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EXPERIMENTAL ARTICLES

Dependence of the Conversion of Chlorophenols by Rhodococci on the Number and Position of Chlorine Atoms in the Aromatic Ring

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Abstract—Study of the conversion of chlorophenols by *Rhodococcus opacus* 1G, *R. rhodnii* 135, *R. rhodochrous* 89, and *R. opacus* 1cp disclosed the dependence of the conversion rate and pathway on the number and position of chlorine atoms in the aromatic ring. The most active chlorophenol converter, strain *R. opacus* 1cp, grew on each of the three isomeric monochlorophenols and on 2,4-dichlorophenol; the rate of growth decreased from 4-chlorophenol to 3-chlorophenol and then to 2-chlorophenol. The parameters of growth on 2,4-dichlorophenol were the same as on 3-chlorophenol. None of the strains studied utilized trichlorophenols. A detailed study of the pathway of chlorophenol transformation showed that 3-chloro-, 4-chloro-, and 2,4-dichlorophenol were utilized by the strains via a modified *ortho*-pathway. 2-Chlorophenol and 2,3-dichlorophenol were transformed by strains *R. opacus* 1cp and *R. rhodochrous* 89 via corresponding 3-chloro- and 3,4-dichlorocatechols, which were then hydroxylated with the formation of 4-chloropyrogallol and 4,5-dichloropyrogallol; this route had not previously been described in bacteria. Phenol hydroxylase of *R. opacus* 1G exhibited a previously undescribed catalytic pattern, catalyzing oxidative dehalogenation of 2,3,5-trichlorophenol with the formation of 3,5-dichlorocatechol but not hydroxylation of the nonsubstituted position 6.

Key words: biodegradation, chlorophenols rhodococci, bioconversion pathways, dehalogenation.

Halogenated phenols are widely known pollutants of the soil and water. They are wastes of the chemical, pharmaceutical, and petroleum industries, and many chlorinated phenols are used as fungicides and disinfectants. In natural environments, halogenated phenols usually occur as a mixture of isomers that are susceptible to different extents to microbial attacks. For example, *para*-substituted halophenols are degraded more readily than *meta*- and, especially, *ortho*-substituted ones; polyhalogenated phenols are less susceptible to microbial attack than monohalogenated phenols. Fluorophenols were reported to be attacked more easily than chlorophenols [1, 2]. It is known that the position and nature of the halogen atom affect not only the rate of decomposition but also its pathway [3, 4], resulting in the formation of various intermediates that are sometimes difficult to predict and can be more toxic and stable than original halophenols [5].

The aim of the present work was to investigate the conversion of various substituted chlorophenols by rhodococci, which were chosen as the subject of investigation due to their abundance in natural environments, their resistance to stress impacts, and their ability to attack a broad range of xenobiotics [6]. The final goal of this work is to develop methods for the bioremediation of soils and waters contaminated with chlorophenols; therefore, the determination of the degradative potential of rhodococci, who belong to the main candidates for the role of chlorophenol biodegraders, seems expedient.

MATERIALS AND METHODS

Phenol from Reakhim (Russia), 2-chloro-, 3-chloro-, 4-chlorophenols, 2,3-dichloro-, 2,4-dichloro-, and 3,4-dichlorophenols from Merck (Germany), and 2,3,5- and 2,4,5-trichlorophenols from Fluka (Switzerland) were used.

The conversion of chlorophenols by 4 rhodococcal strains (*Rhodococcus opacus* 1G, *R. rhodnii* 135, *R. rhodochrous* 89, and *R. opacus* 1cp) was studied. We have already described the isolation of these strains [7, 8].

The capacity for growth on chlorophenols was studied using agarized and liquid media of the same composition of the mineral base (g/l): KH_2PO_4, 1.0; K_2HPO_4, 1.0; NH_4NO_3, 1.0; $MgSO_4 \cdot 7H_2O$, 0.7; $CaCl_2$, 0.02; and saturated solution of $FeCl_3$, 2 drops (pH 7.0–7.2). Chlorophenols were added in portions (40–120 mg/l); trisubstituted chlorophenols were also added in por-

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Table 1. Growth of rhodococci on chlorophenols (estimated according to the five-point system)

Microorganisms	Growth substrates					
	2-chlorophenol	3-chlorophenol	4-chlorophenol	2,3-dichlorophenol	2,4-dichlorophenol	phenol
R. opacus 1G	2	1	–	–	–	5
R. rhodni 135	–	–	–	–	–	5
R. rhodochrous 89	1	1	–	2	–	4
R. opacus 1cp	2	3	5	–	3	5

Note: The sign "–" means absence of growth. None of the strains grew on 2,3,5-trichlorophenol or 2,4,6-trichlorophenol.

tions (40 mg/l). The transformation of the halophenols that were not utilized as the single source of carbon was studied under conditions of cooxidation in the presence of sodium acetate or phenol as growth substrates (2.0 and 0.4 g/l, respectively). Chlorophenols were added in concentrations of 50–150 mg/l.

Analysis of the culture liquid for the presence of intermediates was carried out after 6, 12, and 24 h of cultivation. After centrifugation (8 min, 16000 g, 4°C) the liquid was acidified to pH 2.0, extracted three times with ethyl acetate, and analyzed on DC-Plastikofolien Kieselgel 60F254 plates (Merck, Germany). Plates were developed with a benzene–dioxane–acetic acid (90 : 10 : 2) mixture sprayed with diazotized benzidine and an $AgNO_3$ solution in acetone, and examined under UV light.

After purification, the intermediates were analyzed using a Finnigan MAT-8430 mass spectrometer at an ionizing energy of 70 eV (direct evaporation of the sample); identification of the compounds was performed by comparison with standards and published data [9].

HPLC was carried out on a Spherisorb ODS-2.5 column (250 × 4.5 mm); elution was performed isocratically at a flow rate of 0.8 ml/min using 60% methanol containing 5 mM KH_2PO_4 and acidified with fuming H_2SO_4 to pH 2.0. Detection was carried out at 280 and 298 nm using an LKB Instrument (Bromma Inc.) with a photodiode detector. Peaks of metabolites were identified by comparison with reference compounds.

^1H NMR spectra were recorded on a Bruker AMX–500 spectrometer using deuterated ethyl acetate with tetramethylsilane as an internal standard.

RESULTS

Strains *R. opacus* 1cp, *R. opacus* 1G, *R. rhodnii* 135, and *R. rhodochrous* 89 were tested for the capacity to grow on isomeric monochlorophenols, 2,3-, 2,4-, and 3,4-dichlorophenols, and 2,3,5- and 2,4,5-trichlorophenols; in all, on eight different chlorophenols. Phenol was taken as a nonsubstituted compound. Table 1 and Fig. 1 show data on the growth of rhodococci on agarized and liquid media with chlorinated phenols. From these data it follows that all rhodococci studied showed good growth on phenol. *R. opacus* 1cp could utilize any of the monochlophenols as the sole source of carbon. *R. opacus* 1G grew on agarized medium with 2-chloro- or 3-chlorophenol, but in liquid medium, growth was poor. *R. rhodochrous* 89 grew on agarized medium with 2-chlorophenol but did not grow in liquid medium. *R. rhodnii* 135 utilized none of the monochlorophenols. Among disubstituted chlorophenols, 2,4-dichlorophenol supported the growth of *R. opacus* 1cp on agarized and liquid media, and 2,3-dichlorophenol supported the growth of *R. rhodochrous* 89. Trisubstituted chlorophenols did not support the growth of any of the strains studied; however, they were transformed by rhodococci, both when present as a sole source of carbon and under conditions of cometabolism.

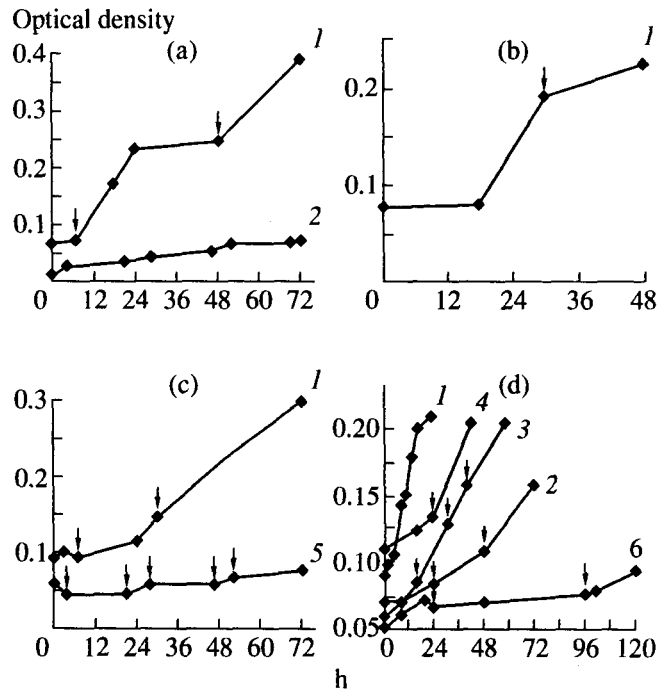


Fig. 1. Curves of growth of (a) *R. opacus* 1G, (b) *R. rhodnii* 135, (c) *R. rhodochrous* 89, and (d) *R. opacus* 1cp on (*1*) phenol, (*2*) 2-chlorophenol, (*3*) 3-chlorophenol, (*4*) 4-chlorophenol, (*5*) 2,3-dichlorophenol, and (*6*) 2,4-dichlorophenol.

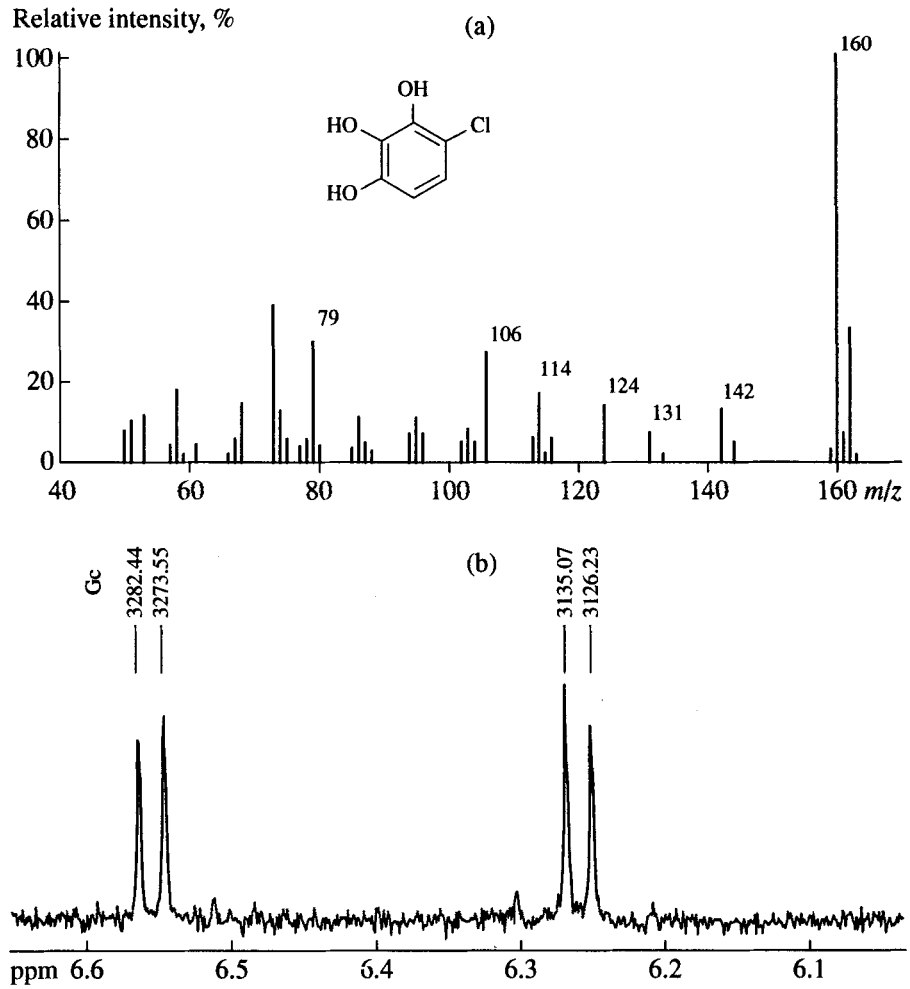


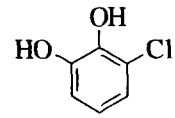
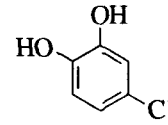
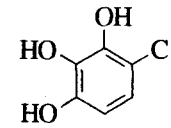
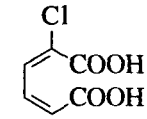
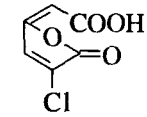
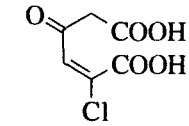
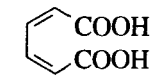
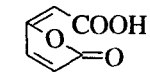
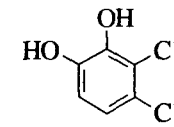
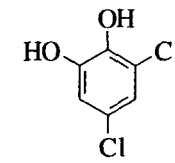
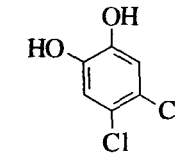
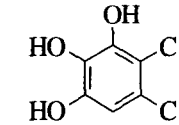
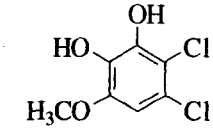
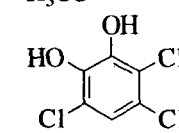
Fig. 2. (a) Mass and (b) NMR spectra of 1,2,3-trihydroxy-4-chlorobenzene (4-chloropyrogallol).

Degradation Pathways of Monosubstituted Chlorophenols

Analysis of the products of monochlorophenol degradation performed using thin-layer chromatography and mass spectrometry revealed a number of intermediates that are listed in Table 2. It should be noted that, in a number of cases when halophenols were utilized very actively, experiments with washed cells had to be carried out for the detection and isolation of intermediates.

Analysis of the conversion products of isomeric chlorophenols showed that 2-chlorophenol was utilized by rhodococci via 3-chlorocatechol as the basic intermediate (Table 2); the formation of 2-chloromuconic acid was also revealed. A new intermediate yielding a positive reaction for phenol hydroxyl was detected in the culture liquid. Due to its rapid disappearance from the culture liquid during growth with 2-chlorophenol, this compound had to be isolated in experiments with washed cells induced with 2-chlorophenol. The isolated compound exhibited an R_f value of 0.25 and an absorption maximum at 270 nm. The mass spectrum shown in Fig. 2a and the high-resolution mass spectrum point to the mass of molecular ion of the 159.9929, which corresponds to the gross-formula $C_6H_5O_3Cl$ and indicates the introduction of a third hydroxyl group into the aromatic ring. After long-term incubation, the compound disappears from the culture liquid. The ^1H NMR spectrum recorded in deuterated acetone showed two doublets at the aromatic area (6.55 ppm) with a ^3JH-H coupling constant of 9.25 Hz (Fig. 2b). These data testify to the existence of two hydrogen atoms in the *ortho*-position of the aromatic ring of the trihydroxylated derivative. In principle, according to NMR data, the formation of two trihydroxylated chlorobenzene isomers—1,2,3-trihydroxy-4-chlorobenzene and 1,2,4-trihydroxy-3-chlorobenzene—is possible. Chemical shifts predicted for the ^1H resonance of these two isomers, relative to tetramethylsilane and according to tables of chemical substitution, are 6.55 and 6.05 ppm for two hydrogen atoms of 1,2,3-trihydroxy-4-chlorobenzene and 6.31 and 6.08 ppm for two hydrogen atoms of 1,2,4-trihydroxy-3-chlorobenzene. The fact that the values actually measured were close to the values calculated for 1,2,3-trihydroxy-

Table 2. Mass spectrometric analysis of the products of degradation of chlorophenols by rhodococci

Compound	Gross formula from HR MS	Main characteristic peaks, *m/z* (%)	Structure
3-Chlorocatechol	–	M^+144(100), 146(34.0), 126(9.0), 128(3.0), 115(7.0), 117(2.0), 98(27.0), 100(9.0), 81(15.0), 63(33.0), 51(19.0)	
4-Chlorocatechol	–	M^+144(100), 146(34.0), 115(5.0), 108(34.0), 98(5.0), 80(44.0), 63(18.0), 52(45.0), 51(28.0)	
4-Chloropyrogallol	$C_6H_5O_3Cl$ 159.9929	M^+160(100), 162(32.3), 142(12.8), 144(4.6), 131(6.5), 133(1.7), 124(12.6), 114(17.0), 116(5.4), 106(26.7), 79(30.1)	
2-Chloromuconic acid	–	M^+176(4.1), 158(3.8), 141(100), 133(18.9), 132(18.8), 131(57.1), 130(47.4), 117(18.1), 95(30)	
2-Chloro-4-carboxymethylene-nebut-2-enolide	$C_6H_3O_4Cl$ 173.9721 $C_5H_4O_3Cl$ 146.9880	M^+174(45.6), 176(13.0), 157(9.9), 159(2.4), 147(100), 149(43.2), 139(21.3), 131(25.9), 130(12.4), 119(18.8), 118(12.2)	
2-Chloromaleyl acetic acid	$C_5H_5O_3Cl$ 147.9894 $C_4H_2O_3Cl$ 132.9687	M^+(–), 148(6.0), 150(2.0), 133(100), 135(35.0), 105(18.0), 104(14.0), 89(39.0), 91(13.0), 69(45.0), 53(50.0), 44(95.0)	
Cis,cis-muconic acid	–	M^+142(2.9), 124(2.9), 97(100), 79(11.1), 69(19.7), 51(14.1), 45(8.9)	
4-Carboxymethylenebut-2-enolide	$C_6H_4O_4$ 140.0111	M^+140(100), 123(12.5), 112(75.9), 95(53.5), 84(89.7), 71(28.7), 69(84.5), 54(68.1)	
3,5-Dichlorocatechol	–	M^+178(100), 180(66.6), 142(31.7), 144(10.5), 114(35.3), 116(12.2), 97(10.4), 86(13.6), 79(30.2)	
3,5-Dichlorocatechol	–	M^+178(100), 180(65.8), 142(15.5), 132(4.1), 114(38.8), 116(12.9), 97(12.3), 86(19.4), 79(20.9)	
4,5-Dichlorocatechol	–	M^+178(100), 180(64.7), 149(6.0), 151(3.0), 132(17.9), 134(10.4), 115(20.4), 117(4.1), 97(42.9), 99(10.6)	
4,5-Dichloropyrogallol	$C_6H_4O_3Cl_2$ 193.9545	M^+194(100), 196(64.9), 176(2.3), 165(5.0), 158(11.2), 130(7.2), 113(16.9), 102(15.0)	
6-Methoxy-3,4-dichlorocatechol	$C_7H_6O_3Cl_2$ 207.9695	M^+208(88.1), 210(57.0), 193(100), 195(63.3), 165(60.8), 167(46.1), 129(26.8), 101(21.6)	
3,4,6-Trichlorocatechol	–	M^+212(10.0), 214(9.6), 216(3.1)	

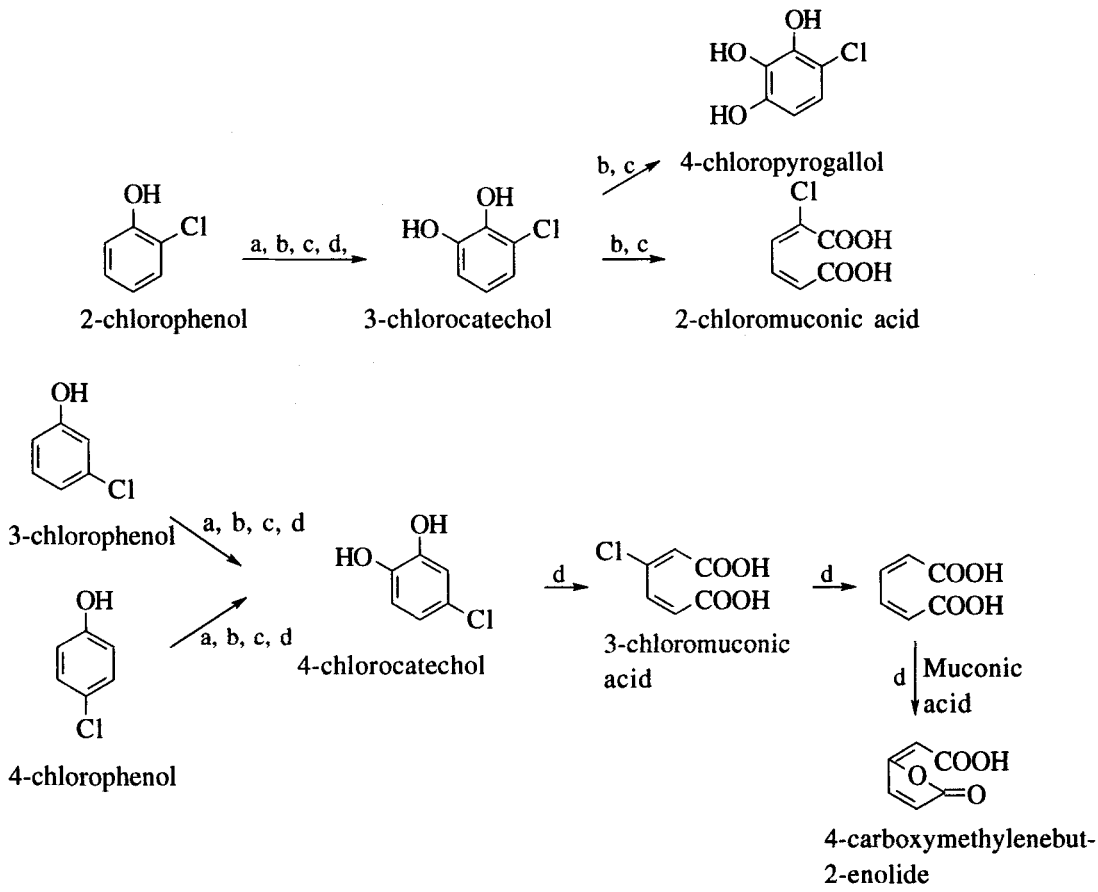


Fig. 3. Scheme of conversion of monochlorophenols by (a) *R. opacus* 1G, (b) *R. rhodnii* 135, (c) *R. rhodochrous* 89, and (d) *R. opacus* 1cp.

4-chlorobenzene shows that the third hydroxyl group of the compound under consideration is in the *ortho*-position relative to the two other hydroxyl groups and in the *para*-position relative to the chlorosubstituent. The product revealed was formed in the conversion of 2-chlorophenol by *R. opacus* 1cp and *R. rhodochrous* 89. *R. rhodnii* 135 accumulated 2-chloromuconate in the reaction mixture (Table 2). Conversion of 2-chlorophenol by *Rhodococcus opacus* 1G was restricted to the transformation of the latter to 3-chlorocatechol.

3-Chlorophenol and 4-chlorophenol were converted by all of the strains investigated to the corresponding chlorocatechol (Table 2). Only strain *R. opacus* 1cp brought about the complete conversion of both chlorophenols via the modified *ortho* pathway involving 3-chloromuconate, dienolactone, chloromaleyl acetate, etc., as intermediates (Table 2). Other strains did not realize the process beyond the formation of 4-chlorocatechol. Thus, the conversion of monochlorophenols by rhodococci may be schematically presented as follows (Fig. 3). The analysis of the data obtained showed that the conversion of monochlorophenols depended on the position of the chlorosubstituent in the aromatic ring. Thus, the conversion rate of isomeric chlorophenols by *R. opacus* 1cp decreased from 4-chlorophenol to 3-chlorophenol and then to 2-chlorophenol, although 2-chlorophenol was attacked by a larger number of the rhodococci studied. Only 2-chlorophenol was subject to subsequent hydroxylation (by *R. opacus* 1cp and *R. rhodochrous* 89) with the formation of chloropyrogallol.

Conversion Pathways of Isomeric Dichlorophenols

Two of the rhodococcal strains studied could use dichlorophenols as a single source of carbon (Table 1, Fig. 1): *R. opacus* 1cp utilized 2,4-dichlorophenol on agarized and liquid media; *R. rhodochrous* 89 utilized 2,3-dichlorophenol. All of the strains studied converted dichlorophenols under cometabolic conditions. When grown on medium with 2,4-dichlorophenol, *R. opacus* 1cp produced 3,5-dichlorocatechol (Table 2), which was then cleaved with the formation of dichloromuconate; the latter compound was converted via the modified *ortho*-pathway, as we have already shown [7]. Under conditions of cometabolism, strain *R. opacus* 1cp converted 2,3-dichlorophenol to 3,4-dichlorocatechol and to a new, previously unknown product. The third isomer, 3,4-dichlorophenol, was also converted by this strain to 4,5-dichlorocatechol; however, in this case, no

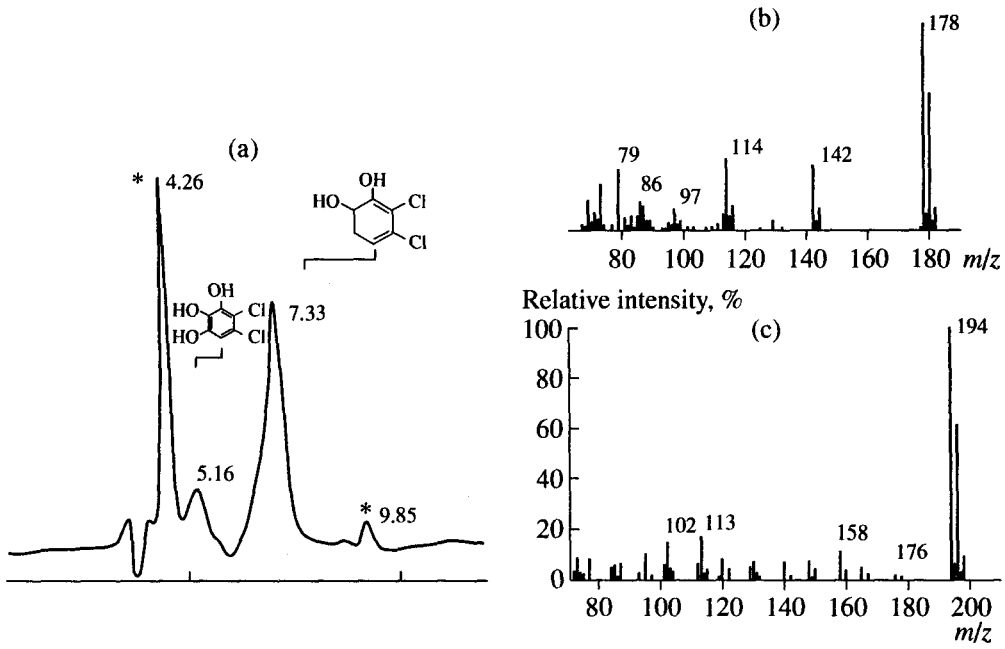


Fig. 4. (a) HPLC elution profile of the ethyl acetate extract of an incubation mixture of *R. rhodochrous* 89 cells with 2,3-dichlorophenol (incubation for 7 h). (b) Mass spectrum of the compound having a retention time of 7.33 min and identified as 3,4-dichlorocatechol. (c) Mass spectrum of the compound having a retention time of 5.16 min and identified as 1,2,3-trihydroxy-4,5-dichlorobenzene. The peaks marked with asterisks were already present in the ethyl acetate extract of the mixture before incubation.

ring cleavage with the formation of chloromuconic acids occurred.

R. opacus 1G converted 2,3-dichloro- and 2,4-dichlorophenols to the corresponding 3,4-dichloro- and 3,5-dichloropyrocatechols. *R. rhodnii* 135 converted 2,3-dichlorophenol to 3,4-dichlorocatechol, and 2,4-dichlorophenol was converted by this strain to 3,5-dichlorocatechol. Corresponding monochlorodienolactone and chloromaleyl acetate were also found in the culture liquid (Table 2). Strain *R. rhodochrous* 89, like *R. opacus* 1cp, also converted 2,3-dichlorophenol to the corresponding 3,4-dichlorocatechol, and then a new, previously unidentified product appeared. In the reaction with diazotized benzidine, the compound behaved like catechol, but it exhibited a lower chromatographic mobility. Figure 4a shows the HPLC elution profile of the ethyl acetate extract of an incubation mixture of *R. rhodochrous* 89 cells with 2,3-dichlorophenol (incubation for 7 h). At this moment, the peak with the retention time of 11.58 min, corresponding to the initial 2-dichlorophenol, was already absent. According to the mass spectrum of the compound obtained after the separation by HPLC (Fig. 4b), the peak with the retention time of 7.33 min might be attributed to 3,4-dichlorocatechol. The peak with the retention time of 5.16 min could be attributed to 3,4-dichlorohydroxycatechol according to the mass spectrum of the compound purified by HPLC (Fig. 4c). The mass spectrum testifies to the introduction of a third hydroxyl group into the aromatic ring (this gives molecular ion M^+ 194). The high-resolution spectrum permits the compound to be assigned to the gross-formula $C_6H_4O_3Cl_2$ (Table 2). The introduction of the third hydroxyl group makes the molecule more resistant to the electron impulse, and the ions produced possess an intensity lower than 17%. This suggests the symmetry of the molecule. In the spectrum, the fragmentation typical to dichloropyrocatechols was observed: $M^+ - H_2O$ (m/z = 176), M^+–HCl (m/z 158), and $M^+ -$ HCl, CO (m/z = 130). The ^{1}H NMR spectrum recorded in deuterated ethyl acetate for the compound preparatively isolated from a silica gel plate showed a singlet at 6.65 ppm (relatively to the internal standard tetramethylsilane). Two possible isomers of trihydroxylated dichlorobenzene—

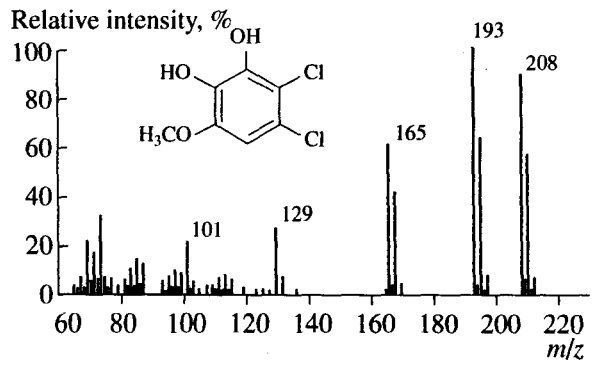


Fig. 5. Mass spectrum of the intermediate of 2,3-dichlorophenol conversion by *R. rhodochrous* 89 that was identified as 6-methoxy-3,4-dichlorocatechol.

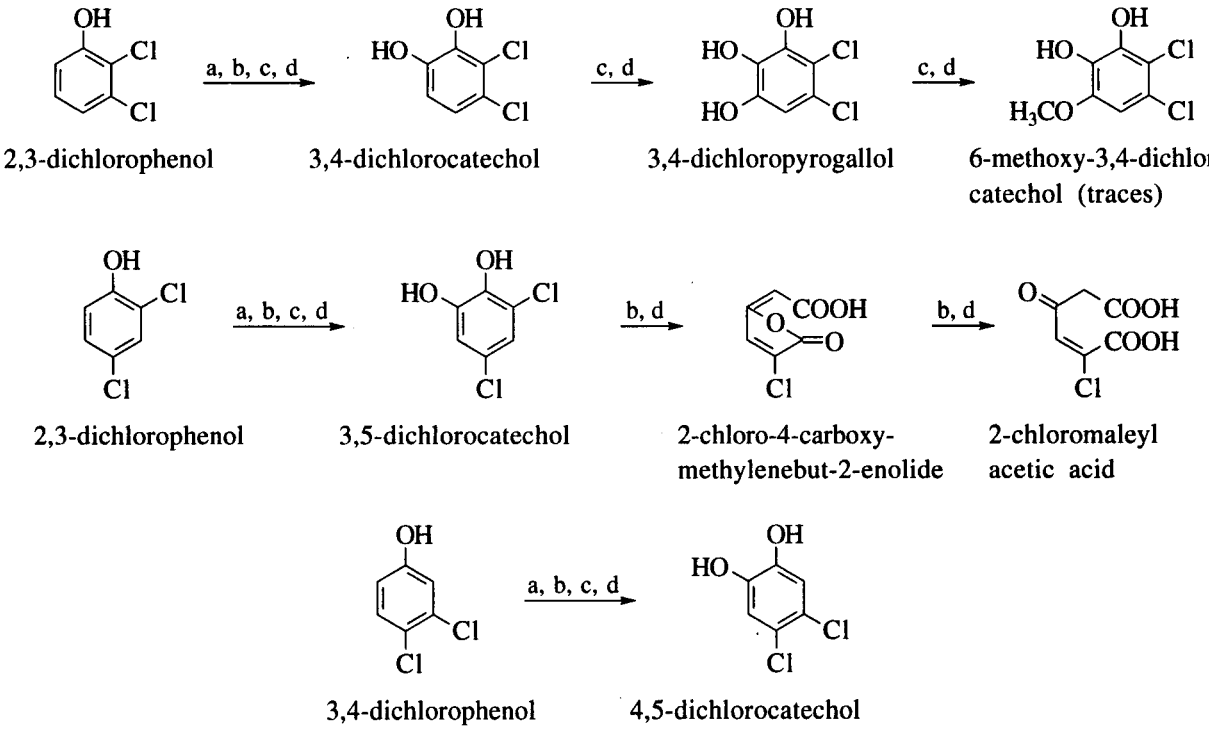


Fig. 6. Conversion of 2,3-dichloro-, 2,4-dichloro-, and 3,4-dichlorophenols by (a) *R. opacus* 1G, (b) *R. rhodnii* 135, (c) *R. rhodochrous* 89, and (d) *R. opacus* 1cp.

1,2,3-trihydroxy-4,5-dichlorobenzene and 1,2,5-trihydroxy-3,4-dichlorobenzene—could be produced. The values of the chemical shift calculated for the ^1H resonance of these two isomers, relative to tetramethylsilane and according to the tables of chemical substitution, are 6.1 and 5.87 ppm for the protons of 1,2,3-trihydroxy-4,5-dichlorobenzene and 1,2,5-trihydroxy-3,4-dichlorobenzene, respectively. Although the value actually measured for our compound was closest to that predicted for 1,2,3-trihydroxy-4,5-dichlorobenzene (4,5-dichloropyrogallol), this cannot be considered ultimate proof of the position of the third hydroxyl group. However, the assignment of the compound under consideration to 4,5-dichloropyrogallol is in agreement with the involvement of the new intermediate 4-chloropyrogallol in the degradation of 2-chlorophenol (in that case, we firmly established the structure of the intermediate in question).

The detection of dichloropyrogallol as an intermediate suggests that the phenol hydroxylase of these strains hydroxylates a nonsubstituted position in the 2,3-dichlorophenol aromatic ring with the formation of dichlorocatechol; then, hydroxylation of another nonchlorinated position takes place with the formation of a trihydroxylated derivative. After the 7-h incubation of *R. opacus* 1cp cells, this product accumulated in an amount approximating 14% of the 3,4-dichlorocatechol revealed; after 20 h of incubation it disappeared, but a methylated derivative—methoxydichlorocatechol—was formed in trace amounts (Table 2, Fig. 5). In the mass spectrum of this compound, two types of fragmentation were reflected: that typical for substituted anisoles—the elimination of the methyl group ($-CH_3$), ($-CO$)—and that typical for chlorosubstituted catechols—HCl (m/z = 129), $-CO$ (m/z = 101). Based on the data obtained, the transformation pathways of isomeric dichlorophenols can be depicted by the following scheme (Fig. 6). It should be noted that all three isomers were hydroxylated in the *ortho*-position (relatively to the already existing hydroxyl group) with the formation of corresponding catechols. Then the catechols were either metabolized via a known modification of the *ortho*-pathway (as in the case of 2,4-dichlorophenol utilization by strain *R. opacus* 1cp) or hydroxylation of the nonsubstituted position occurred with the production of dichloropyrogallol, which was rapidly metabolized by the cultures without being accumulated in the culture liquid. The transformation of dichlorophenols by bacteria via dichloropyrogallol has not been previously described, and a detailed study of this pathway is currently in progress.

Transformation of Trichlorophenols by Rhodococci

None of the strains studied could transform 2,4,5-trichlorophenol, while 2,3,5-trichlorophenol was converted by all cultures. *R. rhodnii* 135 and *R. opacus* 1cp formed trace amounts of 3,5-dichlorocatechol from the latter compound. *R. opacus* 1G and *R. rhodochrous* 89 were able to oxidatively dehalogenate 2,3,5-trichlorophenol with the formation of 3,5-dichlorocatechol (Table 2). Both strains also accumulated 3,4,6-trichlo-

rocatechol in small amounts. The mass of the molecular ion of this compound was M^+ 212 (Table 2). These data testify to the ability of both rhodococci to catalyze oxidative dechlorination and also to the fact that the hydroxylation of the halogenated position of 2,3,5-trichlorophenol prevails over the hydroxylation of the nonhalogenated position.

DISCUSSION

The investigation performed showed that various representatives of the genus *Rhodococcus* are able to utilize phenol and chlorophenols as the sole source of carbon and energy. Virtually all isomeric chlorophenols studied in the present paper were converted by rhodococci. This fact is one more confirmation of the high degradative potential of rhodococci and, hence, their important role in bioremediation of contaminated soils and waters.

A great effect on the susceptibility of chlorophenols to the attack by rhodococci was produced by the number and position of the chlorine substituents in the aromatic ring. As could be expected, the degradability of chlorophenols decreased from monochlorophenols to dichlorophenols and further to trichlorophenols. *Para*-substituted monochlorophenol was utilized by *R. opacus* 1cp more readily than *meta*- and, particularly, *ortho*-derivatives. It should be noted that chlorophenols were transformed both by strain *R. opacus* 1cp, which realizes the *ortho*-pathway of chlorocatechol decomposition for the growth on chlorophenols [7], and by strains *R. rhodnii* 135 and *R. rhodochrous* 89, in which the induction of the modified *ortho*-pathway was not revealed, but a catechol 1,2-dioxygenase with an unusually broad substrate specificity was found (its substrates included 3-catechol) [10].

Another interesting finding of the present study is the ability of rhodococcal phenol hydroxylase to carry out oxidative dechlorination of 2,3,5-trichlorophenol, although it was more logical to expect the hydroxylation of nonsubstituted *para* or *ortho* positions of the ring with the formation of trichlorohydroquinone or trichlorocatechol. The formation of 4-chloropyrogallol and 4,5-dichloropyrogallol from 2-chlorophenol, which was catalyzed by *R. opacus* 1cp and *R. rhodochrous* 89, was also earlier unknown. The fungus *Penicillium simplicissimum* SK 9117 was reported to transform 3-chlorophenol in the presence of phenol with the formation of chlorohydroquinone, 4-chlorocatechol, 4-chloropyrogallol, and 5-chloropyrogallol [4]. The yeast *Candida maltosa*, grown on phenol, transforms 3- and 4-chlorophenols with the formation of 4-chlorocatechol, 5-chloropyrogallol, and 4-carboxymethylenebut-2-en-4-olide [11]. However, neither the fungus nor the yeast grew on chlorophenols, and the objects of transformation were *para*- or *meta*-substituted chlorophenols; the conversion of 2-chlorophenol by the fungus was not studied, and the yeast partially converted this compound to 3-chlorocatechol and then to 2-chloromuconate. The trihydroxylated product revealed by us did not accumulate in the medium and rather rapidly disappeared. According to our preliminary data, this product was subject to *ortho*-decomposition, but this conclusion requires additional examination.

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