'bound' DNA from the cells lead to partial degradation of the DNA to smaller acid-soluble molecules with a simultaneous extraction of carbohydrate. This suggests that the more insoluble DNA fraction is part of a stable complex. If DNA were linked to polysaccharide by ester links between the primary phosphate groups of the DNA and the hydroxyl groups of the sugar residues such a stable complex would be expected. Such esterification of DNA might replace, wholly or in part, electrovalent links to the basic groups of proteins. Purified DNA, extracted from *Haemophilus pertussis*, still contains 33% of carbohydrate (Overend, Stacey, Webb & Ungar, 1951), and carbohydrate is associated

with DNA isolated from rye (Laland, Overend & Webb, 1950). This association of DNA with carbohydrate may be of significance in view of the changes in the serologically active polysaccharide of pneumococci induced by a purified pneumococcal DNA preparation (Avery, MacLeod & McCarty, 1944).

The complex nature of the 'bound' DNA fraction is indicated by the presence of amino-acid and sugar residues. (The finding of purines and pyrimidines, P and deoxypentose is regarded as demonstrating the presence of DNA. This does not, of course, rigidly prove that these components are linked as in DNA). Salton (1952) described the chemical composition of cell walls obtained from *Streptococcus faecalis* by mechanical disintegration. These were largely insoluble in alkali and yielded amino-acids and sugars on hydrolysis. The ultraviolet absorption of the cell-wall suspension gave no evidence for the presence of nucleic acid though the preparation contained 1.8 % P. Our alkali-insoluble residue must contain much somatic material in addition to the cell wall.

There does not seem to be any significant difference in the purine and pyrimidine composition of the two DNA fractions. Both are in the AT (excess adenine and thymine) 'animal' class in contrast to the GC (excess guanine and cytosine) class which Chargaff, Zamenhof, Brawerman & Kerin (1950) have reported for some bacterial DNA preparations.

The chromatographic evidence confirms the validity of the Schmidt & Thannhauser procedure for the separation of the extracted nucleic acids. The finding of ribose in the whole cell is consistent with the presence of a pentose nucleic acid. Most of the P in the 'free' DNA fraction is accounted for by the purines and pyrimidines. The 'bound' DNA has 22 % excess P and the PNA fraction 15 % excess. Mitchell & Moyle (1950) reported that *c.* 70 % and *c.* 93 %, respectively, of the P in two strains of *Strep. faecalis* was accounted for by the nucleic acid ultraviolet absorption.

These observations draw attention to the necessity of examining any alkali-insoluble residue, obtained in the Schmidt & Thannhauser procedure, for the presence of nucleic acid.

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