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Decomposition of silicate minerals by *Bacillus mucilaginosus* in liquid culture

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Abstract

The extraction of K^+ and SiO_2 from silicate minerals by *Bacillus mucilaginosus* in liquid culture was studied in incubation experiments. *B. mucilaginosus* was found to dissolve soil minerals and mica and simultaneously release K^+ and SiO_2 from the crystal lattices. In contrast, the bacterium did not dissolve feldspar. *B. mucilaginosus* also produced organic acids and polysaccharides during growth. The polysaccharides strongly adsorbed the organic acids and attached to the surface of the mineral, resulting in an area of high concentration of organic acids near the mineral. The polysaccharides also adsorbed SiO_2 and this affected the equilibrium between the mineral and fluid phases and led to the reaction toward SiO_2 and K^+ solubilization. These two processes led to the decomposition of silicate minerals by the bacterium.

Introduction

Potassium is an essential macronutrient required by crops. The development of intensively managed agriculture has led to the consumption of increasing amounts of K and many parts of China now have soils that are K-deficient so that the low K supply has become the main yield limiting factor in agriculture. However, there are considerable amounts of insoluble K reserves in many soils, most of which exist in alumino-silicate minerals from which K cannot be absorbed directly by plants. *Bacillus mucilaginosus* has been applied as a biological K fertilizer in some countries for many years and there have been some reports that the bacterium can dissolve K from soil or minerals. For example, Lian (1998) reported the characteristics of a strain of *B. mucilaginosus* isolated from cornfield soil samples. Experiments showed that the bacterium increased the soluble content of K^+ in the culture medium. Vandevivere *et al.* (1994) proposed that *B. mucilaginosus* increases the dissolution rate of silicate and alumino-silicate minerals and releases the K^+ and SiO_2 from the crystal lattice primarily by generating organic acids. However, this hypothesis is controversial and *B. mucilaginosus* is also thought to accelerate the dissolution of a variety of silicates by the production of extracellular polysaccharides (EPS) (Berthelin & Belgy 1979; Malinovskaya *et al.* 1990; Welch *et al.* 1999). The dispute about the mechanism by which *B. mucilaginosus* decomposes silicate minerals and releases K^+ and SiO_2 may have severely limited the use of the organism in agriculture as a form of biological K fertilizer. In this paper we report a laboratory study including the use of the BET–N_2 absorption method to investigate the capacity of *B. mucilaginosus* to

dissolve three types of silicate minerals. We also studied the capsular polysaccharides and organic acids produced by the bacterium and the extent to which polysaccharides absorbed organic acids, K^+ and SiO_2 to elucidate the mechanism by which *B. mucilaginosus* decomposes silicate minerals.

Materials and methods

Bacterial strain and culture media

Bacillus mucilaginosus was obtained from the Chinese Academy of Agricultural Sciences. Agar slants (Institute of Soil Science 1985) were used to culture *B. mucilaginosus*. Two culture media were used to study the dissolution of silicate minerals. Medium A contained (g L^{-1}) sucrose 10.0, Na_2HPO_4 1.0, $(NH_4)_2SO_4$ 0.5, $MgSO_4{\cdot}7H_2O$ 1.0, $CaCO_3$ 1.0, yeast extract 0.2, $FeCl_3$ trace, silicate minerals 10.0; and Medium B contained (g L^{-1}) sucrose 10.0, Na_2HPO_4 1.0, $(NH_4)_2SO_4$ 0.5, $MgSO_4{\cdot}7H_2O$ 1.0, yeast extract 0.20, $FeCl_3$ trace, silicate minerals 10.0.

Minerals

Mica and feldspar were obtained from Nanjing Institute of Geology and Mineral Resources. The feldspar and mica sand were rinsed with deionized water in an ultrasonic bath about 50 times to remove fine particles and further washed with 0.1 mM HCl for several hours to remove fine and very reactive material from the mineral surface. This treatment should not significantly alter the surface chemistry of the minerals as the dissolution of the framework ions Si and Al is approximately stoichiometric at the acid pH (Welch & Ullman 1993). Soil minerals were obtained by boiling the soil with 2 mol L^{-1} HCl (soil: HCl = 1:10) for 20 min then rinsing with deionized water in an ultrasonic bath about 50 times to remove fine particles.

Experimental

Incubation

Incubations were carried out in 250-ml conical flasks containing 50 ml medium at 28 °C in an orbital shaker operating at 180 rev min^{-1}. Samples were taken 5 days after inoculation. There were three experimental treatments.

Analytical methods

Samples were analyzed for pH, Si, organic acids, viscosity and in some cases colony forming units (cfu) of *B. mucilaginosus*. The solution pH was determined with a pH meter. Biomass was determined by counting the colony-forming units. Viscosity measurements of the culture medium were performed in a thermostatic bath at 25 °C using an Ubbelohde capillary viscometer. The organic acids in the culture fluid were analyzed on a Waters Pico-Tag column and confirmed by UV-VIS diode array detector. Samples were passed through a 0.22-μm filter before analysis. The eluent was 0.01 mol/L $(NH_4)_2HPO_4$. The column was maintained at 30 °C. Oxalic, citric and lactic acids were used as external standards for quantitation. The detection wavelength was 210 nm and the reference wavelength was 450 nm. Separation of capsular polysaccharide from the culture fluid was carried out as follows. After bacterial growth, 1% (w/v) phenol was added to the cultures and the mixture was left for 1 h. Cells and precipitated proteins were removed by centrifugation (20 min, 6000 rev min^{-1}). An equal volume of acetone was added to the supernatant and left overnight in the cold (−20 °C). Then 50 mL pH 7.0 NaAc buffer was added and centrifuged (20 min, 6000 rev min^{-1}). Finally, the aqueous solutions of capsular polysaccharide were passed through an Acrocap filter (0.45 μm), dialysed against 0.1 mol L^{-1} $CaCl_2$ overnight and centrifuged. The precipitate was collected, washed twice with 90% (v/v) ethanol and freeze-dried. Infrared spectra of the polysaccharides were recorded with a Nicolet FT-IR Nexus 670 spectrometer in the range 4000–400 cm^{-1} using the KBr disk method. IR spectra of the solution capsular polysaccharides with and without 200 mg mL^{-1} SiO_2 were also recorded. To the culture fluid was added 4% (v/v) H_2O_2 and sterilized at 121 °C for 20 min to decompose the polysaccharides and release the ions absorbed by the polysaccharides, then centrifuged at 10,000×g for 20 min. The concentrations of K^+ in the supernatants were measured by atomic absorption spectrometry. Dissolved SiO_2 was measured by the molybdate blue method. Possible changes in mineral surface characteristics before and after treatment with *B. mucilaginosus* were monitored by low temperature BET–N_2 surface area measurements and Scanning Electron Microscopy (SEM). Samples were passed through a 0.22-μm

filter before analysis of dissolved constituents. Three replicate samples of each treatment were processed.

Adsorption of polysaccharides on SiO_2, K^+ and oxalic acid

Polysaccharide at a concentration of 8% was added to 200 and 500 mg mL^{-1} SiO_2, 50 and 250 mg mL^{-1} K^+, and 200 and 500 mg L^{-1} oxalic acid and the mixtures were stirred overnight and centrifuged at 4000 rev min^{-1} for 20 min. The concentrations of SiO_2, K^+ and oxalic acid in the supernatants were measured. The concentrations of SiO_2, K^+ and oxalic acid in water without polysaccharide were also measured as a control.

Results and discussion

Effect of B. mucilaginosus *on decomposition of feldspar and mica*

The effect of *B. mucilaginosus* on feldspar and mica dissolution in Medium A with mica or feldspar as the sole K source is shown in Table 1. The experiments indicated that there was substantial enhancement of K^+ release in the bacterial treatment in the culture fluid with mica as the sole K source and virtually no enhancement of K^+ release from feldspar. The discrepancy between the two minerals may be due to the differences in their crystal lattices. The structure of feldspar consists of cross-linked, 'double-crankshaft' chains (the *cc* chains) of Si^{4+} and Al^{3+} tetrahedra with charge-compensating such as Na^+, K^+, and Ca^{2+} occupying small cavities in the framework (Bu *et al.* 1998). In contrast, the structure of mica consists of discrete layers of crystal and the K^+ lies between the layers, making the release of K^+ easier from mica than from feldspar.

Table 1. Effect of *B. mucilaginosus* on feldspar and mica dissolution.

Culture	Mica K^+ (mg L^{-1})	Feldspar K^+ (mg L^{-1})
Inoculation	44.5 ± 1.21	51.8 ± 0.66
Control	26.8 ± 0.72	51.6 ± 0.50

Effect of B. mucilaginosus *on decomposition of soil minerals*

Table 2 shows that the concentrations of K^+ and SiO_2 did not increase markedly in Medium A or Medium B compared with the control. However, more K^+ and SiO_2 were released in Medium A than in Medium B and scanning electron micrographs of the surfaces of both fresh and reacted soil minerals in Medium A (Figure 1) show that the reacted mineral surfaces exhibited great variation in surface features. The surfaces of reacted minerals displayed evidence of aggressive mineral dissolution including etch pits and dissolution craters (Figure 1). Furthermore, Table 3 indicates that the surface area per unit weight of leached minerals in Medium A was 266% of that in the control and the average pore diameter was 114% of the control value.

From these results we can conclude that *B. mucilaginosus* led to partial degradation of soil minerals, resulting in release of K^+ and SiO_2, and the degree of degradation was greater in Medium A than Medium B. The viscosity, pH and number of colony-forming units in the two media are compared in Table 4. There was a clear correlation between the capacity for mineral dissolution and the viscosity of the culture liquid. The viscosity of the medium was correlated with the quantity of exopolysaccharides of *B. mucilaginosus* produced and we can infer that polysaccharides of *B. mucilaginosus* may play an important role in the degradation of the silicate minerals.

Organic acids in the culture fluid

It can be seen in Figure 2 that the bacterium produced copious amounts of organic acids in the

Table 2. Effect of *B. mucilaginosus* on dissolution of soil minerals.

Culture	Medium A		Medium B	
	K^+ (mg L^{-1})	SiO_2 (mg L^{-1})	K^+ (mg L^{-1})	SiO_2 (mg L^{-1})
Inoculation	13.4 ± 0.59	216.2 ± 5.29	12.0 ± 0.13	285.8 ± 15.83
Control	12.2 ± 0.18	198.8 ± 2.22	11.6 ± 0.37	225.2 ± 9.05

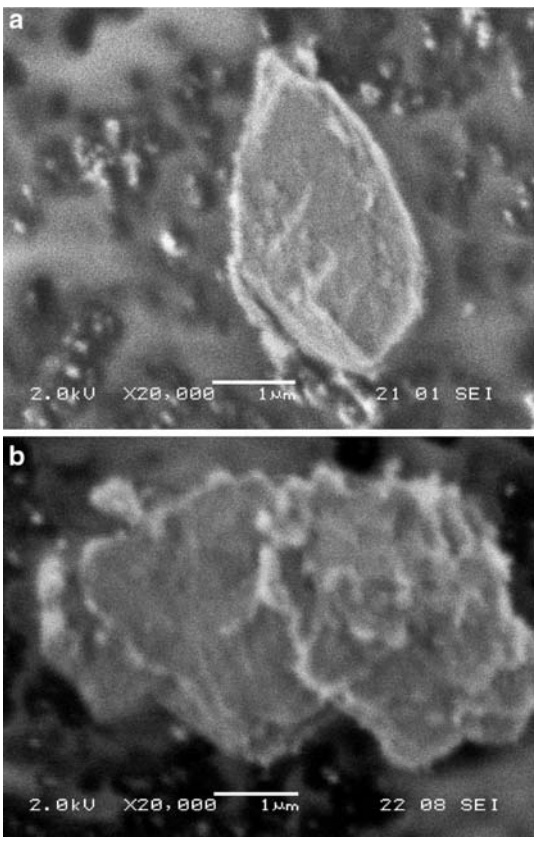

Fig. 1. SEM of the soil minerals (a) before and (b) after bacterial leaching.

culture fluid. The mean concentrations of oxalic, citric and acid lactic acids produced were 76.7, 188 and 124 mg L^{-1}, respectively.

Table 3. Effect of *B. mucilaginosus* on surface area and average pore diameter of soil minerals.

Culture	Surface area (m^2 g^{-1})	Average pore diameter (nm)
Inoculation	3.84 ± 0.19	9.06 ± 0.21
Control	1.44 ± 0.09	7.92 ± 0.06

Table 4. Viscosity and pH of the culture liquid and density of bacteria (colony-forming units: cfu) after growth for 5 days.

Culture medium	Viscosity (m^2 s^{-2})	pH	cfu (10^8)
Medium A	57.54	5.96	1.85
Medium B	5.96	5.73	3.15

Adsorption of bacterial polysaccharides on the K^+, SiO_2 and organic acids

Organic acid was added to the culture fluid to give added concentrations of 100 and 300 mg K^+ L^{-1}, 200 and 500 mg SiO_2 L^{-1}, and 200 and 500 mg L^{-1}, and left to stand for 24 h. The fluids were centrifuged at 6000 rev min^{-1} for 20 min and the concentrations of K^+, SiO_2 and oxalic acid in the supernatant were measured. The results (Tables 4 and 5) show that the culture fluid had strongly adsorbed the oxalic acid and SiO_2 but there was almost no adsorption of K^+.

An analogous process has been found in a number of bacteria as reported, for example, by Mera and Beveridge (1993) who investigated the chemical mechanism of silicate binding to the surface of *Bacillus subtilis* and found that cell wall carboxylates were chemically modified by the addition of an ethylenediamine ligand to reverse their charge. The binding of silicate to the bacterial surfaces can thus be described as outer sphere complex formation because it occurs through electrostatic interaction.

Structure of capsular polysaccharide and interaction with SiO_2

Figure 3 shows the IR spectra of the polysaccharide fractions. The regions 1593.63 cm^{-1} and 3396 cm^{-1} exhibited the obvious characteristic absorption of C=O and –OH of carboxyl groups and this indicates that the polysaccharide includes carboxyl. The infrared spectroscopy of the solution capsular polysaccharide with and without 200 mg SiO_2 mL^{-1} is shown in Figures 4 and 5. From the infrared spectroscopy we can see that there is stronger adsorption at 1076 $cm^{-1}$and 1045 cm^{-1} in Figure 5 than in Figure 4, indicating that the interaction of polysaccharide with SiO_2 involves hydrogen bonds in the adsorption process.

Discussion

The mechanism by which *B. mucilaginosus* releases K^+ or other elements from silicate minerals are complicated. Yakhontova *et al.* (1987) proposed that the intensity of degradation of silicate minerals by the bacterium was dependent on the structure and chemical composition of the mineral.

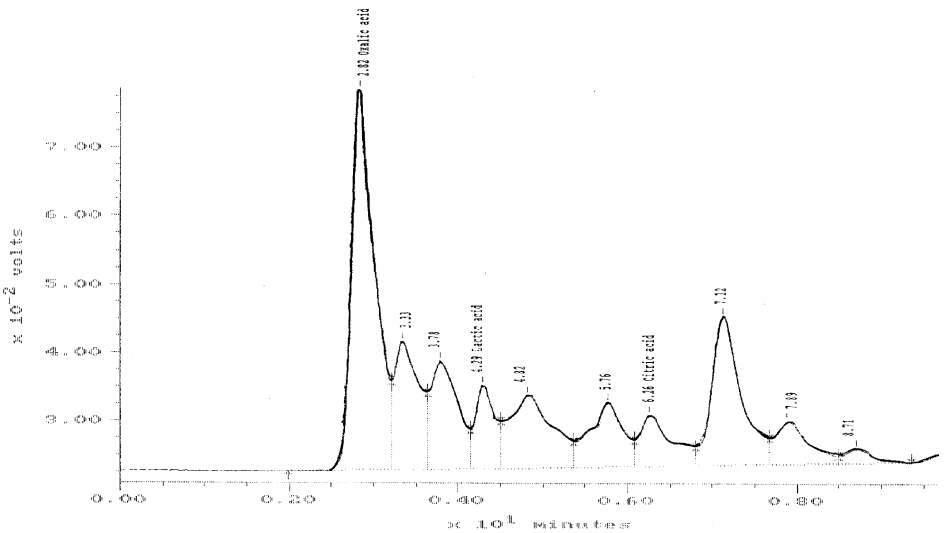

Fig. 2. HPLC of the organic acids in the culture fluid.

Grudev (1987) conducted an experiment that indicated that K^+ can be released from silicate minerals and he proposed that the formation of mucilaginous capsules consisting of exopolysaccharides by the bacteria enhanced mineral dissolution. Vainberg *et al.* (1980) also proposed that dissolution of minerals was caused by the formation of organic acids in the culture media. However, there is a paucity of experimental evidence to support these hypotheses. The present study indicates that leaching of K^+ and SiO_2 from silicate minerals by *B. mucilaginosus* occurs as a result of the participation of both exopolysaccharides and organic acids. *B. mucilaginosus* produces organic acids especially those acids such as oxalate and citrate that can form bidentate complexes with metal ions and which tend to be more effective in enhancing dissolution than monodentate ligands, such as those formed by acetate or propionate (Welch & Ullman 1993) in the process of bacterial reproduction. At the same time the bacterium produces polysaccharides and these can combine with the minerals and form bacterial–mineral complexes. The polysaccharides strongly adsorb the organic acids and an area of high concentration of organic acids is formed near the minerals. Under the effects of organic acids the minerals are partially degraded. On the other hand, the polysaccharides also absorb SiO_2. The resulting alteration of the concentration of SiO_2 will affect the equilibrium between the mineral and fluid phases, leading to a reaction toward SiO_2 and K^+ solubilization, which finally leads to further degradation of the minerals.

Table 5. Absorption of K^+ by the culture medium, of SiO_2 by bacterial polysaccharides, and of oxalic acid by the culture medium.

Added K^+ (mg mL^{-1})	Absorbed K^+(mg mL^{-1})	Absorption rate (%)
100	1.52 ± 0.15	1.52
300	3.73 ± 0.31	1.24
Added SiO_2 (mg mL^{-1})	Absorbed SiO_2 (mg mL^{-1})	Absorption rate (%)
200	130.0 ± 1.05	65.0
500	313.4 ± 9.76	62.7
Added oxalic acid (mg mL^{-1})	Absorbed oxalic acid (mg $mL^{-1)}$	Absorption rate (%)
200	188.0 ± 9.96	94.4
500	483.6 ± 11.12	96.7

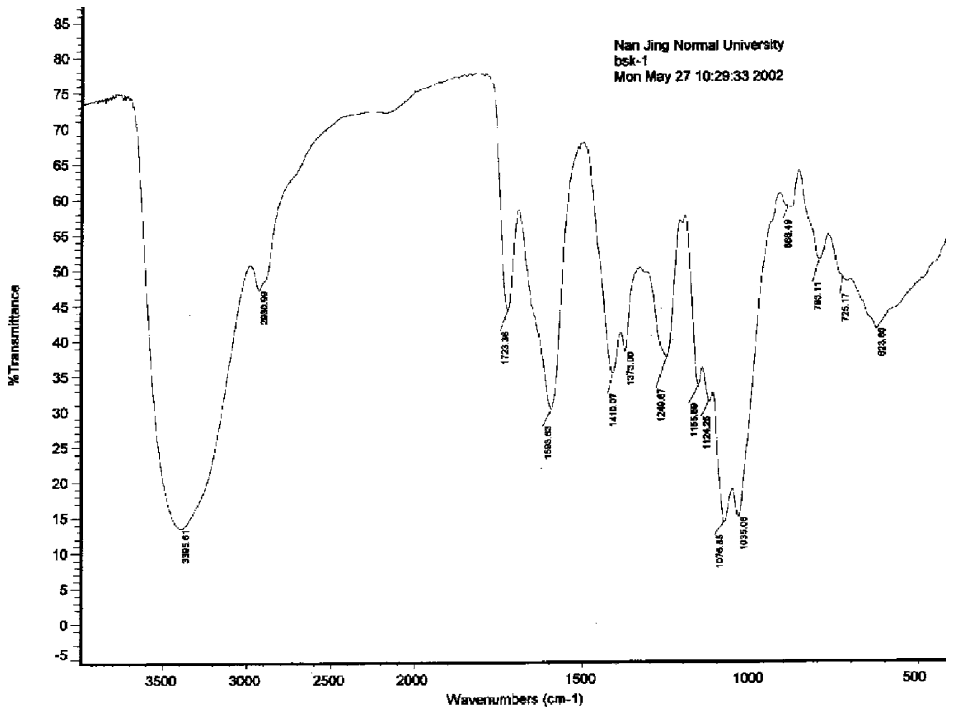

Fig. 3. IR spectra of polysaccharides.

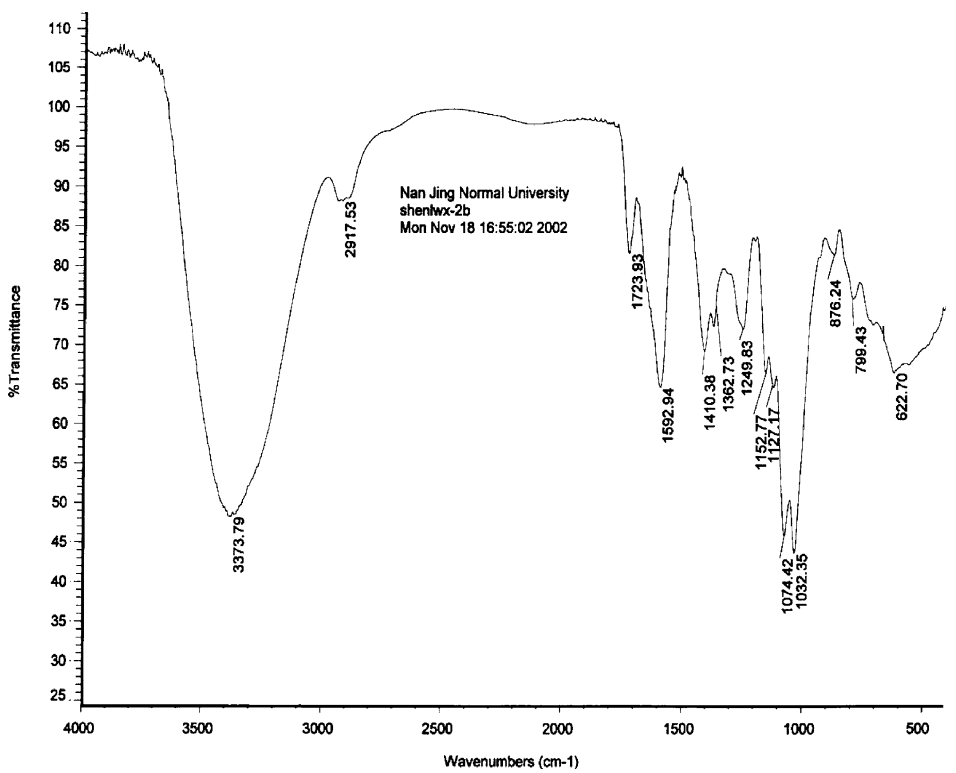

Fig. 4. IR spectra of the solution of polysaccharides.

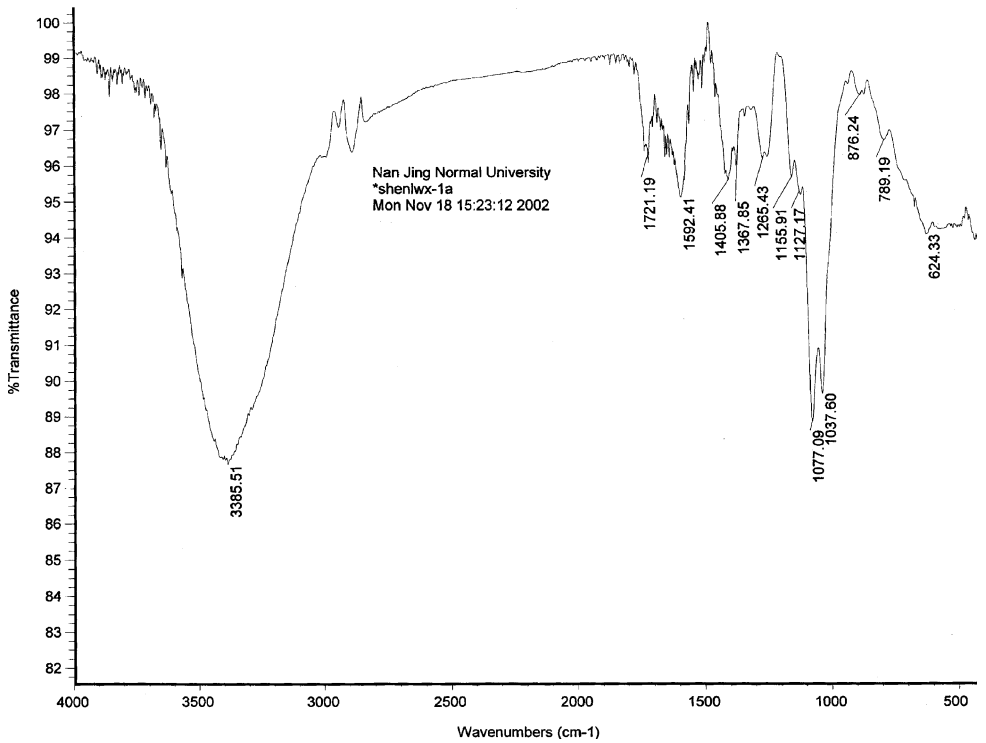

Fig. 5. IR spectra of the solution of polysaccharides after SiO_2 absorption.

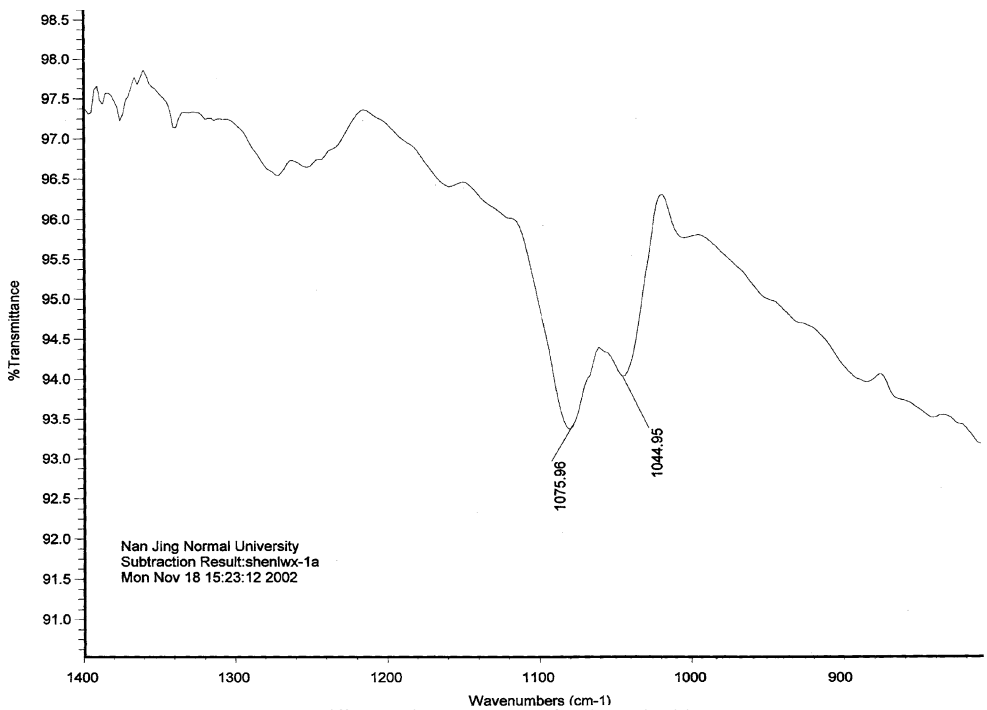

Fig. 6. Difference in IR spectra of polysaccharides.

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