CELL DIVISION IN A SPECIES OF ERWINIA. VII. AMINO SUGAR CONTENT OF DIVIDING AND NONDIVIDING CELLS.[a]

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Received August 15, 1964

Several agents which inhibit cell division affect cell wall synthesis. Tuttle & Gest (1960) reported that several D-amino acids increased cell wall content of amino sugars in Rhodospirillum rubrum. Vancomycin, penicillin, and 5-fluorouracil, all of which inhibit cell division in a species of Erwinia, (Grula & Grula, 1962) cause a reduction in cell wall mucopeptide in gram-positive and gram-negative bacteria (Rogers and Perkins, 1960; Tomasz and Borek, 1962; Reynolds, 1962; Lederberg, 1957; Strominger, Park, and Thompson, 1959; Wylie and Johnson, 1962; Roberts and Johnson, 1962; Collins and Richmond, 1962).

Aim of this study was to determine cell wall content of glucosamine (GA) and muramic acid (MA) of Erwinia cells grown under a wide variety of division inhibiting and reversing conditions and thus to determine if an alteration in amino sugar content or a change in the GA/MA ratio was associated with cell elongation.

Materials and Methods

The defined medium, handling of cultures, growth measurements, etc. have been described (Grula, 1960; Grula and Grula, 1962). Prior to analysis for amino sugars, cells were spun from the growth medium, boiled 15 min. in phosphate buffer (0.05M, pH 7.8), and digested with trypsin for 4 hr at 37 C. The cellular material was then washed 3 times in M NaCl and finally in distilled water (78,000 X G for 30 min.). The washed pellet was hydrolyzed (4N HCl for 4 hrs at 100-105 C in sealed and evacuated tubes), then fractionated for GA and MA by the charcoal column procedure of Park as described by Perkins and Rogers (1959). GA and MA were determined by the Rondle and Morgan procedure (1955) as modified by Crumpton (1959).

[a]This work was supported in part by a research Grant GB-1532 from the National Science Foundation and a Public Health Service research career program award (GM-13,968) from the Division of General Medical Sciences.

Results and Discussion

Several agents that inhibit cell division decrease the amount of GA and MA in the cell wall 33 to 42 per cent (Table I). The ratio of GA to MA does not appear to be significantly altered. It had been previously reported (Grula, 1960) that filamentous cells show evidence of partial spheroplasting. Even though some spheroplasting is evident and filamentous cells contain less GA and MA, these division inhibited cells are not particularly prone to osmotic shock except in relatively rare instances. Therefore, they can be considered to be partial spheroplasts; enough cell wall structure remains to prevent typical complete spheroplasting and sensitivity to osmotic shock.

TABLE I

GLUCOSAMINE AND MURAMIC ACID CONTENT OF FILAMENTOUS CELLS OF ERWINIA SPECIES

Growth Situation	GA*	MA*	(GA + MA)*	MOLAR RATIO GA/MA	PER CENT INHIBITION
Basal medium**	8.0	4.6	12.6	2.4	0.00
+ D-serine	5.4	3.0	8.4	2.5	34
+ DL-serine	4.8	2.4	7.2	2.8	43
+ Vancomycin	5.2	3.2	8.5	2.2	33
+ Penicillin	4.7	3.0	7.7	2.2	39
+ Mitomycin C	5.5	2.9	8.4	2.6	34
+ Ultraviolet light	4.5	2.9	7.4	2.2	42
+ Cycloserine	4.7	2.6	7.3	2.5	42

*Given as μg amino sugar per mg dry wt cells.

**Concentration of division inhibitors: D-serine 1.7 x 10^{-2}M; DL-serine 3.4 x 10^{-2}M; Vancomycin 10 μg/ml; Penicillin 50U/ml; Mitomycin C 0.15 μg/ml; Cycloserine 1.7 x 10^{-5}M; ultraviolet light radiation as reported previously (Grula and Grula, 1962b). The Cycloserine (California Corporation) contains talc and lubricant therefore, we do not know the actual amount of cycloserine used.

Because filamentous cells are partial spheroplasts, we next wished to determine the effect of osmotic protection on cell division. Data given in Table II show that cells divide in a more normal manner in the presence of a proper concentration of a division inhibiting agent when osmotic protection is afforded. In many instances, division appears normal. Only two deviations from this pattern have been observed (penicillin plus either sodium or potassium chloride). In these situations, cell division inhibition is more pronounced.

TABLE II

EFFECTS OF OSMOTIC AGENTS AND PANTOYL LACTONE ON GROWTH, DIVISION, AND LEAKAGE

Additive*	Growth as % of Control†	Cell Length(μ)	Protein‡	Nucleic Acid‡	Keto Acids‡‡
Penicillin (50 U/ml)	100	65% 10-50 35% 50-150	1.72	0.34	273
+ PG 0.66M	200	40% 3-10 60% 10-40	0.39	0.06	240
+ AMDG 0.50M	333	95% 2-5 5% 5-10	0.35	0.06	15
+ NaCl 0.27M	92	20% 10-100 80% 100-300	0.23	0.15	276
+ PL 0.042M	350	70% 3-5 30% 5-10	0.09	0.031	30
Cycloserine 1.7 x 10^{-5}M	100	80% 10-50 20% 50-100	0.52	0.10	134
+ PG 0.66M	121	100% 3-4	0.42	0.036	194
+ AMDG 0.50M	125	100% 2-3	0.18	0.019	12
+ NaCl 0.27M	79	100% 2-3	0.51	0.028	87
Cycloserine 2 x 10^{-5}M	Essentially no growth; Not measurable				
+ PG 0.66M	5600	100% 3-5	0.38	0.038	210
+ AMDG 0.25M	5800	100% 2-5	0.22	0.034	7
+ NaCl 0.27M	3500	100% 2-3	0.62	0.067	90
Ultraviolet light	100	70% 3-20 30%20-70	0.39	0.059	55
+ PG 0.26M	100	100% 3-5	0.32	0.043	76
+ PG 0.66M	14	100% 2-3	1.87	0.20	875
+ AMDG 0.25M	72	100% 3-5	0.46	0.06	38
+ AMDG 0.50M	39	100% 3-5	1.00	0.092	61
+ NaCl 0.27M	28	100% 2-3	0.52	0.10	104
+ NaCl 0.38M	11	100% 2-3	1.20	0.22	262
+ PL 0.042M	86	100% 2-5	0.14	0.037	16
D-serine 1.7 x 10^{-2}M	100	45% 5-50 55%50-100	1.20	0.41	778
+ PG 0.26M	140	100% 3-5	0.78	0.11	574
+ PG 0.66M	92	100% 2-5	0.72	0.073	400
+ AMDG 0.50M	182	80% 3-10 20%10-20	0.66	0.18	44
+ NaCl 0.27M	106	90% 3-10 10%10-50	0.66	0.075	153
+ PL 0.042M	238	100% 2-5	0.26	0.063	137
Vancomycin 10 μg/ml	100	40%10-50 60%50-100	0.64	0.15	348
+ PG 0.26M	105	10% 2-5 90% 5-10	0.68	0.11	273
+ AMDG 0.50M	121	100% 2-5	0.45	0.036	0.00
+ PL 0.042M	105	90% 3-10 10%10-20	0.40	0.074	61
Vancomycin 40 μg/ml	Essentially no growth; Not measurable				
+ NaCl 0.27M	3200	100% 2-6	1.65	0.05	91
+ NaCl 0.38M	1300	100% 2-3	5.7	0.17	72

TABLE II (continued)

Mitomycin C 0.20 μg/ml	100	25% 5-20 75%20-100	2.21	0.50	184
+ PG 0.26M	222	85% 3-10 15%10-20	0.49	0.11	134
+ AMDG 0.50M	116	60% 3-10 40%10-40	0.97	0.18	93
+ NaCl 0.27M	61	50% 3-10 50%10-30	1.87	0.19	182
+ PL 0.042M	117	90% 3-10 10%10-20	0.33	0.084	35

*PG (Propylene glycol); AMDG (α-methyl-D-glucoside); PL (Pantoyl lactone).
†Calculated using optical density measured at 540 mμ after growth for 16 hrs with shaking at 25 C (Grula and Grula, 1962).
‡Calculated from 260/280 readings made on the growth medium after 16 hrs growth. Results are given as mg protein or nucleic acid/mg dry wt cells.
"Given as μg/mg dry wt cells. Pyruvate is the major keto acid in the medium; however, in some instances oxalacetic acid can also be demonstrated as its hydrazone. Technique used for keto acids was that of Haidle and Knight, 1960.

TABLE III

GLUCOSAMINE AND MURAMIC ACID CONTENT OF CELLS GROWN UNDER CONDITIONS OF OSMOTIC PROTECTION OR WITH PANTOYL LACTONE

Growth Situation	GA*	MA*	TOTAL GA + MA*	RATIO GA/MA (MOLAR)
Basal medium	8.0	4.6	12.6	2.4
D-serine (1.4×10^{-2}M)	5.4	3.0	8.4	2.5
+ PG	4.2	2.3	6.5	2.5
+ AMDG	5.4	2.7	8.1	2.8
+ PL	4.4	2.7	7.1	2.2
DL-serine (1.5×10^{-2}M)	4.8	2.4	7.2	2.8
+ PG	4.8	1.5	6.3	4.5
+ AMDG	7.2	3.1	10.3	3.2
+ PL	4.2	2.1	6.3	2.8
Vancomycin (10 μg/ml)	5.2	3.2	8.5	2.2
+ PG	5.8	4.4	10.2	1.8
+ AMDG	5.4	2.8	8.2	2.6
+ NaCl	3.1	1.2	4.2	3.6
+ PL	4.5	3.6	8.1	1.7
Penicillin (50 U/ml)	4.7	3.0	7.7	2.2
+ PL 0.042M	3.4	3.0	6.4	1.6
Mitomycin C(0.15 μg/ml)	5.5	2.9	8.4	2.6
+ PL 0.042M	3.5	2.3	5.8	2.1
Ultraviolet light	4.5	2.9	7.4	2.2
+ PL 0.042M	3.2	1.9	5.1	2.4
Cycloserine (1.7×10^{-5}M)	4.7	2.6	7.3	2.5
+ PL 0.042M	3.7	1.2	4.9	4.3

*Given as μg amino sugar per mg dry wt cells.
PG (propylene glycol 0.66M); AMDG (α- methyl-D-glucoside 0.50M); NaCl (sodium chloride 0.38M); PL (pantoyl lactone 0.042M).

Three additional parameters are reported in Table II. These also relate to possible cell membrane damage (protein and nucleic acid, resulting from leakage or lysis, and keto acid content of the growth medium). Pantoyl lactone in all cases significantly lowers the medium content of protein, nucleic acid, and keto acids while causing good cell division activity. Regarding osmotic protectors, nucleic acid release is lowered in all situations except when division activity is inhibited by ultraviolet light.

It is further seen (Table II) that increased growth and cell division activity can be correlated positively in all situations except where ultraviolet light is the division inhibitor or, in most instances, where sodium chloride is used for osmotic protection. However, at high levels of cycloserine or vancomycin wherein no growth will occur at 16 hours, addition of protective levels of sodium chloride allows for relatively good growth and excellent cell division activity.

Because osmotic protectors or pantoyl lactone restored active cell division, it was of interest to determine if this restoration could be correlated with increased synthesis of mucopeptide. In all instances, the amino sugar content of the cell wall continues to be lowered in the presence of the osmotic agents. (Table III)

Thus, our data reveal that osmotic protection allows cells to divide even though cell wall synthesis remains impaired. Under conditions of abnormal wall synthesis osmotic protection allows the invagination and separation phases of cell division to proceed, probably by preventing secondary membrane damage.

Although some effects of pantoyl lactone are similar to those of osmotic protective agents, pantoyl lactone is not acting osmotically. It is effective at a concentration one-tenth those of any of the osmotic agents; the latter are not active at such a low concentration.

We wish to thank Dr. J. T. Park for providing us with a sample of muramic acid.

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