The members of the genus *Arthrobacter* are Gram-positive, catalase-positive and pleomorphic rods, and belong to the order *Actinimycetales* (Stackebrandt et al., 1997). According to a list of bacterial names with standing in nomenclature (URL: http://www.bacterio.cict.fr/) (Euzéby, 1997), the number of species accounted for in this genus is 30, eliminating the species reidentified as other taxa than the genus *Arthrobacter*. The species of the genus *Arthrobacter* are separated into two major groups, the *Arthrobacter globiformis*/*A. citreus* group and the *Arthrobacter nicotianae* group by their chemotaxonomic features (Jones and Keddie, 1992; Keddie et al., 1986). The peptidoglycan type of the *A. globiformis*/*A. citreus* group is A3α, whereas that of the *A. nicotianae* group is A4α. In addition, their menaquinone systems and cell-wall polymers differ from each other. The species of the *A. globiformis*/*A. citreus* group have MK-9(H2) and neutral polysaccharides, whereas those of the *A. nicotianae* group have MK-8 and/or MK-9 and teichoic acid. As an exception, *A. crystallopoietes* in the *A. globiformis*/*A. citreus* group has teichoic acid.

Recently, the phylogenetic relationship of the genus *Arthrobacter* was studied, and it was found that each of the species of the *A. globiformis*/*A. citreus* group and the *A. nicotianae* group is placed in several clusters by the 16S rRNA gene sequence (Tanaka et al., 2001). Additionally, the genus *Arthrobacter* is closely related to other genera, particularly to the genus *Micrococcus* (Koch et al., 1994).

Approximately 250 *Arthrobacter* strains are available from culture collections around the world. These strains were isolated by the use of various media from environments, especially soils for the screening of useful substance-producing bacteria (Brim et al., 1999; Nakajima-Kambe et al., 1995; Stevenson, 1967; Westerberg et al., 2000) and for research into microbiological diversity (Balkwill and Ghiorse, 1985; Crocker et al., 2000; Eppard et al., 1996; Gardener and Bruijn, 1998), and from clinical specimens (Funke et al., 1996; Hou et al., 1998; Wauters et al., 2000). A considerable number of *Arthrobacter* strains have been identified, but the others have been remained unidentifed at the species level. Additionally, some *Arthrobacter* species consist of only one strain. Probably, the media used for the above isolations were not focused on the isolation of a variety of *Arthrobacter* strains, and the strains were isolated by chance. Therefore, *Arthrobacter* strains available from culture collections are rather limited to certain species, and a considerable number of the strains come from the same origins. New *Arthrobacter* strains are needed for the further taxonomic study of the genus *Arthrobacter*. As part of the study, media for the isolation of *Arthrobacter* strains should be designed in consideration of their character-
In a previous paper, Tanaka et al. (2000) reported the design of a defined medium for the cystite formation of *Arthrobacter* strains, CT medium, and mentioned the cystite formation due to the nutritional stress caused by the imbalance of concentrations of K\(^+\) and Mg\(^{2+}\). Further, they defined the cystites of *Arthrobacter* strains as being aberrant cell forms, drumstick and oval in the cell shape, in a range of 1.5 to 3.5 mm in a diameter of a colony) on R agar because vegetative cells might die with a long cultivation in CT liquid medium (Tanaka et al., 2000).

Of 14 strains isolated by the use of CT agar medium, five strains (C1, C2, C3, C4, and C5) formed cystites in CT liquid medium. Of 18 strains isolated by the use of nutrient agar, one strain (N1) formed cystites, and of 14 strains by Mulder’s agar medium, two strains (M1 and M2) formed cystites. The vegetative cells of the above cystite-forming strains were Gram-positive by the staining, catalase-positive, and pleomorphic rods (rod-coccus cycle), and did not contain diaminopimelic acid in the cell walls. Diaminopimelic acid was detected by thin-layer chromatography (Komagata and Suzuki, 1987). Further, 16S rRNA gene sequences were determined, and a phylogenetic tree was constructed (Lisdiyanti et al., 2000).

Soils were collected at several places in Japan, and used for isolation sources. Serial decimal dilutions were made with saline, 0.1 ml dilutions (10\(^{-1}\), 10\(^{-2}\), and 10\(^{-3}\)) were spread on agar plates, and cultivated at 30°C for 3 days. Visible colonies grown on CT agar medium, nutrient agar, and Mulder’s agar medium were picked up at early phase of growth (more than 1 mm in a diameter of a colony) on R agar because vegetative cells might die with a long cultivation in CT liquid medium (Tanaka et al., 2000).

Further, the phylogenetic relationship of the cystite-forming isolates was deduced on the basis of 16S rRNA gene sequences. As shown in Fig. 1, *Arthrobacter* strains of the described species and isolates were grouped into 14 clusters by 16S rRNA gene sequences; the *Arthrobacter oxydans* cluster, the *Arthrobacter globiformis* cluster, the *Arthrobacter nictiana* cluster, the *Arthrobacter ureafaciens* cluster, the *Arthrobacter citreus* cluster, the *Arthrobacter albus* cluster and eight clusters consisting of two strains or a
single strain. Cystite-forming strains were separated into the \textit{Arthrobacter oxydans} cluster, the \textit{Arthrobacter globiformis} cluster, the \textit{Arthrobacter ureafaciens} cluster, the \textit{Arthrobacter citreus} cluster, and a cluster consisting of two strains (\textit{Arthrobacter} sp. JCM 1339 and M1) and clusters consisting of a single strain such as the \textit{Arthrobacter crystallopoietes} cluster and the \textit{Arthrobacter atrocyaneus} cluster. On the other hand, cystite-non-forming strains were separated into the \textit{Arthrobacter nicotianae} cluster, the \textit{Arthrobacter albus} cluster, and the clusters consisting of a single strain of \textit{Arthrobacter chlorophenolicus}, \textit{Arthrobacter psychrolactophilus}, \textit{Arthrobacter rhombi}, \textit{Arthrobacter woluensis}, and \textit{Arthrobacter agilis}. \textit{A. agilis} IAM 14192 did not grow in CT liquid medium.

In this study, the cystite-forming isolates from CT agar medium (C1, C2, C3, C4, and C5) and from nutrient agar (N1) were located at the \textit{Arthrobacter globiformis} cluster. In contrast, strain M2 formed yellow colonies, and was placed in the \textit{Arthrobacter oxydans} cluster. Therefore, cystite-forming isolates belonging to the \textit{A. globiformis}/\textit{A. citreus} group were isolated from the soil samples. In addition, \textit{Arthrobacter} sp. JCM 1339 was closely related to the strain M1 isolated from 2002 Isolation of \textit{Arthrobacter} strains

Fig. 1. Phylogenetic relationships of the cystite-forming strains and cystite-non-forming strains of the genus \textit{Arthrobacter} based on 16S rRNA gene sequences.

The scale bar represents 1 nucleotide substitution per 100 nucleotides. Numerals indicate the bootstrap value derived from 1,000 replications. Isolates are shown by underbars. \textcircled{1}: Cystite-forming strain. \textcircled{2}: Cystite-non-forming strain. NG: Did not grow in CT liquid medium. \textsuperscript{a} Data from Tanaka et al. (2001). \textsuperscript{b} Not tested for cystite formation.
Mulder’s agar medium, and they were accommodated to a cluster separate from the *Arthrobacter globiformis* cluster. The cystite formation of *Arthrobacter* sp. JCM 1339 was first reported by Jensen (1934), and the peptidoglycan type of this strain was A3α (Fiedler et al., 1973).

The ratio of cystite-forming strains to all isolates from CT agar medium was 36%; nutrient agar was 6%; and Mulder’s agar medium was 14%. Cystite-forming isolates from CT agar medium were located only at the *Arthrobacter globiformis* cluster. Therefore, CT agar medium was the most useful for the isolation of the strains of the *A. globiformis* cluster. Additionally, the cystite formation reflects phylogenetic relationships, and may be a useful characteristic for the identification of *Arthrobacter* strains.

Considering the above data, strains of such clusters other than the *Arthrobacter globiformis* cluster, as the *Arthrobacter citreus* cluster, the *Arthrobacter oxydans* cluster, the *Arthrobacter ureafaciens* cluster, and others, would be isolated by the use of modified CT agar medium containing vitamins and amino acids because the strain M2 was isolated by the use of Mulder’s agar medium containing yeast extract and Casamino Acids. Therefore, the isolation of *Arthrobacter* strains was attempted from soils by the use of modified CT media. CTV agar medium contained 1 mg of thiamine·HCl, riboflavin, niacin, Ca-pantothenate, pyridoxine·HCl, and pyridoxal·HCl each, 0.2 mg of p-amino benzoic acid, and 0.01 mg of folic acid and biotin each in 1,000 ml of CT agar medium. CTB agar medium contained 0.01 mg of biotin in 1,000 ml of CT agar medium because some *Arthrobacter* strains require biotin for their growth (Jones and Keddie, 1992; Keddie et al., 1986). CTA agar medium contained 200 mg of L-alanine, L-arginine·HCl, L-aspartic acid, and L-lysine·HCl each, 100 mg of L-cysteine·HCl, glycine, L-histidine·HCl, L-isoleucine, L-leucine, L-methionine, L-phenylalanine, L-proline, L-threonine, L-tyrosine, and L-valine each, 50 mg of L-serine and L-tryptophan each, and 500 mg of L-sodium glutamate in 1,000 ml of CT agar medium. Solutions of vitamins and amino acids were filter-sterilized, and added to CT agar. All the above-mentioned media contained 50 μg/ml cycloheximide for the inhibition of the growth of yeasts and molds.

Of 31 strains newly isolated by the use of CT agar medium, 15 strains formed cystites in CT liquid medium; of 30 strains isolated by the use of CTV agar medium, 18 strains formed cystites; and of 38 strains isolated by the use of CTB agar medium, 20 strains formed cystites. In addition, four isolates from CTV agar medium and CTB agar medium required vitamin(s) for the cystite formation in CT liquid medium. The ratio of cystite-forming strains to all isolates from CT agar medium, CTV agar medium, and CTB agar medium was 48%, 60%, and 53%, respectively. Therefore, not only biotin but also other vitamin(s) in CT agar medium were effective for the isolation of cystite-forming strains. Some cystite-forming isolates from CTV agar medium and CTB agar medium would be placed in the *Arthrobacter citreus* cluster, the *Arthrobacter oxydans* cluster, and the *Arthrobacter ureafaciens* cluster. Further, strains belonging to other clusters than the known clusters have probably been isolated. In fact, some isolates from CTV agar medium and CTB agar medium formed pink, orange, or creamy colored colonies. Interestingly, 45 strains isolated from CTA agar medium did not form cystites. Some of cystite-non-forming isolates from CT, CTV, CTB, and CTA agar media were Gram-positive by the staining, catalase-positive, and irregular rods. Detailed characteristics of the isolates will be reported elsewhere.

As mentioned above, the genus *Arthrobacter* was separated into the *A. globiformis/A. citreus* group and *A. nicotianae* group by their chemotaxonomic characteristics. Data obtained in this study would indicate a correlation between the cell wall structure of A3α and the cystite formation. It seemed likely that the cystite-forming *Arthrobacter* strains were limited to those with A3α, which would be strains in the *Arthrobacter globiformis/A. citreus* group. The genus *Arthrobacter* consists of 14 clusters including clusters comprising two strains or a single strain on the basis of the 16S rRNA gene sequence. Therefore, a large number of new isolates are needed for the reconstruction and further systematic study of the genus *Arthrobacter*.

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**References**


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