

Supplementary Material: Periodontal condition and subgingival microbiota characterization in subjects with Down syndrome.

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Supplementary Material

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Supplementary Table S1. Presumptive identification of bacterial species in culture.

Tables S1a. Bacteria *Aggregatibacter actinomycetemcomitans* and *Eikenella corrodens* were identified by biochemical tests contained in the RapID™ NH System KIT (REMEL OXOID-Thermo Fisher Scientific). Results are expressed as a series of positive percentages for each test of the system. Identifications were made based on test results on RapID NH panels along with other laboratory information (Gram stain, catalase, or growth on a differential or selective medium).

Bacterial species	Culture medium	Incubation	colony morphology	Gram-staining	catalase reaction	PRO	GGT	ONPG	GLU	SUC	EST	RES	PO4	ORN	URE	NO3	IND
<i>Aggregatibacter actinomycetemcomitans</i>	Dentaid-1	3 days in air with 5% CO2 at 37°C	Small colony, 1 mm in diameter, with a dark border and a “star” or “crossed cigars” shaped inner structure	negative	+	46	98	1	95	16	59	39	58	1	0	79	0
<i>Eikenella corrodens</i>	Blood Agar Base, with haemine, menadione and 5% sterile horse blood	7-14 days of anaerobic incubation (80% N2, 10% CO2 and 10% H2)	Small and greyish, with a greenish discoloration of the underlying agar, with a typically depression (or “pit”) in the agar	negative	-	86	0	9	0	0	0	23	0	97	0	93	0

PRO: Proline α -nitroanilide; GGT: γ -glutamyl α -nitroanilide; ONPG: σ -nitrophenyl, β , D-galactoside; GLU: Glucose; SUC: Sucrose; EST: Fatty acid ester; RES: Resazurin; PO4: α -nitrophenyl phosphate; ORN: Ornithine; URE: Urea; NO3: Nitrate; IND: Tryptophan.

Table S1b. Bacterial identification using biochemical tests contained in the RapID™ ANA II System KIT (REMEL-OXOID-Thermo Fisher Scientific). Results are expressed as a series of positive percentages for each system test. Identifications are made based on test results on RapID ANA II panels along with other laboratory information (Gram stain, catalase, or growth on a differential or selective medium).

Bacterial species	Culture medium	Incubation	Colony morphology	Gram-staining	Catalase reaction	URE	BLTS	αARA	ONPG	αGLU	B	αGAL	αFUC	NAG	PO4	LGY	GLY	PRO	PAL	ARG	SER	PYR	IND
<i>Porphyromonas gingivalis</i>	Blood Agar Base, with haemine, menadione and 5% sterile horse blood	7-14 days of anaerobic incubation (80% N ₂ , 10% CO ₂ and 10% H ₂)	Small and round, smooth, shiny, convex with colour from green, reddish to brown	negative	-	0	2	0	2	0	0	0	0	99	95	96	19	0	5	93	27	27	99
<i>Prevotella intermedia</i>			Big shiny and smooth black pigmented colonies, rounded, flat and darker, with an irregular outline	negative	+	0	0	0	0	99	0	0	93	0	98	98	4	0	0	96	6	0	99
<i>Tannerella forsythia</i>			Pale pink and speckled, circular, convex, and may have a depressed centre (donut-shaped)	negative	-	0	78	0	95	99	50	0	99	99	99	98	12	0	31	99	81	0	98
<i>Parvimonas micra</i>			Small, rough or smooth, dome-shaped, bright white, with α - haemolytic halo colonies	positive	-	0	0	0	0	4	0	0	0	5	93	95	98	92	88	98	96	86	0

<i>Fusobacterium nucleatum</i>			Large, flat, irregular, whitish to pinkish and slightly grainy colonies	negative	-	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	45	0	36	99
<i>Campylobacter rectus</i> (formerly <i>Wolinella</i> spp.)			Small colonies, growing inside agar, translucent, slightly pink colour, round and convex. The center is higher than the rest, with shiny lines and points which are the limits of the “valley”	negative	-	0	0	0	0	0	0	5	0	0	0	5	0	0	0	0	88	0	0	0
<i>Capnocytophaga</i> spp.			Gliding and translucent reddish colonies	negative	-	0	42	0	89	96	86	2	1	78	76	99	99	90	99	99	95	13	0	
<i>Actinomyces odontololyticus</i>			Small, irregular and convex colonies with dark centre (reddish) and white ring around it. They are called “bull-eyes”	positive	-	0	4	15	86	96	46	5	92	78	76	99	99	90	99	99	95	13	0	

URE: Urea; BLTS: α -nitrophenyl- β , D-disaccharide; α ARA: α -nitrophenyl- α , L-arabinoside; ONPG: σ -nitrophenyl- β , D-galactoside; α GLU: α -nitrophenyl- α , D-glucoside; β GLU: α -nitrophenyl- β , D-glucoside; α GAL: α -nitrophenyl- α , D-galactoside; α FUC: α -nitrophenyl- α , L-fucoside; NAG: α -nitrophenyl-n-acetyl β , D-glucosaminide; PO4: α -nitrophenylphosphate; LGY: Leucyl-glycine- β -naphthylamide; GLY: Glycine- β -naphthylamide; PRO: Proline- β -naphthylamide; PAL: Phenylalanine- β -naphthylamide; ARG: Arginine- β -naphthylamide; SER: Serine- β -naphthylamide; PYR: Pyrrolidonyl- β -naphthylamide; IND: Tryptophan.

Table S1c. Additional tests in the identification of *P. gingivalis*, *P. intermedia*, *P. micra*, *T. forsythia*.

Bacterial species	B	I	G
<i>P. gingivalis</i>	positive	positive	negative
<i>P. intermedia</i>	negative	positive	positive
<i>P. micra</i>	negative	negative	negative
<i>T. forsythia</i>	positive	negative	positive

BANA test: N- α -Benzoyl-DL-arginine-2-Naftilamide);

Indole test: Kovacs reactive;

α -Glucuronidase: β -naphthyl- α -D-glucopironidase.

P. gingivalis: Porphyromonas gingivalis; *P. intermedia*: Prevotella intermedia; *P. micra*: Parvimonas micra; *T. forsythia*: Tannerella forsythia.

Supplementary Table S2. Demographic characteristics for the complete study population.

	ALL SUBJECTS (n=124)			
AGE	mean	SD	min	max
age	23.2	7.5	6	42

GENDER	fem	male	%fem	%male
gender	55	68	44.7%	55.3%

SYSTEMIC CONDITION	none	one	≥2	%none	%one	%≥2
n systemic conditions	61	42	21	49.2%	33.9%	16.9%

SPECIFIC CONDITIONS	n (no)	n (yes)	% (no)	% (yes)
cardiovascular diseases	123	1	99.2%	0.8%
cardiopathies	108	16	87.1%	12.9%
hypothyroidism	80	44	64.5%	35.5%
levothyroxine intake	82	42	66.1%	33.9%

SD, standard deviation; min, minimum; max, maximum, fem, female.

Supplementary Table S3. Periodontal clinical outcomes, expressed as means and standard deviations (SD), for the complete study population, and for each study group.

	ALL SUBJECTS (n=124)			
	sampling sites		Ramfjord teeth	
	mean	SD	mean	SD
probing depth	2.36	0.77	2.27	0.40
probing depth (proximal)			2.39	0.48
gingival recession	0.09	0.42	0.04	0.16
clinical attachment level	2.45	0.91	2.06	0.53
bleeding on probing	26.0%	30.4%	19.1%	18.9%
plaque index	61.0%	33.7%	60.5%	31.6%
plaque index (proximal)			66.2%	32.0%

	PERIODONTAL HEALTH (H) (n=62)				GINGIVITIS (G) (n=34)				PERIODONTITIS (P) (n=28)			
	sampling sites		Ramfjord teeth		sampling sites		Ramfjord teeth		sampling sites		Ramfjord teeth	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
probing depth	2.03	0.33	2.09	0.20	2.20	0.54	2.19	0.30	3.28	0.96	2.76	0.43
probing depth (proximal)			2.15	0.24			2.31	0.34			3.00	0.52
gingival recession	0.09	0.42	0.03	0.08	0.01	0.09	0.02	0.05	0.18	0.63	0.10	0.29
clinical attachment level	2.12	0.55	1.88	0.27	2.21	0.56	1.76	0.34	3.46	1.16	2.80	0.47
bleeding on probing	7.8%	16.2%	5.7%	6.8%	38.2%	29.7%	29.1%	17.2%	50.9%	30.8%	35.8%	17.7%
plaque index	46.7%	34.0%	46.0%	30.6%	72.8%	31.0%	74.0%	27.6%	77.7%	21.9%	75.0%	23.9%
plaque index (proximal)			52.9%	33.2%			79.3%	26.1%			78.5%	24.6%

Probing depth, probing depth (proximal), gingival recession, clinical attachment level, expressed in mm.

Supplementary Table S4. Microbiological findings (quantitative polymerase chain reaction, qPCR), with PCR counts expressed as means and standard deviations (SD), and frequencies of detection as percentages, for the complete study population, and for each study group.

	ALL SUBJECTS (n=124)				
qPCR counts	mean	SD	n	(+)	prev.
<i>A. actinomycetemcomitans</i>	6857	41713	124	11	8.87%
<i>P. gingivalis</i>	64064	458060	124	15	12.10%
<i>T. forsythia</i>	342644	1755259	124	65	52.42%

	PERIODONTAL HEALTH (n=62)					GINGIVITIS (n=34)					PERIODONTITIS (n=28)				
qPCR counts	mean	SD	n	(+)	prev.	mean	SD	n	(+)	prev.	mean	SD	no	(+)	prev.
<i>A. actinomycetemcomitans</i>	406	2077	62	4	6.45%	5601	22125	34	3	8.82%	22668	83438	24	4	14.29%
<i>P. gingivalis</i>	5591	35883	62	6	9.68%	6396	33297	34	4	11.76%	263564	947768	23	5	17.86%
<i>T. forsythia</i>	88497	249908	62	30	48.39%	45934	99763	34	16	47.06%	1265688	3569224	9	19	67.86%

n, number of samples; (+), samples with pathogen detection; prev., frequency of detection of the target pathogen.

A. actinomycetemcomitans: *Aggregatibacter actinomycetemcomitans*; *P. gingivalis*: *Porphyromonas gingivalis*; *T. forsythia*: *Tannerella forsythia*.

Supplementary Table S5. Microbiological findings (culture), with counts (colony forming units per mL) expressed as means and standard deviations (SD), and frequencies of detection expressed as percentage, for the complete study population, and for each study group.

Culture counts	ALL SUBJECTS (n=124)				
	mean	SD	n	(+)	prev.
Total anaerobic	2832524	2995872	124	124	100.0%
<i>A. actinomycetemcomitans</i>	0	0	124	0	0.0%
<i>P. gingivalis</i>	24860	136809	124	26	21.0%
<i>P.intermedia</i>	8469	34782	124	51	41.1%
<i>T. forsythia</i>	2790	15321	124	6	4.8%
<i>P.micra</i>	1606	12498	124	6	4.8%
<i>F. nucleatum</i>	38830	56858	124	116	93.5%
<i>C. rectus</i>	4694	44963	124	9	7.3%
<i>E. corrodens</i>	5802	20211	124	25	20.2%
<i>Capnocytophaga</i> spp.	2758	11568	124	16	12.9%
<i>A. odontolyticus</i>	20726	140018	124	7	5.6%

Culture counts	PERIODONTAL HEALTH (n=62)					GINGIVITIS (n=34)					PERIODONTITIS (n=28)				
	mean	SD	n	(+)	prev.	mean	SD	n	(+)	prev.	mean	SD	n	(+)	prev.
Total anaerobic	2026694	2352873	62	62	100.0%	3056088	3139308	34	34	100.0%	4345393	3522364	28	28	100.0%
<i>A. actinomycetemcomitans</i>	0	0	62	0	0.0%	0	0	34	0	0.0%	0	0	28	0	0.0%
<i>P. gingivalis</i>	4292	14613	62	11	17.7%	20015	87615	34	7	20.6%	76286	267689	28	8	28.6%
<i>P.intermedia</i>	3082	11140	62	22	35.5%	13818	51280	34	14	41.2%	13900	43400	28	15	53.6%
<i>T. forsythia</i>	97	762	62	1	1.6%	4412	14604	34	3	8.8%	6786	27763	28	2	7.1%
<i>P.micra</i>	2244	16507	62	4	6.5%	1765	8694	34	2	5.9%	0	0	28	0	0.0%
<i>F. nucleatum</i>	37845	59718	62	60	96.8%	47663	65393	34	29	85.3%	30286	35602	28	27	96.4%
<i>C. rectus</i>	806	4168	62	3	4.8%	15324	85673	34	4	11.8%	393	1892	28	2	7.1%
<i>E. corrodens</i>	2677	13408	62	8	12.9%	5300	18295	34	6	17.6%	13329	31052	28	11	39.3%
<i>Capnocytophaga</i> spp.	1968	9377	62	6	9.7%	5588	17785	34	7	20.6%	1071	3150	28	3	10.7%
<i>A. odontolyticus</i>	12258	54153	62	5	8.1%	10000	58310	34	1	2.9%	52500	277804	28	1	3.6%

SD, standard deviation; (+), samples with pathogen detection; prev., frequency of detection of the target pathogen.

A. actinomycetemcomitans: *Aggregatibacter actinomycetemcomitans*; *P. gingivalis*: *Porphyromonas gingivalis*; *T. forsythia*: *Tannerella forsythia*; *P. micra*: *Parvimonas micra*; *F. nucleatum*: *Fusobacterium nucleatum*; *C. rectus*: *Campylobacter rectus*; *E. corrodens*: *Eikenella corrodens*; *A. odontolyticus*: *Actinomyces odontolyticus*.

Supplementary Table S6. Microbiological findings (culture), with proportions of total anaerobic microbiota in percentage, expressed as means and standard deviations (SD), for the complete study population, and for each study group.

	ALL SUBJECTS (n=124)	
Proportions	mean	SD
<i>A. actinomycetemcomitans</i>	0.00%	0.00%
<i>P. gingivalis</i>	0.60%	2.22%
<i>P.intermedia</i>	0.31%	1.08%
<i>T. forsythia</i>	0.06%	0.36%
<i>P.micra</i>	0.15%	1.24%
<i>F. nucleatum</i>	2.20%	3.11%
<i>C. rectus</i>	0.10%	0.70%
<i>E. corrodens</i>	0.22%	0.79%
<i>Capnocytophaga</i> spp.	0.16%	0.62%
<i>A. odontololyticus</i>	0.99%	6.27%

	PERIODONTAL HEALTH (n=62)		GINGIVITIS (n=34)		PERIODONTITIS (n=28)	
Proportions	mean	SD	mean	SD	mean	SD
<i>A. actinomycetemcomitans</i>	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
<i>P. gingivalis</i>	0.25%	0.96%	0.39%	1.27%	1.63%	4.13%
<i>P.intermedia</i>	0.22%	0.81%	0.38%	1.37%	0.44%	1.23%
<i>T. forsythia</i>	0.01%	0.10%	0.12%	0.57%	0.11%	0.39%
<i>P.micra</i>	0.25%	1.72%	0.10%	0.44%	0.00%	0.00%
<i>F. nucleatum</i>	2.42%	3.27%	2.35%	3.40%	1.53%	2.25%
<i>C. rectus</i>	0.04%	0.19%	0.26%	1.30%	0.04%	0.14%
<i>E. corrodens</i>	0.12%	0.66%	0.24%	0.74%	0.41%	1.06%
<i>Capnocytophaga</i> spp.	0.16%	0.79%	0.22%	0.48%	0.06%	0.19%
<i>A. odontololyticus</i>	1.50%	8.44%	0.36%	2.09%	0.63%	3.35%

A. actinomycetemcomitans: *Aggregatibacter actinomycetemcomitans*; *P. gingivalis*: *Porphyromonas gingivalis*; *T. forsythia*: *Tannerella forsythia*; *P. micra*: *Parvimonas micra*; *F. nucleatum*: *Fusobacterium nucleatum*; *C. rectus*: *Campylobacter rectus*; *E. corrodens*: *Eikenella corrodens*; *A. odontolyticus*: *Actinomyces odontolyticus*.