Occurrence of teichurono-peptide in cell walls of group 2 alkaliphilic *Bacillus* spp.

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Cell walls were prepared from four strains belonging to group 2 alkaliphilic *Bacillus* spp. Non-peptidoglycan components were extracted, with trichloroacetic acid, from the cell wall preparations and isolated by DEAE-cellulose column chromatography. All the components were acidic, and were composed of amino acids and sugars. Several components with different compositions were detected. The cell walls commonly contained teichurono-peptide, composed of polyglucuronic acid and a polypeptide of acidic amino acids.

Introduction

Gram-positive bacteria possess cell walls composed of peptidoglycan and other specific polymers, such as proteins and polysaccharides. The cell walls of several alkaliphilic strains of the genus *Bacillus* also have specific polymers. These strains have been divided into three groups on the basis of the chemical composition of their walls and other physiological characteristics (Aono & Horikoshi, 1983). The walls of group 2 organisms contain large amounts of acidic amino acids and uronic acids. Although most strains in group 2 can grow at a neutral pH, the same acidic amino acids and uronic acids are found in much smaller quantities in the cell wall preparations when the bacteria are grown in this way. This indicates that the acidic components in the outermost cell layer of the group 2 bacteria might have a function in supporting growth at an alkaline pH.

The structural components of the cell wall of the alkaliphilic *Bacillus* strain C-125 have already been described. It contains A1 γ -peptidoglycan, teichuronic acid and teichurono-peptide (Aono *et al.*, 1984; Aono, 1985, 1989). The teichuronic acid is composed of galacturonic acid, glucuronic acid and *N*-acetylglucosamine (Aono & Uramoto, 1986); the teichurono-peptide is a complex of polyglutamic acid and polyglucuronic acid. Similar components are suspected to be present in other group 2 alkaliphilic *Bacillus* strains, as large

amounts of uronic acids and acidic amino acids have been found in their cell walls (Aono, 1987). However, no structural analysis of the cell walls of these bacteria has been reported.

This paper describes the isolation and partial characterization of the acidic structural components of the cell wall of group 2 alkaliphilic *Bacillus* strains.

Methods

Cultivation of organisms and preparation of cell walls. The alkaliphilic strains of *Bacillus* spp. used in this study, A-59, C-3, C-11 and Y-25 (Aono & Horikoshi, 1983), were grown aerobically at 30 °C in 6–18 litres of the following medium: (g per litre of deionized water): K₂HPO₄, 13.7; KH₂PO₄, 5.9; citric acid, 0.34; MgSO₄·7H₂O, 0.05; Na₂CO₃, 10.6; glucose, 5; peptone, 5; yeast extract, 0.5. The pH of this medium was about 10. Cells in the early stationary phase of growth (OD₆₆₀ 2.0) were harvested by centrifugation (8000 g, 10 min, 4 °C). The cells were resuspended in 2% (w/v) SDS/0.1 M-NaCl and incubated at 80 °C for 30 min to inactivate autolytic enzymes. The cells were recovered by centrifugation (8000 g, 20 min, 20 °C), washed with 0.1 M-NaCl/0.1% NaN₃ and stored at –20 °C.

Cell walls were prepared from the frozen cells as described previously (Aono & Horikoshi, 1983).

Isolation of the non-peptidoglycan components. Each cell wall preparation (2–3 g dry wt) was suspended in 200 ml 5% (w/v) trichloroacetic acid (TCA). The suspension was gently shaken at 30 °C for 10–12 h. Non-peptidoglycan components, solubilized with TCA, were recovered in the supernatant fluid obtained after centrifugation (20000 g, 20 min, 4 °C). The insoluble fraction of the cell wall preparation was extracted twice more with TCA. The extracts were then combined and thoroughly dialysed against running water (Spectra/Por 6 dialysis tubing, *M_r* cut-off 3500; Spectrum Medical Industries).

After the dialysis, a solution containing the non-diffusible material was diluted twofold with 100 mM-acetic acid/NaOH buffer (pH 5.0). The solution was loaded on a DE-52 DEAE-cellulose column

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Abbreviation: TFMS, trifluoromethanesulphonic acid.

(2.5 × 50 cm; Whatman) which had been equilibrated with 50 mM-acetic acid/NaOH buffer (pH 5.0). The column was washed with 500 ml of the buffer at a flow rate of 120 ml h⁻¹. The column was eluted, at the same rate, with 250 ml of the buffer containing 0.2 M-NaCl, followed by a linear gradient elution from 0.2 to 0.6 M-NaCl in the buffer (700 ml) at a flow rate of 60 ml h⁻¹. Fractions (15 ml) containing uronic acids and amino compounds were pooled, dialysed against deionized water and concentrated in a rotary evaporator at 43 °C.

The acidic polymers fractionated by DEAE-cellulose column chromatography, were applied on a column (2.5 × 100 cm) of Fractogel HW-55S (separation range M_r 1000–200000 for dextran; Merck) or 65S (M_r 10000–1000000 for dextran; Merck) which had been equilibrated with 0.2 M-NaCl. The column was eluted with 0.2 M-NaCl at a flow rate of 30 ml h⁻¹. Fractions (8.5 ml) containing uronic acids and amino compounds were pooled, dialysed against water and freeze-dried (Aono, 1985).

Trifluoromethanesulphonic acid (TFMS) treatment of the teichuronopeptides. The acidic polymer preparation (1 mg dry wt) was placed in a glass tube and dried *in vacuo* over solid NaOH at 43 °C. Methoxybenzene (0.1 ml) and cold TFMS (0.2 ml) (Wako Pure Chemical) were added to the dried sample (Edge *et al.*, 1981). Nitrogen gas was substituted for air in the vessel by flushing. The reaction mixture was gently shaken at 26 °C for 4.5 h and frozen overnight at -20 °C, after addition of 6 ml 10% (v/v) *n*-hexane in diethyl ether. The precipitate that appeared during the freezing process was recovered by centrifugation (2500 g, 10 min, room temperature), washed with *n*-hexane/diethyl ether (1:10, v/v) three times and given a final wash with diethyl ether. The precipitate was dried in air and dissolved in 0.2 M-NaH₂PO₄/NaOH buffer (pH 7.2). The solution was then dialysed (M_r cut-off 3500) against distilled water (Aono, 1987).

Hydrazinolysis of the teichuronopeptides. The sample (1 mg dry wt) was suspended in 0.4 ml anhydrous hydrazine (Takara Shuzo Co.) and heated at 100 °C for 3 h. After removal of the hydrazine by evaporation *in vacuo* over concentrated H₂SO₄ (Takasaki *et al.*, 1982), the residue was dissolved in 0.2 ml 50 mM-acetic acid/NaOH buffer (pH 5.0). The solution (70 µl) was loaded on a column (1.5 × 85 cm) of Sephadex G-15 equilibrated with the same buffer. The column was eluted with the same buffer, at a flow rate of 20 ml h⁻¹, and fractions (1 ml) were collected. Void elution volume fractions (total 3 ml) were combined and retained (Aono, 1989).

Analyses. (i) *Amino acids and amino sugars.* Samples were hydrolysed in 4 M-HCl in sealed tubes at 100 °C for 16 h. Amino compounds were determined by the ninhydrin method (Spies, 1957) with glutamic acid as a reference. Amino sugars were determined by the Elson-Morgan reaction with glucosamine as a reference (Spiro, 1966; Wheat, 1966). Where necessary, amino compounds were analysed with an amino acid analyser. L-Glutamic acid in the hydrolysate was determined with L-glutamate dehydrogenase (EC 1.4.1.3) and diaphorase (Beutler & Michal, 1974). L-Aspartic acid was determined with aspartate aminotransferase (EC 2.6.1.10) (Möllerling, 1985). The D-isomer content of the acidic amino acids was estimated by subtraction of the L-form acid content from the total content of glutamic acid or aspartic acid determined with the amino acid analyser. L-Alanine was determined with L-alanine dehydrogenase (EC 1.4.1.1) (Williamson, 1985); D-Alanine was determined with D-amino acid oxidase (EC 1.4.3.3) (Grassl & Supp, 1985). All the enzymes for the assays were purchased from Boehringer Mannheim.

(ii) *Uronic acids and neutral sugars.* Uronic acids were determined by the carbazole reagent method with glucuronic acid as a reference (Davidson, 1966; Spiro, 1966). Glucuronic acid was estimated with the thioglycolic acid/mannose/sulphuric acid reagent (Dische, 1947). Glucose was determined by the anthrone reagent method (Spiro, 1966).

(iii) *Sodium ions.* Sodium ions were determined with an atomic absorption spectrometer.

(iv) *Dry weight.* Dry weights of samples were measured by heating to constant weight at 43 °C *in vacuo* over NaOH.

(v) *M_r values.* M_r values were estimated for the samples by HPLC gel filtration on columns of Shodex WS802.5F (Showa Denko) (Aono, 1987). The column was eluted with 0.1 M-NaH₂PO₄/NaOH buffer (pH 7.2) at a flow rate of 1.0 ml min⁻¹. The elution pattern was monitored by measuring the differential refractive index. Pullulans (Shodex P-82; Showa Denko) were used as M_r standards to calibrate the system (Aono, 1987).

Thin-layer chromatography (TLC). Ascending Avicel cellulose thin-layer chromatograms were run in the following solvents: (A) ethyl acetate/pyridine/water/acetic acid (5:5:3:1, by vol.) at room temperature; (B) phenol/1% aqueous ammonia water (5:2, by vol.) at 43 °C. For the separation of amino acid isomers, samples were developed on a CHIR plate (Merck) in solvent (C) acetonitrile/methanol/water (4:1:1, by vol.), together with authentic isomers, at room temperature. The compounds were located with an alkaline silver nitrate (Trevelyan *et al.*, 1950) or ninhydrin (Stepka, 1957) spray.

Paper electrophoresis. Samples were spotted in the middle of a strip of Whatman no. 1 filter paper, 30 cm in length, and electrophoresed in 10% (v/v) acetic acid/pyridine buffer (pH 2.5) at 12.5 V cm⁻¹ for 2 h (Aono, 1985). The compounds were located as described above.

Results

Preparation of cell walls and extraction of acidic polymers

During the stationary phase of growth, the pH of the cultures decreased rapidly. The cells were harvested at the early stationary phase of growth to avoid exposure to the lowered pH. When the cells were harvested, the pH of the cultures was 9.1–9.5 (results not shown). Therefore, the cell walls described below should be representative of the alkaliphilic bacteria grown at alkaline pH.

Cell wall fractions were prepared from SDS-treated cells of four strains, A-59 (80 g wet wt), C-3 (78 g), C-11 (45 g) and Y-25 (58 g), and were found to contain 820, 510, 620 and 1240 µmol of uronic acids, respectively. TCA extraction solubilized 89–99% of the uronic acid residues originally found in each cell wall preparation. Most of the uronic acid was retained during thorough dialysis. Therefore, it appeared that these residues were present as constituents of some high- M_r polymers bound to peptidoglycan through acid-labile linkages.

Fractionation of non-peptidoglycan components

The non-diffusible materials obtained from the cell walls of strains C-3 and Y-25 were separated into two fractions by DEAE-cellulose column chromatography (Fig. 1*b, d*). One fraction (C-3-1 or Y-25-1) eluted from the column at 0.3–0.4 M-NaCl and the other (C-3-2 or Y-25-2) at 0.4–0.5 M-NaCl. All four fractions contained uronic acids and amino compounds as major components. The TCA extracts from strains A-59 and C-11 each yielded a single

Fig. 1. Elution profile of DEAE-cellulose chromatography of the non-dialysable materials of TCA extracts from strains (a) A-59, (b) C-3, (c) C-11 and (d) Y-25. Chromatography was carried out as described in Methods. Fractions (15 ml) were assayed for uronic acids (●), amino compounds (▲) and NaCl (□). The concentration of NaCl was determined from the refractive index of the fractions.

peak, again composed of uronic acids and amino compounds (Fig. 1a, c). These two fractions eluted at 0.4–0.5 M-NaCl from the column, and were designated A-59 and C-11.

These six fractions were further purified by gel chromatography. Each fraction yielded a single overlapping peak of uronic acids and amino compounds (results not shown). Thus, these six acidic polymers appeared to be almost purified by the DEAE-cellulose chromatography. The acidic polymer obtained by dialysis and lyophilization from each fraction was designated substance A-59, C-3-1, C-3-2, C-11, Y-25-1 or Y-25-2 respectively.

Chemical composition of the acidic polymers

The six substances obtained by ion-exchange and gel-filtration chromatography were hydrolysed with HCl under various conditions. The hydrolysates from 5 μ g of each substance were subjected to cellulose TLC mainly in solvent (A). Alkaline silver nitrate revealed several spots of R_F 0.65 (1), 0.35 (2), 0.32 (3), 0.12 (4) and 0.11 (5), as shown in Table 1. Spots 1 and 4 were obtained from all the samples except Y-25-1. Spots 2 and 5 were obtained only from Y-25-1. Spot 3 was produced only by C-3-1. Spot 2 also reacted with ninhydrin. Ninhydrin spray revealed further spots of R_F 0.12 (6), 0.08 (7), 0.07 (8), 0.06 (9) and 0.03 (10). Spots 6 and 7 were obtained only from C-3-1. Spot 8 occurred in all the hydrolysates except C-3-1. Spot 9 was obtained only from C-3-2. Spot 10 was produced by A-59, C-3-1 and C-3-2. Some fainter spots revealed by each spray were considered to be oligomers of the compounds.

The reducing compounds 1, 3, 4 and 5 corresponded to authentic glucuronolactone, glucose, glucuronic acid and galacturonic acid, respectively. These identifications were confirmed by cellulose TLC in solvent (B) and by paper electrophoresis. The ninhydrin-positive compounds, 6, 7, 8, 9 and 10, corresponded to alanine, serine, glutamic acid, glycine and aspartic acid. These identifications were also confirmed by paper electrophoresis and with the automatic amino acid analyser. Small amounts of serine and glycine were detected in A-59 and a small amount of serine was detected in Y-25-2 with the analyser. Compound 2 did not correspond to any commercially available authentic compounds on the basis of R_F values. This compound was thought to be an amino sugar because of its reducing ability and ninhydrin reactivity. This amino sugar was purified from the hydrolysate of a large amount of C-3-1, and identified as D-quinovosamine (Ito *et al.*, 1993).

Isomers of alanine, aspartic acid and glutamic acid were determined enzymically. Aspartate aminotransferase did not react on the aspartic acid from any substance under conditions which permitted the enzyme to react with authentic L-aspartic acid (Möllerling, 1985). Therefore, the aspartic acid was present in the D-form. Alanine found in C-3-1 was oxidized by L-alanine

Table 1. *Composition of acidic non-peptidoglycan components purified by DEAE-cellulose and gel chromatography*

The experimental details are described in Methods. Abbreviations: Ala, alanine; Asp, aspartic acid; Glu, glutamic acid; Gly, glycine; Ser, serine; Glc, glucose; GalN, galactosamine; FucN, fucosamine; QuiN, quinovosamine; GalU, galacturonic acid; GlcU, glucuronic acid. The number indicated under these abbreviations is the spot number of the respective compound detected by cellulose TLC and designated tentatively in this paper. A blank indicates not detected. Data on teichuronic acid (TUA) and teichurono-peptide (TUP) of C-125 are from Aono (1985).

Acidic polymer	Component [$\mu\text{mol (mg polymer)}^{-1}$]													M_r^*
	D-Asp 10	L-Glu 8	D-Glu 8	Gly 9	L-Ala 6	L-Ser 7	Glc 3	GalN	FucN	QuiN 2	GlcU 1, 4	GalU 5	Na	
A-59	1.46	1.75	1.45	0.07		0.09		0.20			0.88		3.1	21000
C-3-1	0.92				0.79	0.35	1.17	0.02			0.55		3.4	
C-3-2	1.45	1.48	1.44	1.39				0.09			0.81		4.7	21000
C-11		2.16	2.31					0.10			0.95		2.1	20000
Y-25-1		0.96						0.02		1.08		2.17	2.8	
Y-25-2		2.29	2.31			0.05		0.10			0.87		2.5	21000
C-125 TUA		0.004							1.3		1.6	1.5	2.8	
C-125 TUP		4.4						0.04			0.96		4.2	21000

* M_r estimated for each acidic polymer by gel chromatography.

Table 2. *Recoveries of components of macromolecules obtained by the deglycosylation with TFMS*

The teichurono-peptides (1 mg) were treated with TFMS at 26 °C for 4.5 h. The deglycosylated products were washed with 10% (v/v) *n*-hexane/diethyl ether and then dialysed against distilled water. The non-diffusible polypeptides were assayed for the constituents found in the original acidic polymers. Each determination is presented as μmol , with percentage recovery given in parentheses. The recovery was calculated taking the content in the original untreated polymer as 100%. See Table 1 for abbreviations. A blank indicates not detected.

Acidic polymer	Recovery [$\mu\text{mol (%)}$]				M_r^*
	Asp	Glu	Gly	GlcU	
A-59	0.87 (60)	2.0 (63)		0.015 (2)	13000
C-3-2	0.83 (57)	1.7 (58)	0.76 (55)	0.042 (5)	15000
C-11		3.0 (67)		0.024 (3)	12000
Y-25-2		3.3 (72)		0.026 (3)	12000
Polyglutamate†		6.1 μmol		0.015 μmol	14000

* M_r value of the polypeptide preparation.

† For polyglutamate, the results shown are μmol of each constituent per 1 mg of the product from the C-125 teichurono-peptide deglycosylated at 0 °C for 5 h, then at 22 °C for 2 h (Aono, 1987).

dehydrogenase but not by D-amino acid oxidase, indicating that the alanine was in the L-form (Grassl & Supp, 1985; Williamson, 1985). With the exception of C-3-1 and Y-25-1, the samples contained almost equimolar D- and L-glutamic acid. The serine found in C-3-1 was examined by chelating chromatography on the CHIR plate. The R_F value of the serine derived from C-3-1 (0.45) corresponded to authentic L-serine (R_F 0.46) but not to D-serine (R_F 0.42) in solvent (C).

These identifications for constituents of the acidic components are summarized in Table 1. The analyses accounted for a considerable part of the dry weight of each preparation, as follows: A-59, 87%; C-3-1, 59%; C-3-2, 93%; C-11, 85%; Y-25-1, 73%; Y-25-2, 84%. It

is probable that most of the components were identified and determined, except in the case of C-3-1 and Y-25-1.

Identification of teichurono-peptide among the acidic polymers

The above analyses did not indicate the chemical nature of the acidic polymers. The teichuronic acid of strain C-125 eluted at 0.29–0.35 M-NaCl and the teichurono-peptide at 0.40–0.48 M-NaCl from the column under the conditions also used in this study (Aono, 1985). All the highly acidic polymers which eluted from the DEAE-cellulose column at 0.4–0.5 M-NaCl (Fig. 1) were composed of acidic amino acids and glucuronic acid. Molar

Table 3. Recoveries of components of macromolecules obtained by hydrazinolysis

The samples (1 mg) were incubated in anhydrous hydrazine at 100 °C for 3 h. After removal of hydrazine, one-third of each product was fractionated by Sephadex G-15 gel chromatography. Macromolecules eluted at the void volume were assayed for glucuronic acid and amino compounds. The recovery of each compound was calculated taking the content in the original untreated polymer as 100%. Abbreviations: GlcU, glucuronic acid; TUP, teichuronopeptide; AC, total amino compounds.

Acidic polymer	Recovery (%)		M_r^*
	AC	GlcU	
A-59	8	17	5000
C-3-2	3	26	4300
C-11	8	18	4700
Y-25-2	13	28	5400
C-125 TUP	12	26	4500
Polyglucuronate†	0.26 µmol	5.1 µmol	4800

* M_r value of the macromolecule obtained from the teichuronopeptide by hydrazinolysis.

† For polyglucuronate, the results shown are µmol of each constituent per 1 mg of the product purified from the C-125 teichuronopeptide, treated with hydrazinolysis at 100 °C for 5 h, by gel and DEAE cellulose chromatography (Aono, 1989).

ratios of glucuronic acid to total acidic amino acids were 1:4.7–5.4. These ratios resembled that of 1:4.6 previously found in the teichuronopeptide of strain C-125 (Table 1). Furthermore, M_r values of these substances were similar to that of C-125 teichuronopeptide. These similarities suggested that the four substances (A-59, C-3-2, C-11 and Y-25-2) resembled the teichuronopeptide of strain C-125. However, the other polymers (C-3-1 and Y-25-1) that eluted at a lower concentration of NaCl did not appear to be teichuronic acid on the basis of their chemical composition.

Polypeptide moieties prepared by deglycosylation of teichuronopeptides with TFMS

The four polymers (A-59, C-3-2, C-11 and Y-25-2) were deglycosylated with TFMS. Macromolecular products were assayed for the residues found in the original polymers (Table 2); the proportions of amino acids were unchanged by this treatment. The molar ratio of aspartic acid:glutamic acid was 1:2.3 in the original A-59, and 1:2.1 in the polypeptide obtained after the TFMS treatment. The ratio of aspartic acid:glutamic acid:glycine was 1:2.1:0.92 in the original C-3-2 and 1:2.0:0.96 in the polypeptide. However, the content of glucuronic acid decreased to 0.5–2.0% of the acidic amino acids. This suggested that TFMS treatment released almost all the glucuronic acid residue from the original substances. The M_r values of the resulting polypeptides (12000–15000) were similar to that (14000) of the polyglutamate

prepared from the C-125 teichuronopeptide by the same method (Aono, 1987).

Release of polyglucuronic acid by hydrazinolysis of teichuronopeptide

The four substances were treated with anhydrous hydrazine. Macromolecular products were analysed (Table 3). In order to enhance recovery of the glucuronic acid moieties, the substances were treated with hydrazine for a short time. Therefore, cleavage of peptide bonds was not complete and the resulting products might have contained a proportion of peptides. These peptides are removable from the polyglutamic acid preparation by DEAE-cellulose chromatography (Aono, 1989). The M_r values (4300–5400) of the products were similar to that (4500) of the product prepared from the C-125 teichuronopeptide and to the size (4800) of the polyglucuronic acid previously prepared from the C-125 teichuronopeptide by hydrazinolysis (Aono, 1989).

Discussion

Teichuronic acid and teichuronopeptide were effectively extracted with TCA from the cell walls of the four strains belonging to the group 2 alkaliphilic *Bacillus* spp. The methods used in this study were previously developed for extraction of teichuronic acid and teichuronopeptide from the alkaliphilic *Bacillus* strain C-125 (Aono, 1985). The results indicated that the methods extracted non-peptidoglycan components from the cell walls of the group 2 strains. The acidic non-peptidoglycan components extracted with TCA were fractionated by DEAE-cellulose column chromatography. This method also seemed effective in separating acidic components (Fig. 1).

The acidic substances isolated from the organisms were divided into two groups on the basis of the NaCl concentration required to elute them from the DEAE-cellulose column (Fig. 1; Aono, 1985). The higher anionic substances, A-59, C-3-2, C-11, C-125-2 (teichuronopeptide) and Y-25-2, eluted at a higher concentration of NaCl, and were mainly composed of glucuronic acid and acidic amino acids. Molar ratios of glucuronic acid and amino acids were 1:4.6–5.4 (Table 1). M_r values of these substances were estimated as 20000–21000 by gel chromatography. These results suggested that these substances were similar to one another in their chemical structure.

TFMS has been used to deglycosylate several glycoproteins (Edge *et al.*, 1981). This method was applied to the anionic polymers in order to remove glucuronic acid residues. The TFMS treatment removed most of the glucuronic acid (Table 2). The residues appeared to be

intact polypeptides. M_r values of these products were 12000–15000. Hydrazinolysis has been used to prepare saccharide chains from glycoproteins (Takasaki *et al.*, 1982). M_r values of the products prepared from these anionic substances by hydrazinolysis ranged between 4300 and 5400 (Table 3).

If one polyglucuronate moiety and one polypeptide moiety were covalently bound in these substances, as in the C-125 teichuronopeptide, the M_r of sodium salts of the polypeptide or polyglucuronate moiety should be 15600–17200 or 3800–4400 on the basis of the chemical analyses shown in Table 1. These values are in close agreement with the experimental value of 12000–15000 obtained after the TFMS treatment, or 4300–5400 after the hydrazinolysis. M_r values of the polyglutamic acid and polyglucuronic acid prepared from C-125 teichuronopeptide were 14000 and 4800 (Aono, 1987, 1989). Thus, the substances can be concluded to be complex copolymers consisting of polypeptide and polyglucuronate moieties. Their chemical natures are similar to that of the C-125 teichuronopeptide.

The term 'teichuronopeptide' was previously proposed for a complex copolymer isolated from the cell walls of the alkaliphilic *Bacillus* strain C-125 and composed of poly-L-glutamate and polyglucuronate (Aono, 1989). Substances A-59, C-3-2, C-11 and Y-25-2 are variants of teichuronopeptide. Therefore, the results described here indicate that a type of teichuronopeptide is present in the cell walls of a range of group 2 alkaliphilic *Bacillus* spp.

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