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Characteristics of *Arthrobacter cumminsii*, the Most Frequently Encountered *Arthrobacter* Species in Human Clinical Specimens

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During a 2-year period, 10 strains of *Arthrobacter cumminsii* were isolated in or received by a Swiss routine clinical bacteriology laboratory, and 5 further isolates were referred to a Swedish bacteriology reference center over a 5-year period, making *A. cumminsii* the most frequently encountered *Arthrobacter* species in these two laboratories. The present report outlines the clinical features of the 15 *A. cumminsii* strains and presents an extended biochemical characterization of this microorganism. *A. cumminsii* exhibits a unique cellular fatty acid pattern with the consistent presence of $C_{14:0i}$ and $C_{14:0}$ fatty acids as well as relatively large amounts of $C_{16:0i}$ and $C_{16:0}$ fatty acids usually not seen in other *Arthrobacter* spp. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was found to be a useful tool for confirmation of the identification of *A. cumminsii*. The MICs of 39 antimicrobial agents were determined, and it was demonstrated that aminoglycosides and quinolones had only weak activities against *A. cumminsii* strains, in contrast to their activities against most other coryneform bacteria. As a result of the extended characterization of *A. cumminsii*, an emended description of this species is presented. Due to the lack of *A. cumminsii* in established identification systems, it is most likely that this species is underdiagnosed in many routine clinical bacteriology laboratories.

In recent years, some genera (e.g., *Aureobacterium*, *Cellulomonas*, and *Microbacterium*) belonging to the coryneform bacteria (i.e., aerobically growing, asporogenous, irregular, non-partially acid-fast, gram-positive bacteria) were shown not only to be present in environmental specimens but also to be derived from human clinical material and to cause infections in selected patients (10). The genus *Arthrobacter* is another genus which is very widely distributed in the environment, especially in soil (12), and it has only been recently described as being isolated from human clinical specimens (2, 7, 11). It was demonstrated that there is an enormous heterogeneity within *Arthrobacter* strains isolated from humans, suggesting that many strains are representatives of separate individual yet undescribed species (7). However, 3 of 11 clinical *Arthrobacter* strains in the study by Funke et al. (7) were shown to be *Arthrobacter cumminsii* strains, whereas all the other *Arthrobacter* strains were single representatives of different taxa. This indicated for the first time that *A. cumminsii* might be the most frequently encountered *Arthrobacter* species in clinical specimens. The present report from two different European laboratories confirms this initial observation. In addition, an extended characterization of *A. cumminsii* comprising 15 new strains is presented. The extended characterization has led to a slightly emended description of *A. cumminsii.*

MATERIALS AND METHODS

Strains and culture conditions. Workers in the Department of Medical Microbiology at the University of Zürich (DMMZ) isolated from patients' material 9 of the 15 strains listed in Table 1 by established methods (3). The nine Swiss patients were all hospitalized in the Zürich metropolitan area. Only strain DMMZ 2279 was referred from abroad to DMMZ for identification. The other five strains listed in Table 1 were referred from various laboratories in Sweden to the Culture Collection of the University of Göteborg (CCUG) for identification. All strains were subcultured on Columbia agar (Difco, Detroit, Mich.) supplemented with 5% sheep blood (SBA) at 37°C in a 5% CO_2 atmosphere before they were used for further testing.

Biochemical tests. All isolates were initially screened for their biochemical reactions according to the system outlined by von Graevenitz and Funke (17). The commercial API Coryne system (bioMérieux, Marcy l'Etoile, France) was used according to the manufacturer's instructions except that the strips were incubated and read after up to 7 days at 37°C in ambient air.

Chemotaxonomic investigations. The diamino acid of the cell wall peptidoglycan was determined by thin-layer chromatography analysis (7). The cellular fatty acid (CFA) patterns were generated by using the Sherlock Microbial Identification System (Microbial ID, Inc., Newark, Del.) (18).

Susceptibility tests with antimicrobial agents. The MICs (always given in micrograms per milliliter) of 39 antimicrobial agents were determined by a microdilution method with the Merlin MIC system (Merlin Diagnostics, Bornheim-Hersel, Germany) with H medium (Merlin Diagnostics), as outlined previously (5). β-Lactamase activity was tested for by using the chromogenic substrate nitrocefin (14). MICs were interpreted by using the criteria for staphylococci (which are not as strict as the criteria for streptococci) established by the National Committee for Clinical Laboratory Standards (13), although it must be emphasized that the National Committee for Clinical Laboratory Standards has not explicitly published guidelines for coryneform bacteria.

PAGE of whole-cell proteins. The strains were grown on horse blood agar (Columbia base) at 37°C in the presence of 5% CO_2. Polyacrylamide gel electrophoresis (PAGE) of whole-cell proteins was performed as described by Pot et al. (15). For densitometric analysis, normalization and interpretation of protein patterns, the Gelcompar (version 3.1) software package (Applied Maths, Kortrijk, Belgium) was used. The levels of similarity between pairs of traces were expressed by the Pearson product moment correlation coefficient converted for convenience to a percentage value.

RESULTS AND DISCUSSION

The 10 DMMZ *A. cumminsii* strains included in this study (Table 1) were isolated or received between January 1996 and December 1997, whereas the 5 CCUG *A. cumminsii* strains were referred to CCUG for identification between June 1991 and May 1996. Workers in DMMZ isolated or received as reference cultures seven other *Arthrobacter* strains during the 2-year period described above, and none of those was identical to any other strain or belonged to any of the other presently established *Arthrobacter* species. CCUG had received three

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TABLE 1. Origins of the *A. cumminsii* strains studied

Strain no.	Patient's sex,[a] age (yr)	Material or clinical diagnosis	Other bacteria isolated
DMMZ 1490 (CCUG 38876)	f, 28	Urinary tract infection	
DMMZ 2279 (CCUG 38877)	f, 66	Urinary tract infection	Members of the family *Enterobacteriaceae*, enterococci
DMMZ 2358 (CCUG 38878)	f, 45	Chronic otorrhea	*Staphylococcus aureus*
DMMZ 2554 (CCUG 38879)	m, 73	Urinary tract infection	
DMMZ 2558 (CCUG 38880)	f, 17	Infected amniotic fluid	
DMMZ 2591 (CCUG 38881)	f, 33	Vaginal swab	Lactobacilli
DMMZ 3147 (CCUG 38882)	f, 40	Calcaneus osteomyelitis	*Corynebacterium striatum*
DMMZ 3406 (CCUG 38883)	f, 23	Urinary tract infection	
DMMZ 3419 (CCUG 38884)	m, 70	External otitis	Miscellaneous gram-negative bacteria
DMMZ 3452 (CCUG 38885)	m, 61	Deep tissue infection, upper leg	
CCUG 28647	f, 36	Chronic cervicitis	
CCUG 28802	ND,[b] 70	ND	
CCUG 33745	m, 47	Blood culture, urosepsis	
CCUG 35241	m, 83	Blood culture	
CCUG 35863	f, 81	Leg wound	

[a] f, female; m, male.
[b] ND, no data.

other clinical *Arthrobacter* strains during the 5-year period mentioned above. Therefore, *A. cumminsii* was the most frequently encountered *Arthrobacter* species isolated from clinical specimens in these two European laboratories.

Of the 14 patients for whom data were available, 9 were female and 5 were male. Their mean age was 50.2 years (age range, 17 to 83 years). We did not observe multiple isolates from the same patients. None of the patients was epidemiologically linked. Additional clinical data were, unfortunately, not available for the patients from whom the five CCUG strains were isolated. We observed three patients with urinary tract infections, and *A. cumminsii* grew (10^4 to $>10^5$ CFU/ml) in pure cultures from samples from these patients. In a sample from one patient with a deep tissue infection, *A. cumminsii* grew in pure culture to approximately 10^3 to 10^4 CFU/ml; Gram staining showed a moderate leukocyte response, but no microorganisms were seen. Short, small gram-positive rods were seen in the direct Gram stain of a sample of infected amniotic fluid. *A. cumminsii* was recovered together with multiple other bacteria from two patients with external otitis. It was also not grown in pure culture from a sample from a patient with calcaneus osteomyelitis or in a pure culture from a vaginal swab. In summary, *A. cumminsii* seems to be a microorganism with a rather low level of pathogenicity since cases of severe, life-threatening infections were not observed in our series of patients. However, *A. cumminsii* might be the bacterial agent responsible for selected cases of urinary tract infections. In the initial study of Funke et al. (7) on *A. cumminsii*, two of the three strains also came from patients with urinary tract infections, and a third strain was isolated from a patient with a skin infection. It seems not unlikely that *A. cumminsii* is part of the normal human skin and mucous membrane flora, in particular, in the genitourinary tract, although these anatomical sites have not been systematically screened for *A. cumminsii*. Up to now, humans have not been described as reservoirs for *Arthrobacter* spp. (12).

A. cumminsii colonies are smaller than 2 mm in diameter after 24 h of incubation at 37°C either in a 5% CO_2-enriched atmosphere or in ambient air. In contrast, all other presently defined *Arthrobacter* spp. which are able to grow at 37°C exhibit colonies which are larger than 2 mm in diameter after 24 h of incubation (4, 7). Colonies of *A. cumminsii* are more grayish than those of other *Arthrobacter* spp. and are also less convex. Like the other *Arthrobacter* spp., *A. cumminsii* does not give off the typical cheese-like smell which is observed in the biochemically closely related *Brevibacterium* spp. (6, 7). Gram stains of colonies from SBA cultures (24 h of incubation at 37°C) showed ordinary (i.e., not branching), relatively small (1 to 2 μm in length) coryneform bacteria. A typical rod-coccus growth cycle was not observed for *A. cumminsii* strains on SBA.

According to the biochemical typing system for coryneform bacteria developed by von Graevenitz and Funke (17), we observed the following characteristics for all 15 strains studied: catalase positive, oxidative metabolism, nonmotile, nitrate reduction negative, esculin hydrolysis negative, no acid formation in cystine Trypticase agar medium from glucose, maltose, sucrose, mannitol, and xylose, CAMP reaction negative, and no lipophilism. Lysine was detected as the cell wall diamino acid in all 15 strains. When extended morphological characterization as well as extended biochemical typing based on the API Coryne system were performed, we found some features which had not been observed for the three *A. cumminsii* strains included in the original description of this species (7): (i) 4 of 15 strains had a sticky consistency, (ii) two strains were urease positive (which was confirmed by the observation of urease activity in Christensen's medium), (iii) all 15 strains produced acid from ribose after 7 days of incubation, and (iv) 2 of 15 strains also produced acid from glucose (but more weakly than from ribose).

Numerous API Coryne codes were observed: after 24 h, 0000004 ($n = 2$ isolates), 2101004 ($n = 1$), 4002004 ($n = 2$), 6000004 ($n = 3$), 6002004 ($n = 5$), 6101004 ($n = 1$), and 6102004 ($n = 1$); after 48 h, 0000004 ($n = 1$), 0002004 ($n = 1$), 2103004 ($n = 1$), 4002004 ($n = 2$), 6000004 ($n = 1$), 6002004 ($n = 7$), 6102004 ($n = 1$), and 6103004 ($n = 1$); and after 7 days, 0002204 ($n = 2$), 2103204 ($n = 1$), 4002204 ($n = 1$), 4002304 ($n = 1$), 6002204 ($n = 7$), 6002304 ($n = 1$), 6102204 ($n = 1$), and 6103204 ($n = 1$). It is noteworthy that *A. cumminsii* is not included in the database (version 2.0) of the API Coryne system but that *Arthrobacter* spp. are included (9). However, because up to eight different numerical profiles were observed for *A. cumminsii* strains at each time that the strips were read, it might be difficult to include this species in the system's database. Eleven of the 15 strains exhibited pyrazinamidase activity and 12 of 15 exhibited pyrrolidonyl arylamidase activity. Weak alkaline phosphatase activity was present

in 3 of 15 strains. Gelatinase activity was consistently observed in all strains after 7 days.

The CFA patterns of the *A. cumminsii* strains are given in Table 2. It is obvious that $C_{15:0ai}$ and $C_{15:0i}$ are not as dominant in *A. cumminsii* as they are in other *Arthrobacter* spp. In contrast to other *Arthrobacter* spp., $C_{14:0i}$ and $C_{14:0}$ are always detectable in *A. cumminsii*. In addition, larger amounts of $C_{16:0i}$ and $C_{16:0}$ are present in *A. cumminsii* than in most other *Arthrobacter* spp. In nearly all *Brevibacterium* spp., the amounts of $C_{15:0ai}$ and $C_{17:0ai}$ together are greater than 75% of the total CFAs (6), whereas these CFAs represent only 50 to 60% of the total CFAs in *A. cumminsii* strains. In contrast, significantly more $C_{16:0}$ is found in *A. cumminsii* than in *Brevibacterium* spp. In summary, *A. cumminsii* strains exhibit a very unique CFA pattern which is not observed in any other coryneform bacterium (1, 18).

The antimicrobial susceptibility patterns of the *A. cumminsii* strains are given in Table 3. With the exception of ceftibuten, the β-lactams showed good activity against *A. cumminsii*. Rifampin exhibited an excellent activity, with MICs of ≤0.01 μg/ml, and all *A. cumminsii* strains tested were susceptible to glycopeptides, as has been reported before (7). Only strains DMMZ 2358, CCUG 35241, and CCUG 35863 were resistant to tetracyclines, and no strain was resistant to macrolides. Aztreonam and fosfomycin MICs for all 15 strains were relatively high, but the MICs for *A. cumminsii* were slightly lower than those for other coryneform bacteria, especially true corynebacteria (4, 8). Surprisingly, for all isolates the MICs of the aminoglycosides, in particular, those of netilmicin and tobramycin, were relatively high. The MICs of the following other aminoglycosides were also elevated for *A. cumminsii*: kanamycin (MIC at which 50% of strains are inhibited [MIC_{50}], 64 μg/ml; MIC_{90}, 256 μg/ml), streptomycin (MIC_{50}, 16 μg/ml; MIC_{90}, 64 μg/ml), spectinomycin (MIC_{50}, 32 μg/ml; MIC_{90}, 64 μg/ml), apramycin (MIC_{50}, 64 μg/ml; MIC_{90}, >128 μg/ml), ribostamycin (MIC_{50}, >128 μg/ml; MIC_{90}, >128 μg/ml), and livitomycin (MIC_{50}, >128 μg/ml; MIC_{90}, >128 μg/ml). Surprisingly, high quinolone MICs were also observed, in particular, those of norfloxacin and ofloxacin. The MICs of the following other quinolones were also elevated: enoxacin (MIC_{50}, >32 μg/ml; MIC_{90}, >32 μg/ml), fleroxacin (MIC_{50}, 16 μg/ml; MIC_{90}, >32 μg/ml), and pefloxacin (MIC_{50}, 32 μg/ml; MIC_{90}, >32 μg/ml). Poor activities of aminoglycosides and quinolones combined with good activities of most beta-lactams as well as those of macrolides and tetracyclines have so far not been observed for any other taxon of coryneform bacteria (4, 8, 10, 16). β-Lactamase activity was not detected in any of the 15 strains tested. The better activity of amoxicillin-clavulanic acid compared to that of amoxicillin (Table 3) is probably due to an intrinsic activity of clavulanic acid against *A. cumminsii* strains. Whether the resistance against aminoglycosides and quinolones is acquired or intrinsic is not clear at present, but its presence in all strains tested makes an intrinsic mechanism (e.g., complicated or blocked transport through the bacterial cell wall) more likely.

The whole-cell protein profiles of 10 randomly chosen strains included in this study were examined by sodium dodecyl sulfate (SDS)-PAGE. A dendrogram derived from a numerical analysis of the protein profiles is presented in Fig. 1. All 10 strains grouped together with the type strain of *A. cumminsii*, strain CCUG 36788 (DSM 10493). In addition, they formed a distinct branch with a within-group correlation level of >65%. This indicated that the strains examined represent a homogeneous group. It has been established that comparative quantitative whole-cell protein profiles and quantitative DNA-DNA hybridizations correlate very well (15). It is further evident from the SDS-PAGE analysis that the clinical strains were distinct

TABLE 2. CFA patterns of *A. cumminsii* and phenotypically related taxa

Taxon	% total CFAs[a]											
	13:0*ai*	14:0*i*	14:0	15:0*i*	15:0*ai*	16:0*i*	16:0	17:0*i*	17:0*ai*	18:1ω9*c*	18:1ω7*t*	18:0	Feature 6[b]
A. cumminsii[c]	1 ± 1 (0–2)	3 ± 1 (1–5)	3 ± 2 (1–7)	3 ± 1 (2–5)	37 ± 6 (26–49)	13 ± 2 (9–19)	13 ± 5 (6–26)	1 ± 1 (1–2)	18 ± 5 (11–28)	2 ± 1 (1–4)	1 ± 1 (0–2)	3 ± 2 (1–7)	1 ± 1 (0–2)
Other *Arthrobacter* spp.[d]		0–4	0–3	3–29	34–75	3–15	2–10	0–11	6–25	0–3		0–4	
Brevibacterium spp.[e]				4–11	36–72	2–13	1–5	0–4	17–47				

[a] Values are means ± standard deviations (ranges) or ranges.
[b] Feature 6 includes 18:2ω6*c*, 18:2ω9*c*, and 18:0 ANTE.
[c] Results are based on results for all 15 strains included in the present study.
[d] Data are based on references 4 and 7.
[e] Data are based on references 4 and 6.

TABLE 3. Antimicrobial susceptibility pattern of *A. cumminsii*

Antimicrobial agent	MIC (μg/ml)		
	Range	50%	90%
Amikacin	4–64	16	64
Amoxicillin	≤0.06–2	≤0.06	1
Amoxicillin-clavulanic acid	≤0.06–0.25	≤0.06	≤0.06
Azithromycin	0.06–4	0.125	0.5
Aztreonam	1–>64	16	>64
Cefaclor	≤0.06–0.5	≤0.06	0.125
Ceftazidime	0.5–8	1	2
Ceftibuten	1–>64	4	>64
Chloramphenicol	1–64	2	32
Ciprofloxacin	0.5–>32	2	16
Clarithromycin	≤0.03–0.5	≤0.03	0.06
Clindamycin	0.03–1	0.125	1
Doxycycline	0.25–8	0.5	1
Erythromycin	≤0.03–1	≤0.03	0.125
Fosfomycin	16–>256	32	>256
Fusidic acid	0.125–0.5	0.25	0.25
Gentamicin	2–>128	8	16
Imipenem	≤0.03–0.125	0.06	0.125
Loracarbef	≤0.125–0.5	≤0.125	≤0.125
Netilmicin	4–>128	64	>128
Norfloxacin	8–>64	32	>64
Ofloxacin	1–32	4	32
Oxacillin	≤0.03–4	0.125	2
Penicillin	≤0.01–0.5	≤0.01	0.5
Piperacillin	≤0.125–4	≤0.125	4
Rifampin	≤0.01	≤0.01	≤0.01
Teicoplanin	0.125–0.5	0.5	0.5
Tetracycline	1–64	1	16
Tobramycin	16–>128	64	>128
Vancomycin	0.5–1	1	1

from other *Arthrobacter* spp. and phylogenetically related taxa examined. We acknowledge that the use of SDS-PAGE for the identification of species of coryneform bacteria is reserved for a reference laboratory, especially since the laboratory must at first create a large database for later comparative studies and since the technique is labor-intensive. However, once this has been established, SDS-PAGE is a useful tool for the identification of coryneform bacteria to the species level, in particular, for clustering analysis.

Although the pathogenic potential of *A. cumminsii* seems to be rather low compared to those of other gram-positive bacteria, we believe that the present report will make clinical bacteriologists more aware of *A. cumminsii* strains. For the routine clinical laboratory we recommend that nonfermenting, short, gram-positive rods without a typical corynebacterial Gram staining result (10) but with whitish-grayish colonies of <2 mm in diameter should raise the suspicion of *A. cumminsii*. On the basis of the data presented above, an emended description of *A. cumminsii* is given.

Emended description of *Arthrobacter cumminsii* Funke, Hutson, Bernard, Pfyffer, Wauters, and Collins 1996. The following description is based on the studies of 18 strains (3 strains were from the study of Funke et al. [7] and 15 are from the present study).

The cells are coryneform bacteria without irregular branching. No spores are formed. The cells are nonmotile. The organism is obligately aerobic. Colonies are whitish-grayish, smooth, slightly convex, and either of creamy or of sticky consistency and are of less than 2 mm in diameter after 24 h of incubation at 37°C in 5% CO_2 on SBA. Catalase activity is detected, but nitrate reductase activity is not. Urease activity is variable. Esculinase activity is not detected. DNase and gelatinase activities are observed within 10 days. Acid is produced from ribose but not from maltose, sucrose, mannitol, xylose, lactose, or glycogen. Acid production from glucose is variable. The CAMP reaction is negative. The following other enzyme activities are

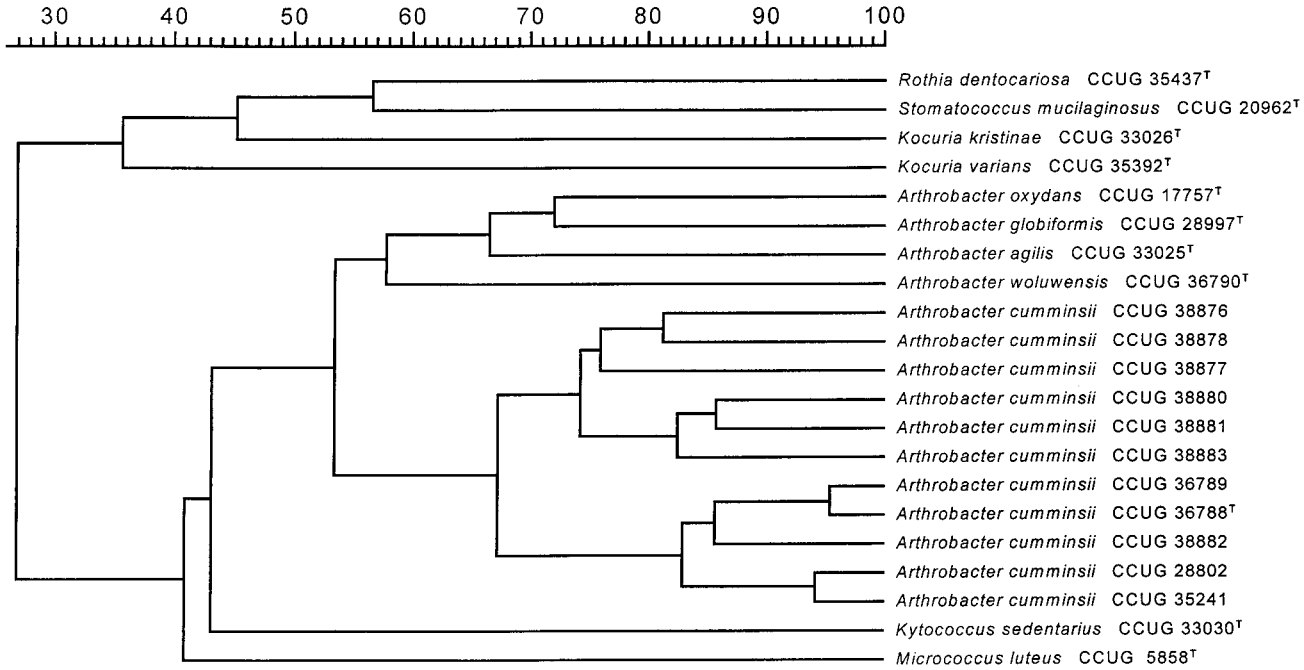

FIG. 1. Similarity dendrogram based on whole-cell protein patterns of *A. cumminsii* and phylogenetically related taxa. Levels of correlation were expressed as percentages of similarity for convenience.

detected: esterase (C_4), esterase lipase (C_8), leucine arylamidase, acid phosphatase, and phosphoamidase. The activities of pyrazinamidase, pyrrolidonyl arylamidase, alkaline phosphatase, and cystine arylamidase are variable. Chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase are not present. The peptidoglycan type is L-lysine-L-serine-L-glutamic acid. The DNA base composition ranges from 60 to 62 mol% G+C. The organism is isolated from human clinical specimens. The type strain is strain DSM 10493 (CCUG 36788), which has been deposited in the German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany, and in the Culture Collection of the University of Göteborg, Göteborg, Sweden.

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