Bacillus as a potential diagnostic marker for yellow tongue coating

Supporting Information Appendix

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Supporting Figures

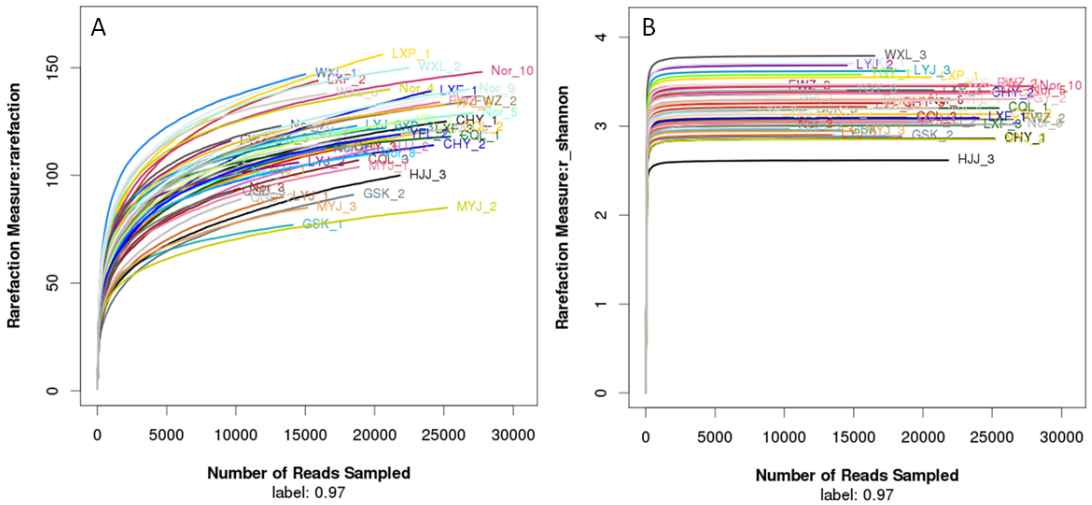

Fig.S1. Rarefaction curves (A) alpha diversity of all 43 samples based on species-level OTUs; (B) Shannon rarefaction curves of 43 samples.

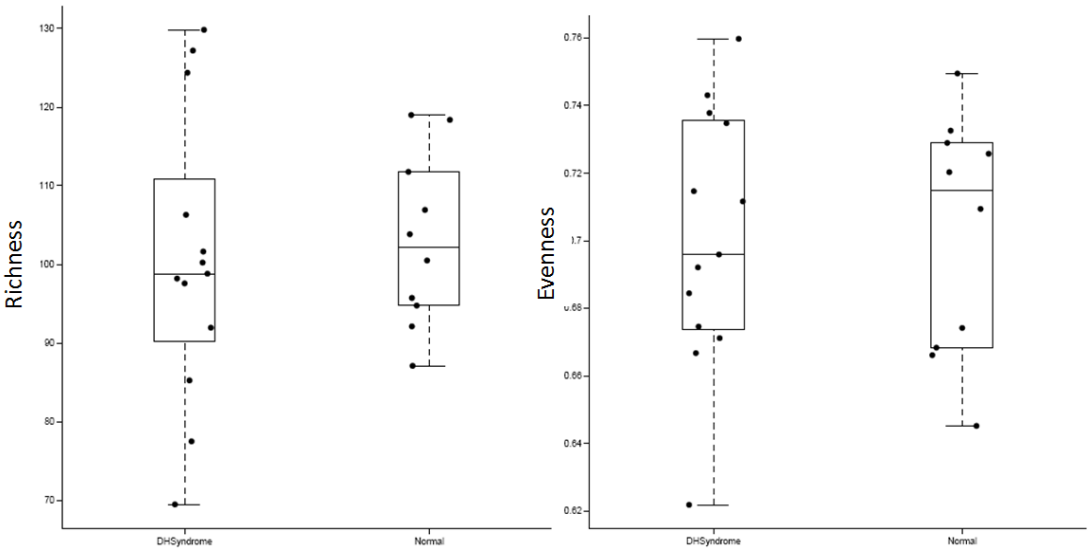

Fig.S2. Richness and evenness for the OUTs observed in this study for CEG yellow tongue caoting group (n=13) and healthy control (n=10) . By the Student's t-test, the two groups had no significant difference in richness and evenness(*P*=0.126 and *P*=0.381 respectively).

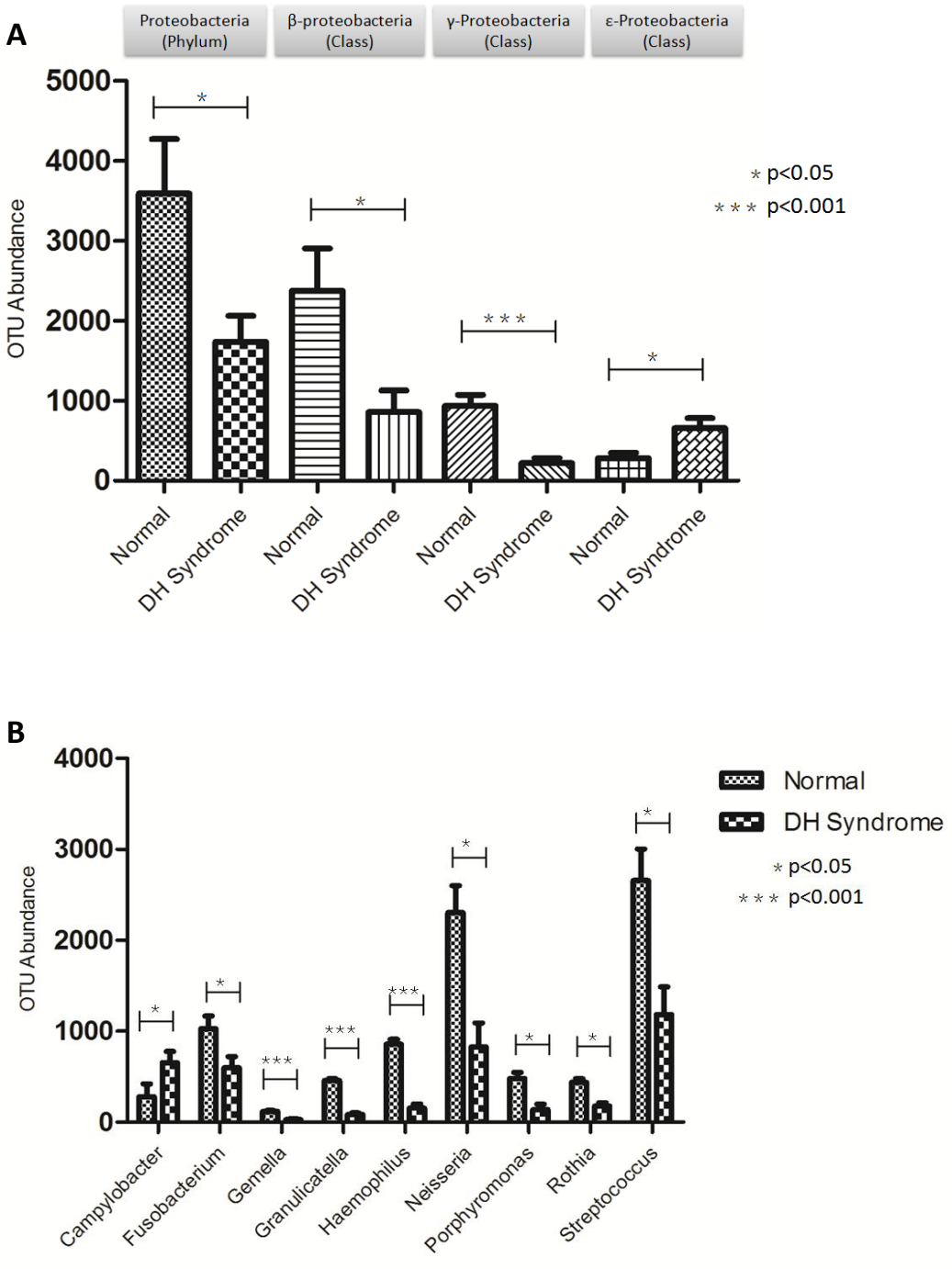

Fig.S3. Abundance of OTUs in patients with CEG yellow tongue coating and healthy controls. A:at Pylum level; B:at genus level Student's *t*-test was uesd; mean ± S.E.M. ("YTC":yellow tongue coating)

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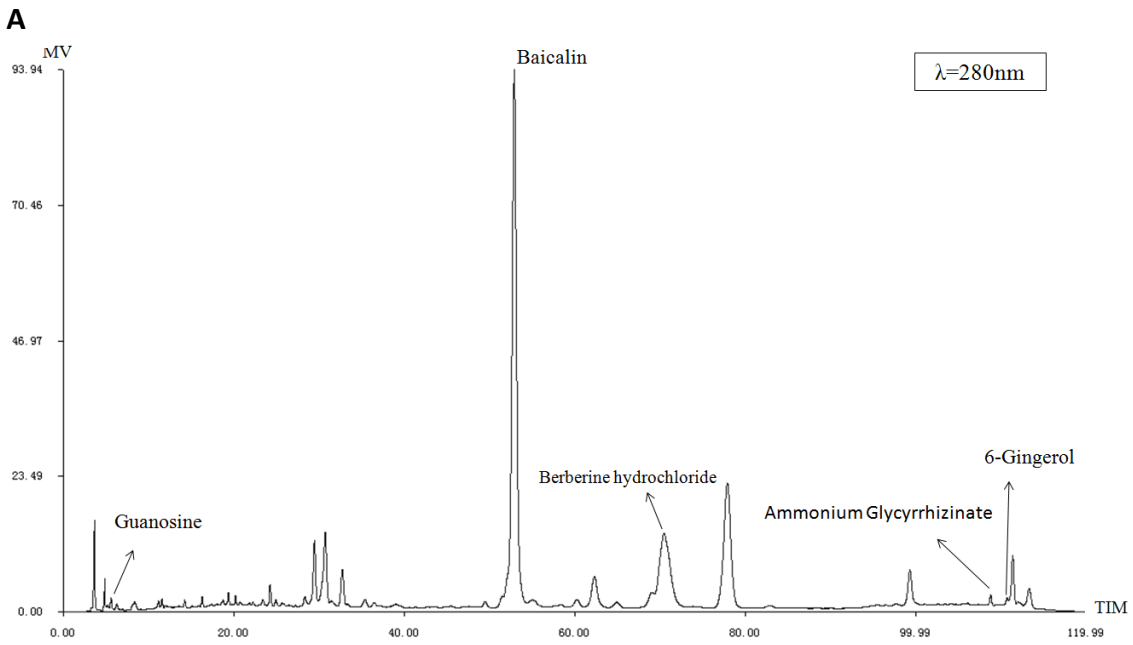
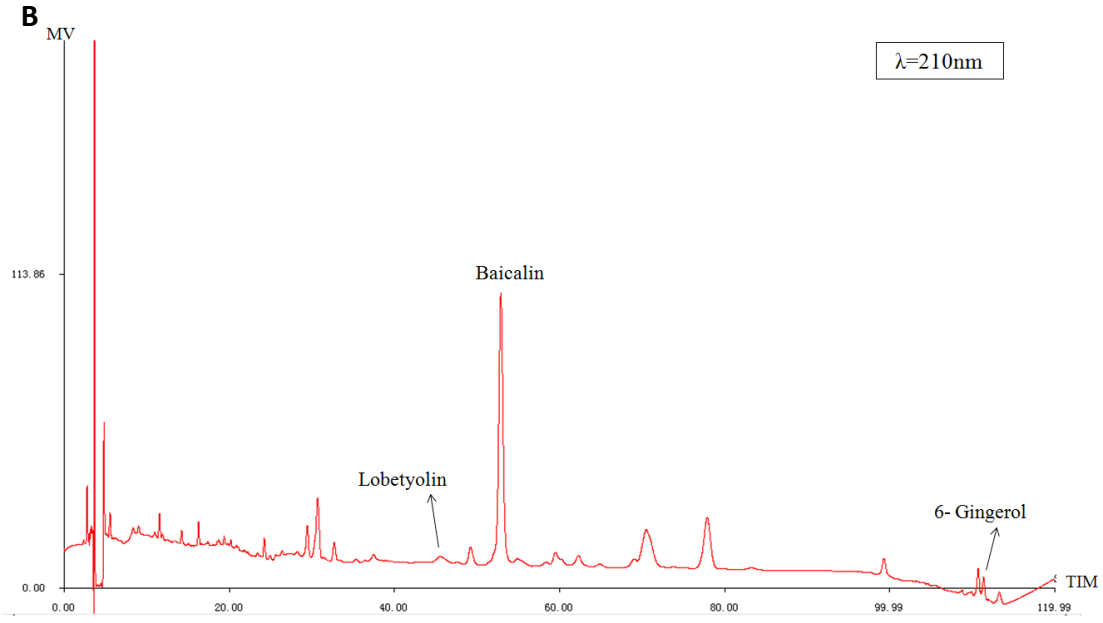

A
MV
93.94
70.46
46.97
23.49
0.00
Baicalin
λ=280nm
Berberine hydrochloride
6-Gingerol
Ammonium Glycyrrhizinate
Guanosine
TIM
0.00
20.00
40.00
60.00
80.00
99.99
119.99

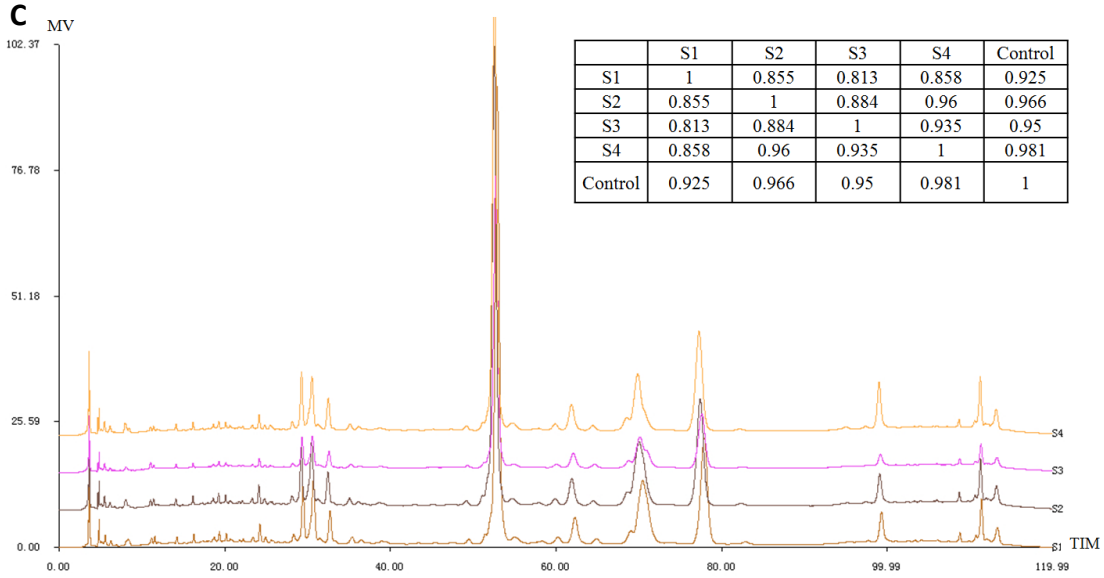
70

71


B
MV
λ=210nm
113.86
0.00
Baicalin
Lobetyolin
6- Gingerol
TIM
0.00
20.00
40.00
60.00
80.00
99.99
119.99

72

C



	S1	S2	S3	S4	Control
S1	1	0.855	0.813	0.858	0.925
S2	0.855	1	0.884	0.96	0.966
S3	0.813	0.884	1	0.935	0.95
S4	0.858	0.96	0.935	1	0.981
Control	0.925	0.966	0.95	0.981	1

Fig.S4. Quality control of Ban Xia Xie Xin decoction. A： HPLC graphs of main chemicals in extract preparations at 280nm; B : HPLC graphs of main chemicals in extract preparations at 210nm; C: HPLC graphs of Ban Xia Xie Xin water extract preparations after 7 days of storage and between 2 batches. (S1: the first batch herbs water extract; S2:S1 stored for 7 days at 4℃ ; S3: the second batch herbs water extract; S4: S3 stored for 7 days at 4℃.) The table shows the similarity of each group.

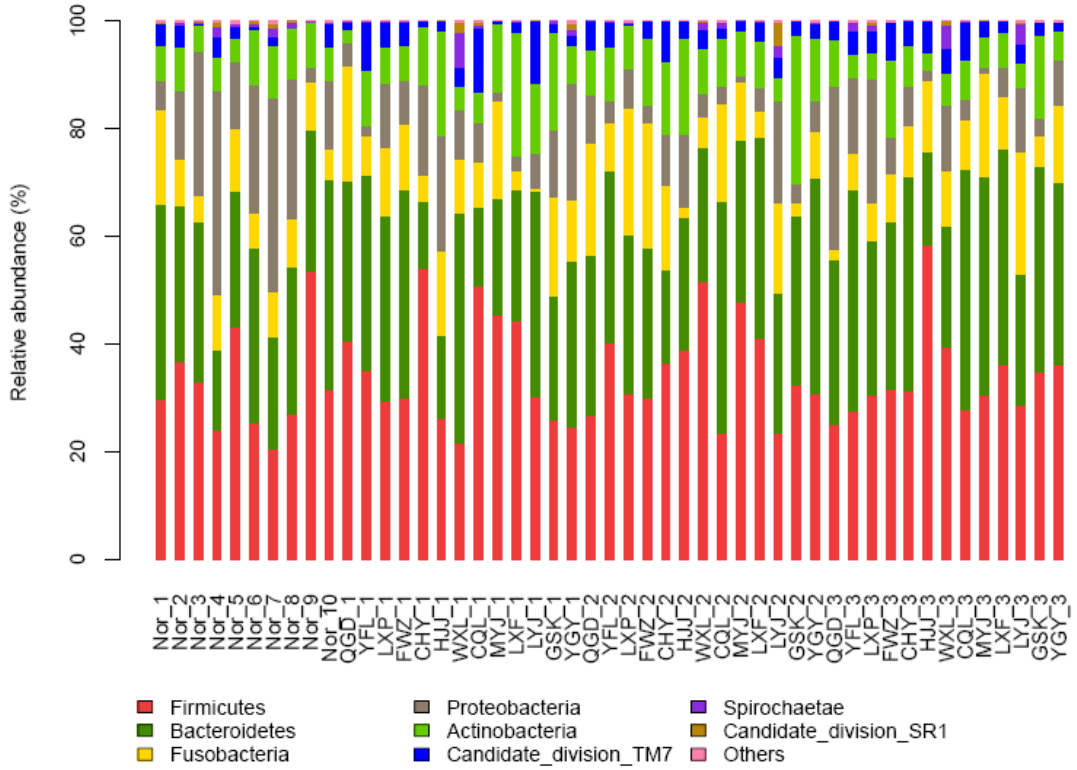


Fig.S5. 16S rRNA sequencing analysis and taxonomy classification of the tongue coating microbiome at the phylum level(All 4 groups)

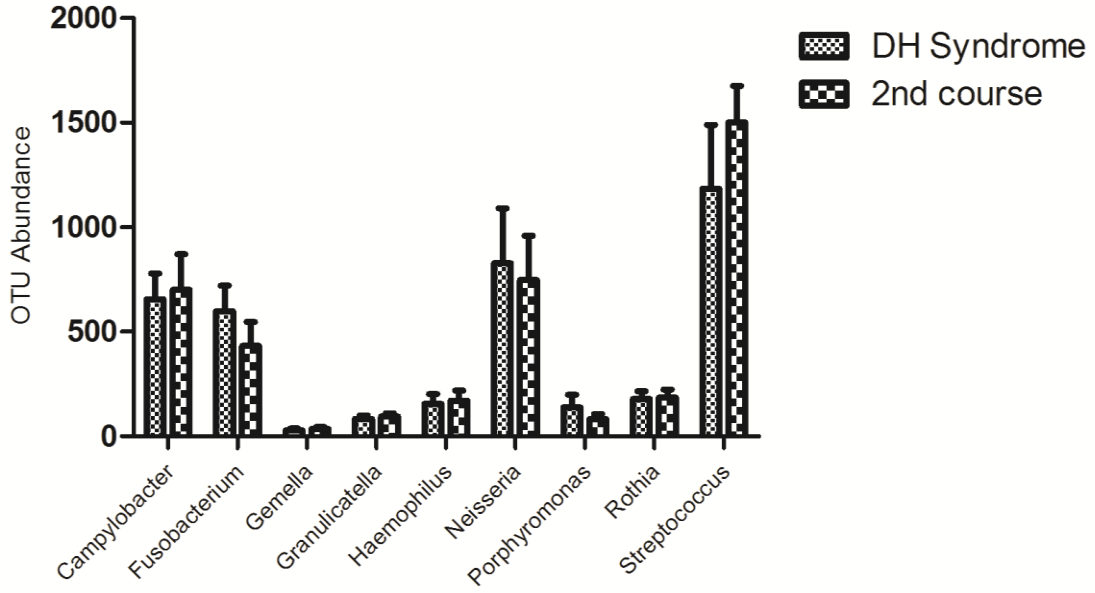


Fig.S6. Abundance of OTUs in patients with CEG yellow tongue coating and 2nd course treatment group (No significance were found)("YTC":yellow tongue coating)

Supporting Tables

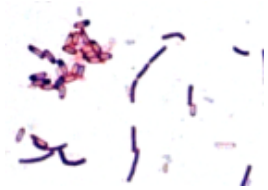
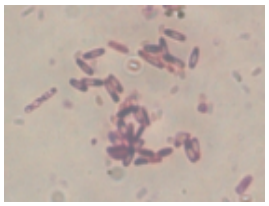
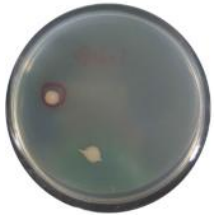
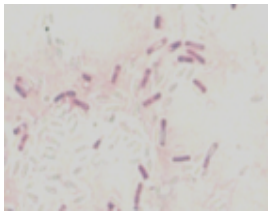
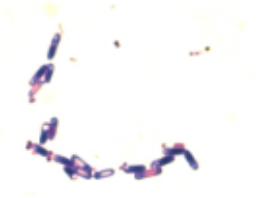
Table S1. Demographic information of the study participants in discover phase

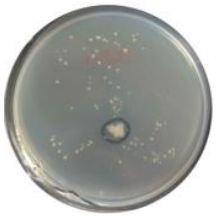
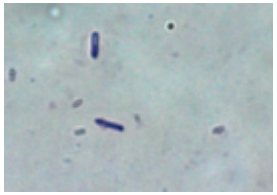
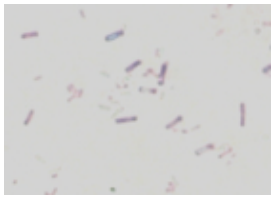
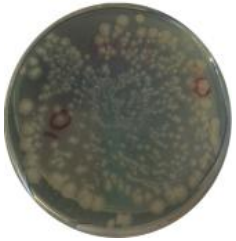
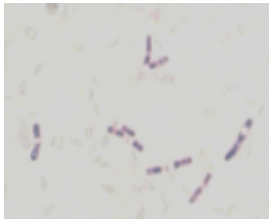
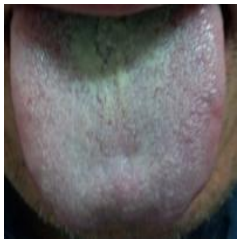
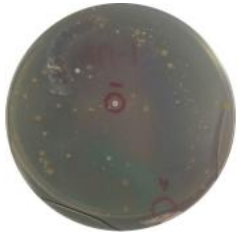
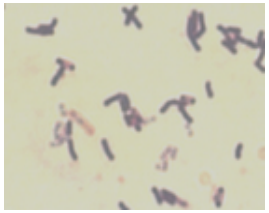
Demographic variable	Healthy volunteers **(n=10)**	CEG patients with yellow tongue coating **(n=13)**
Age(Mean±SD)	27.7±7.9	52.4±11.6
Male(%)	3(30)	7(53.8)
Female(%)	7(70)	6(46.2)
Smoking(%)	0	2
Helicobacter pylori（%）	0	0

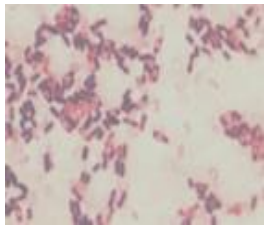
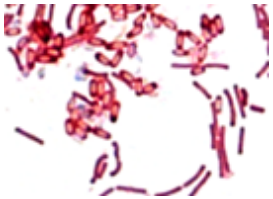
Table S2 Demographic information of the study participants in validation phase

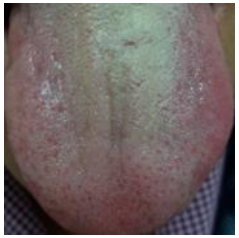
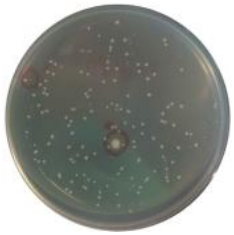
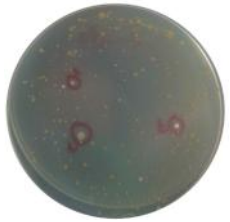
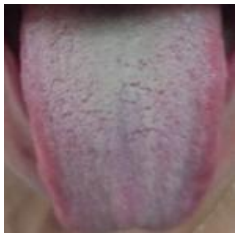
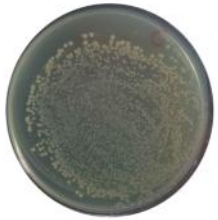
Demographic variable	Healthy volunteers **(n=11)**	Patients from Gastroenterology dept **(n=26)**		Patients from Cardiovascular dept **(n=14)**	
	White tongue coating only	Yellow tongue coating (n=16)	White tongue coating (n=10)	Yellow tongue coating (n=6)	White tongue coating (n=8)
Age(Mean±SD)	54.9±5.1	58.6±10.3	56.6±9.1	65.8±11.7	68.6±13.9
Male(%)	1（9.09）	5（31.25）	6（60）	3（50）	2（25）
Female(%)	10（90.91）	11（68.75）	4（40）	3（50）	6(75)
Smoking(%)	0	3(18.75)	0	0	2(25)

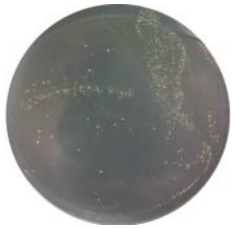
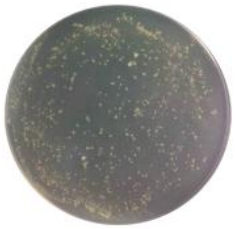
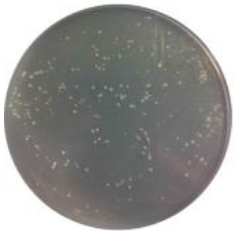
Table S3 The results of staining microscopy

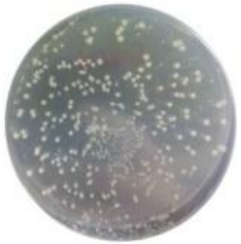
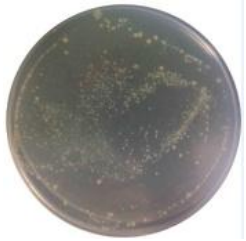
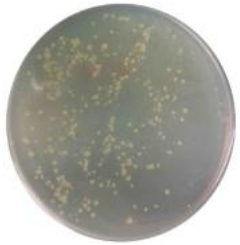
NO.	Disease	Tongue coating figure	Tongue coating color	Phe agar	Gram-staining	Bacillus positive/negative
GI-1	chronic atrophic gastritis		yellow			positive
GI-2	gastritis, bile reflux		yellow			positive
GI-3	chronic gastritis		yellow			positive
GI-4	chronic erosive gastritis		yellow			positive

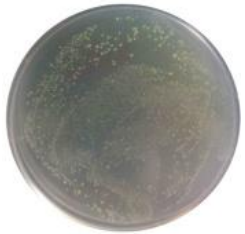
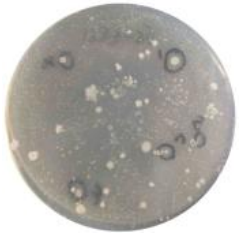
GI-5	chronic atrophic gastritis		yellow			positive
GI-6	chronic atrophic gastritis		yellow			positive
GI-7	chronic atrophic gastritis		yellow			positive
GI-8	chronic atrophic gastritis		yellow			positive

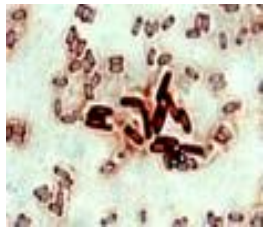
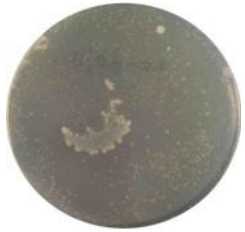
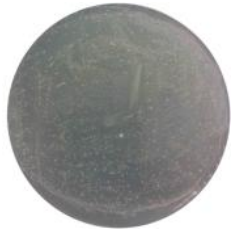
GI-9	chronic erosive gastritis		yellow			positive
GI-10	chronic atrophic gastritis		yellow			positive
GI-11	superficial gastritis		yellow			positive
GI-12	gastric polyposis,duodenal bulbar inflammation		yellow			positive

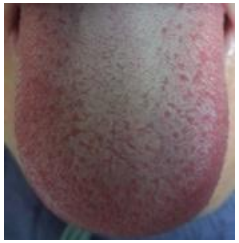
GI-13	chronic atrophic gastritis		yellow		\	negative
GI-14	chronic erosive gastritis		yellow		\	negative
GI-15	chronic atrophic gastritis		yellow		\	negative
GI-16	chronic erosive gastritis		yellow		\	negative

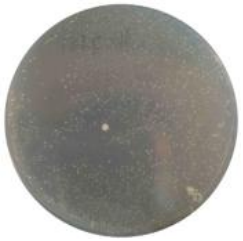
GI-17	superficial gastritis,gallbladder polyps		white		\	negative
GI-18	bile reflux.Ileocecal resection		white		\	negative
GI-19	superficial gastritis		white		\	negative
GI-20	gastric ulcer		white		\	negative

GI-21	chronic atrophic gastritis		white		\	negative
GI-22	enteritis		white		\	negative
GI-23	chronic atrophic gastritis		white		\	negative
GI-24	chronic atrophic gastritis		white		\	negative

GI-25	chronic atrophic gastritis		white		\	negative
GI-26	gastric ulcer		white		\	negative
Ca-1	Coronary heart disease		yellow			positive
Ca-2	Coronary heart disease		yellow			positive

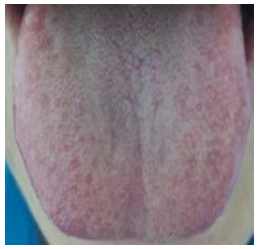
Ca-3	chronic stroke		yellow			positive
Ca-4	Coronary heart disease		yellow			positive
Ca-5	Coronary heart disease		yellow		\	negative
Ca-6	Coronary heart disease		yellow		\	negative

Ca-7	Coronary heart disease		white		\	negative
Ca-8	miocardial infarction		white		\	negative
Ca-9	Coronary heart disease		white		\	negative
Ca-10	hyperuricemia		white		\	negative

Ca-11	arrhythmia		white		\	negative
Ca-12	Coronary heart disease		white		\	negative
Ca-13	Coronary heart disease		white		\	negative
Ca-14	Coronary heart disease		white		\	negative

H-1	\		white		\	negative
H-2	\		white		\	negative
H-3	\		white		\	negative
H-4	\		white		\	negative

H-5	\		white		\	negative
H-6	\		white		\	negative
H-7	\		white		\	negative
H-8	\		white		\	negative

H-9	\		white		\	negative
H-10	\		white		\	negative
H-11	\		white		\	negative

Note:”GI-”:Samples from Gastroenterology department; “Ca-”:Samples from Cardiovascular department; “H-”:Samples from healthy volunteers