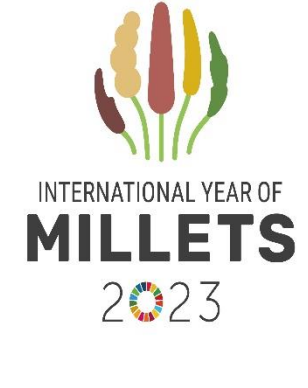


γ-aminobutyric acid (GABA) producing Lactobacilli exhibited anti-Alzheimer potential in an animal model

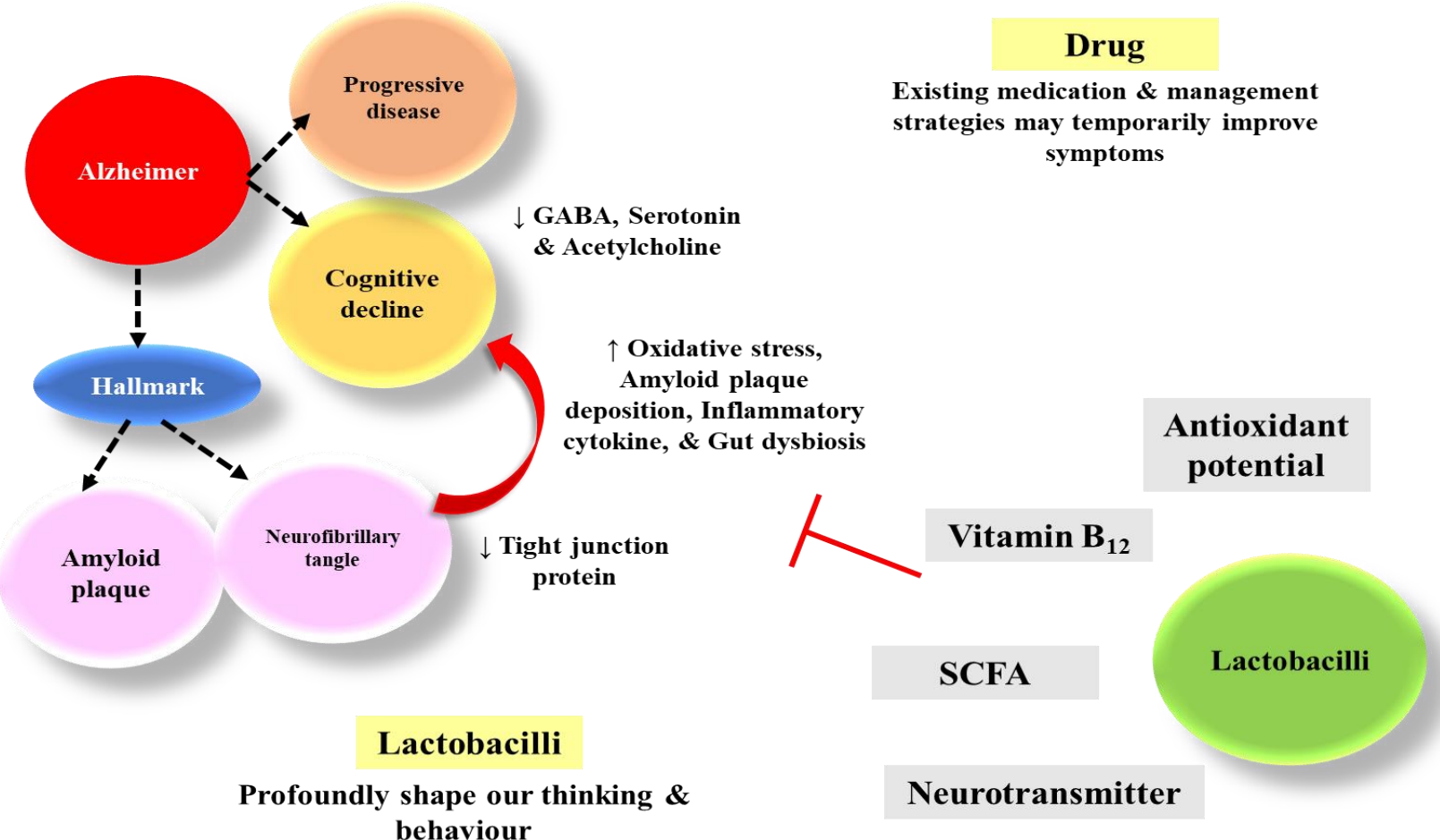


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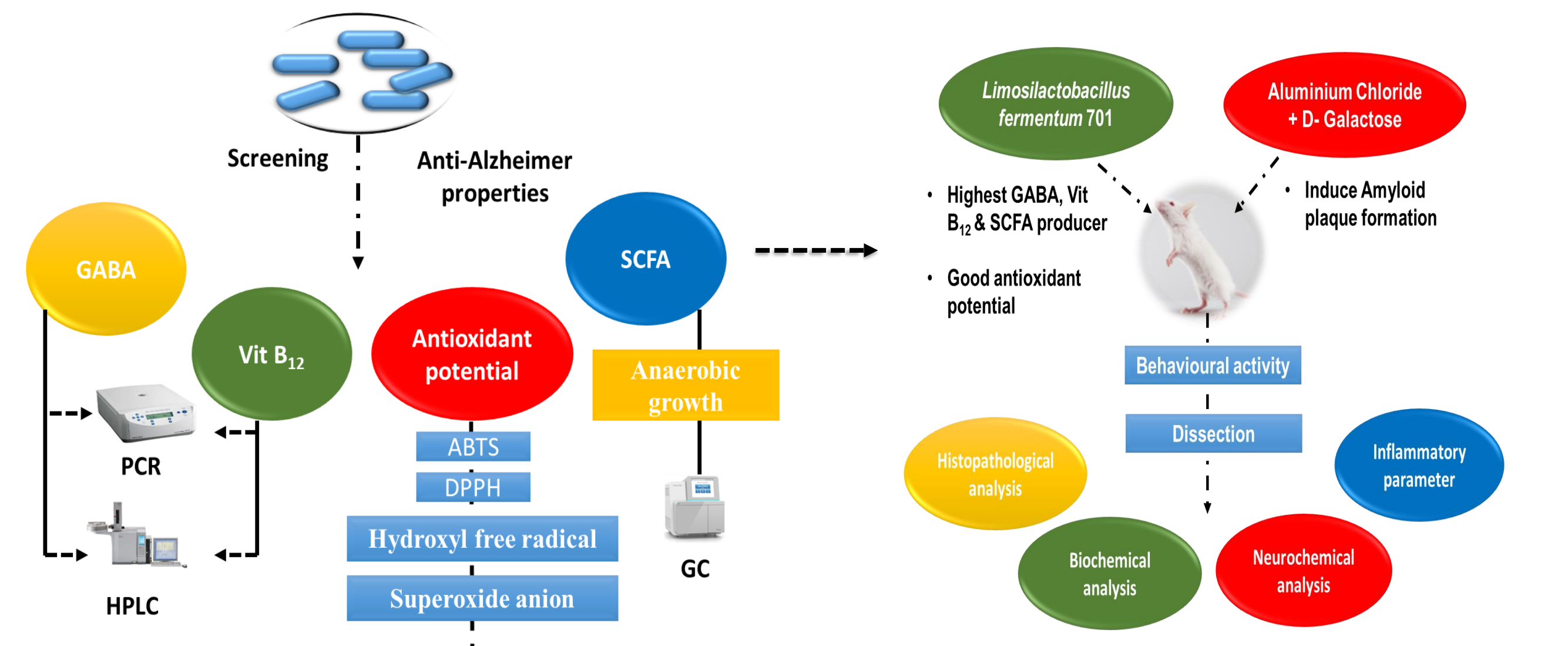
Introduction

>50 million people are affected by AD and number is expected to double until 2050



Alzheimer's patients commonly exhibit reduced brain tissue size, which is attributed to the formation of tangles resulting from abnormal twisting of 'tau' protein strands. Additionally, erratic folding of amyloid-beta peptides leads to the creation of plaques. An effective solution to counteract the side effects of commercially available drugs could be the use of GRAS status bacteria, such as probiotics.

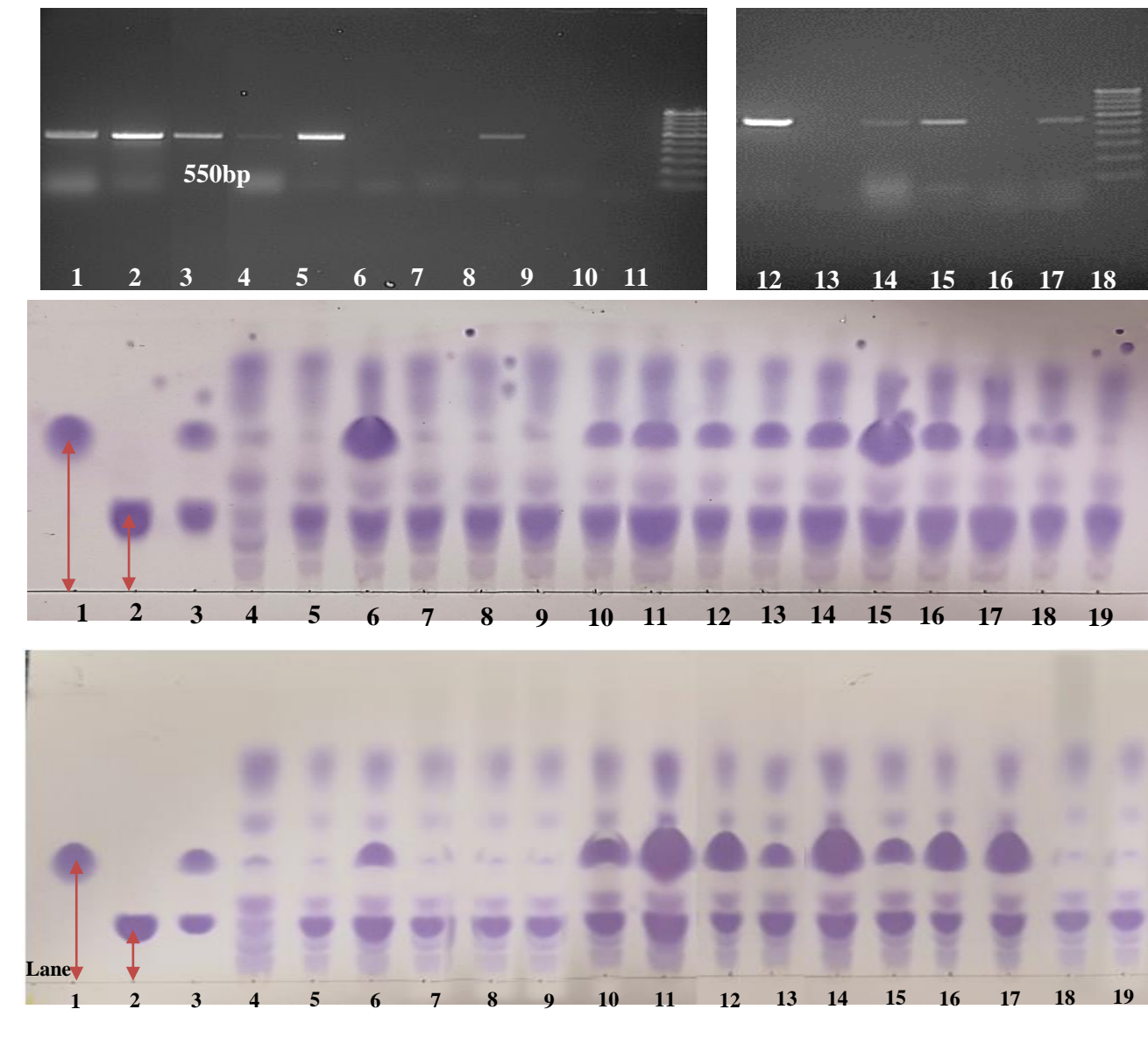
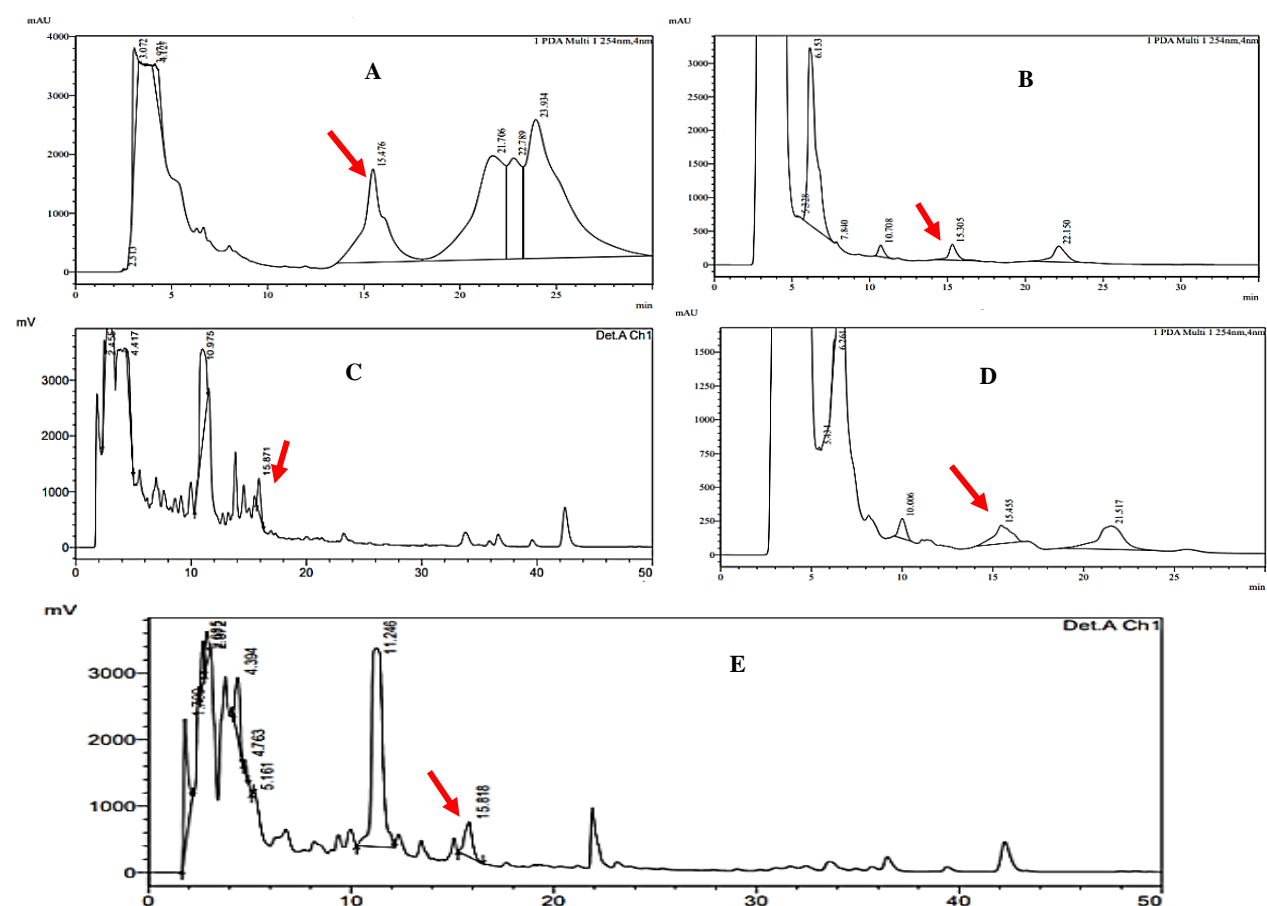
Material & Method



Result & Discussion

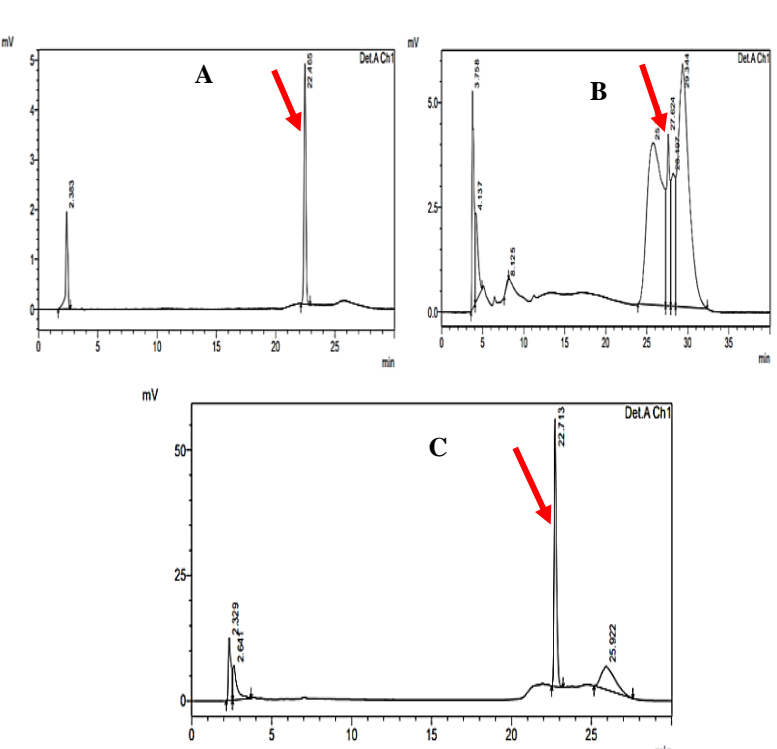
1. Screening of GABA:

Confirmation of high GABA producer in Lactobacilli using HPLC



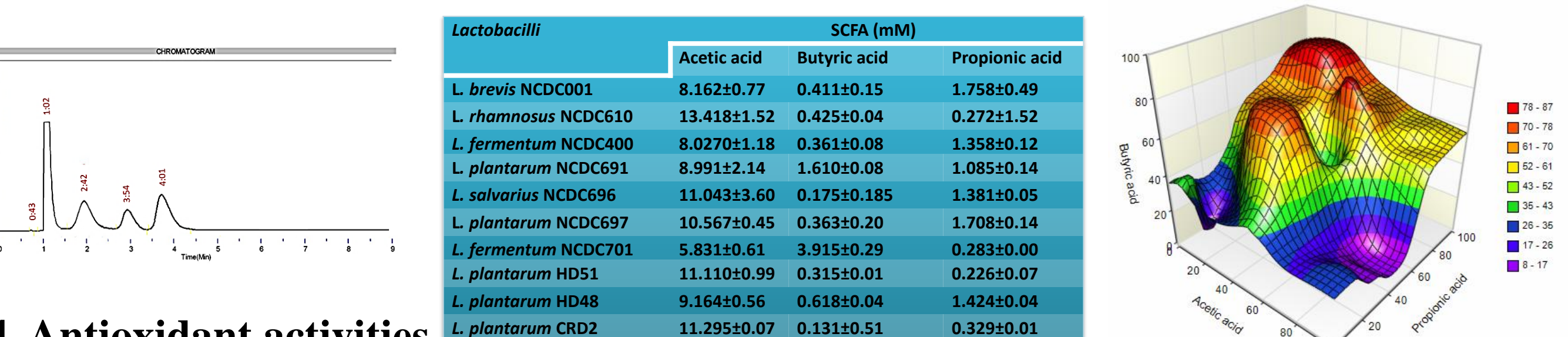
L. brevis NCDC001, *L. fermentum* NCDC400, *L. plantarum* CRD2, *L. plantarum* HD48, *L. plantarum* HD51, *L. rhamnosus* NCDC610, *L. fermentum* NCDC701, *L. plantarum* NCDC697, *L. plantarum* NCDC691 and *L. plantarum* NCDC696 were 85.29±47, 86.83±52, 85.59±49, 73.37±80, 91.11±50.5, 55.20±50.5, 99.43±51, 35.75±39, 40.03±55.5 and 50.17±60.5mM (P<0.0001) respectively

2. Screening on the basis of vitamin B₁₂ production

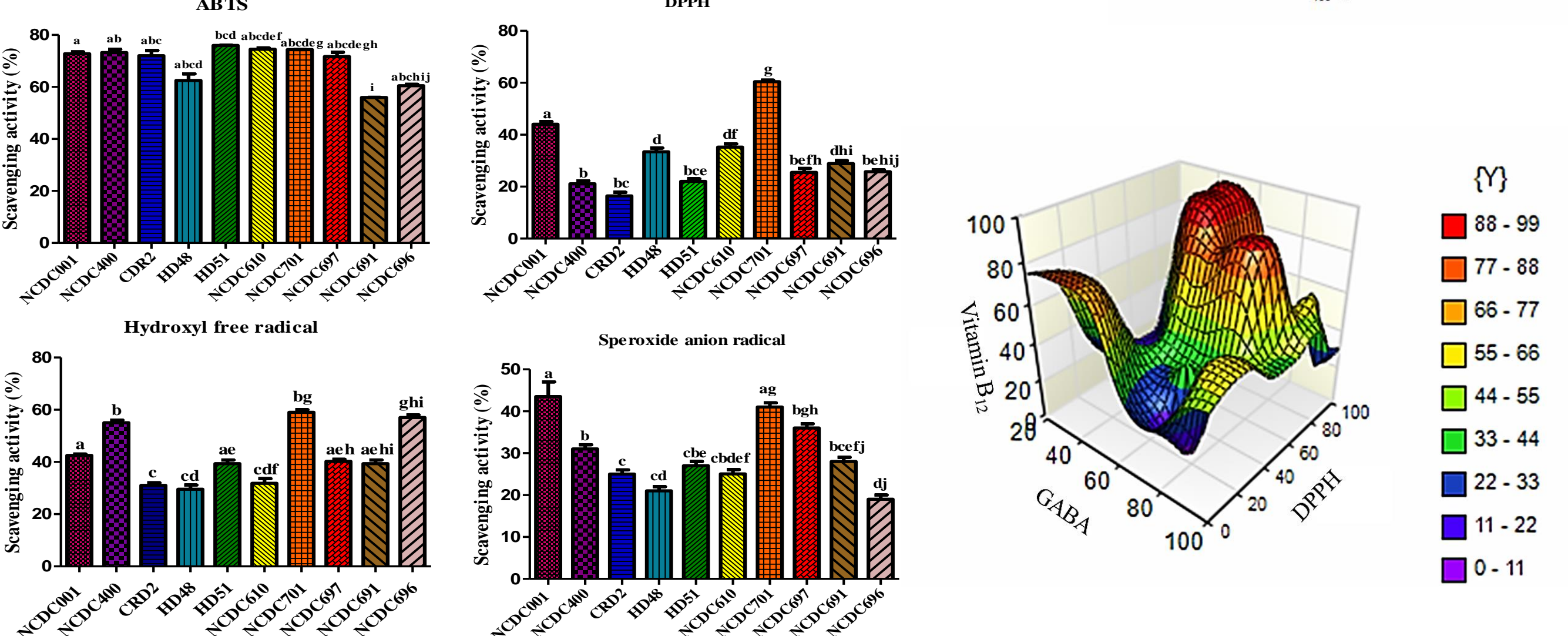


Lactobacillus species	Vitamin B ₁₂ (µg/mL)	
	Intracellular	Extracellular
<i>L. fermentum</i> NCDC400	1.160±49	2.762±27.5
<i>L. rhamnosus</i> NCDC610	0.5384±12	0.623±80.5
<i>L. fermentum</i> NCDC701	0.7120±50	2.780±67.5
<i>L. plantarum</i> NCDC691	1.184±17	2.044±21.5
<i>L. plantarum</i> HD48	2.643±10	Nil
<i>L. plantarum</i> HD51	0.315±6.5	Nil

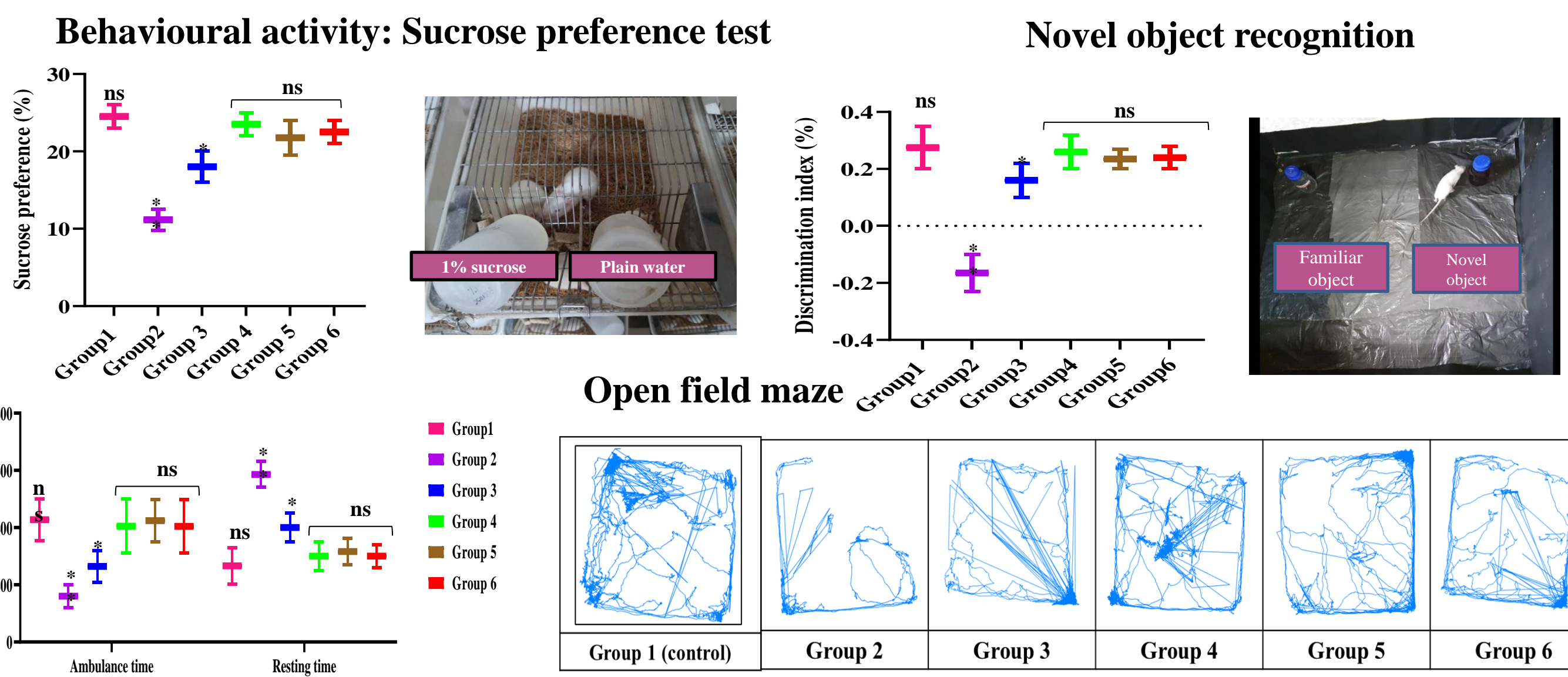
3. Estimation of SCFA by GC



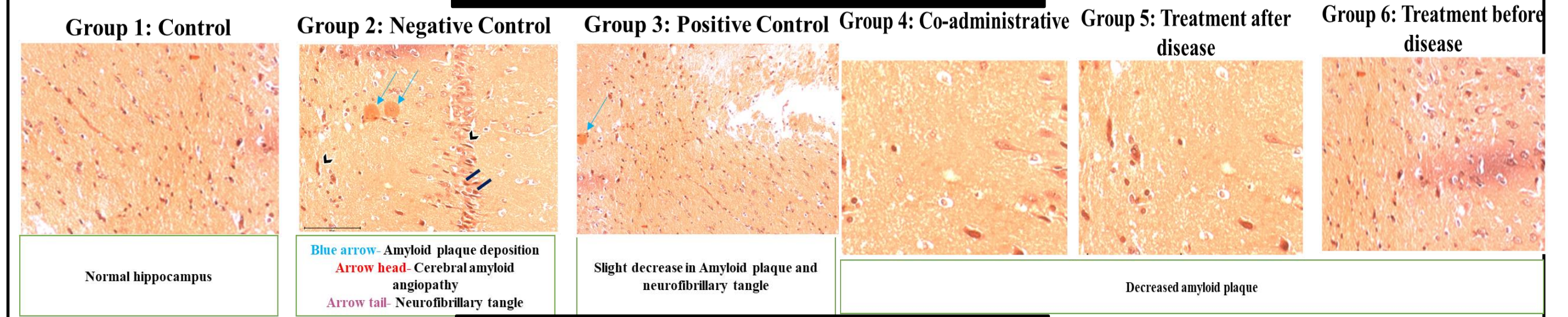
4. Antioxidant activities



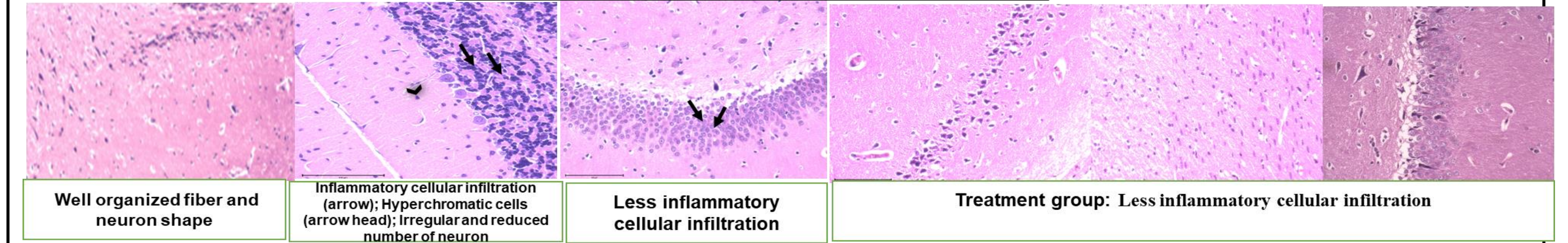
5. Effect of probiotic treatment on AICl₃/D-gal induced Alzheimer rat model



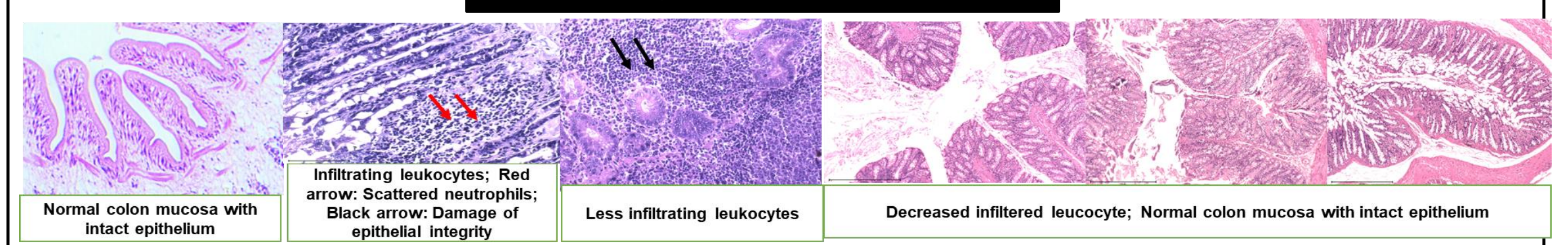
Histopathology- Congo red staining of brain



Haematoxylin and eosin stain- Brain



Haematoxylin and eosin stain- Colon



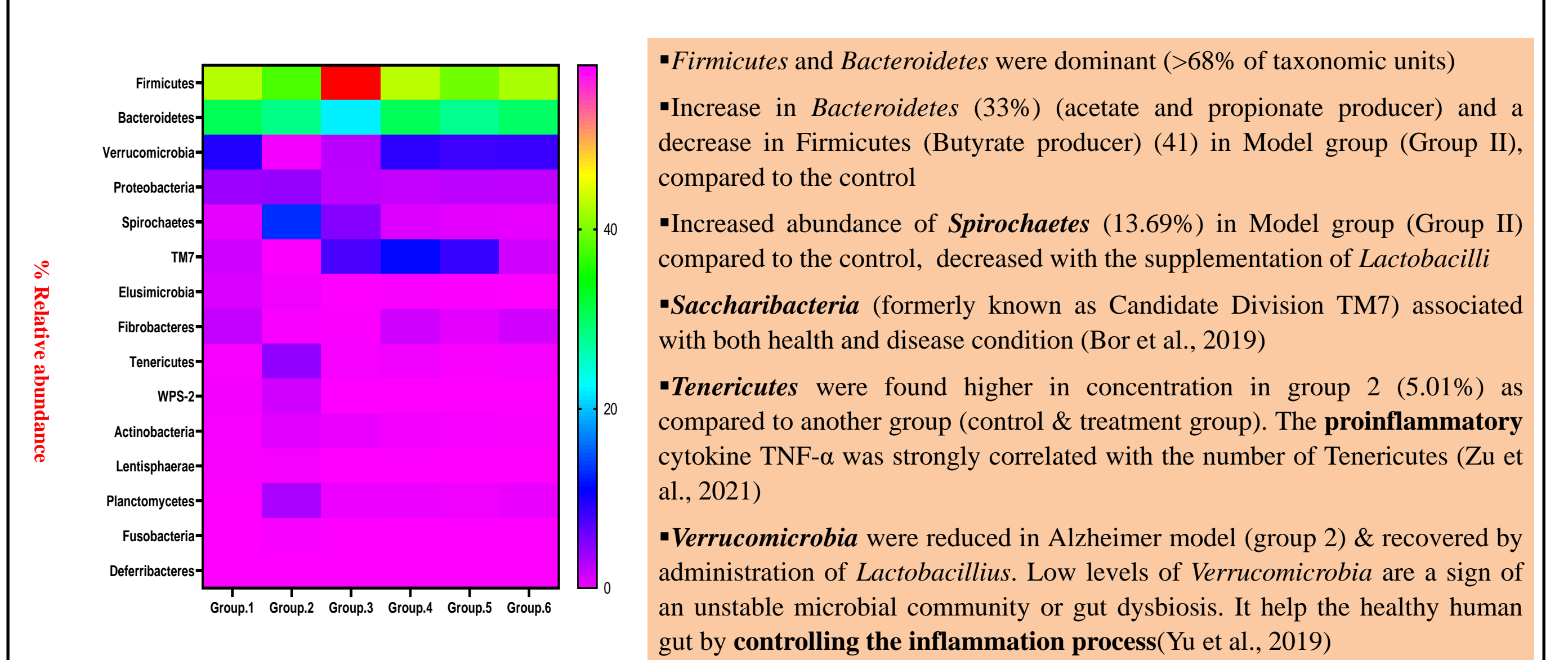
Neurochemical & inflammatory parameter

Parameter	Groups	
	Alzheimer model (Group 2)	Probiotic treatment (group 4,5,6)
B-amyloid plaque	Increase	Decrease
GABA	Decrease	Increase
Acetylcholine	Decrease	Increase
Serotonin	Decrease	Increase
IL-10	Decrease	Increase
TNF-α	Increase	Decrease
IL-6	Increase	Decrease

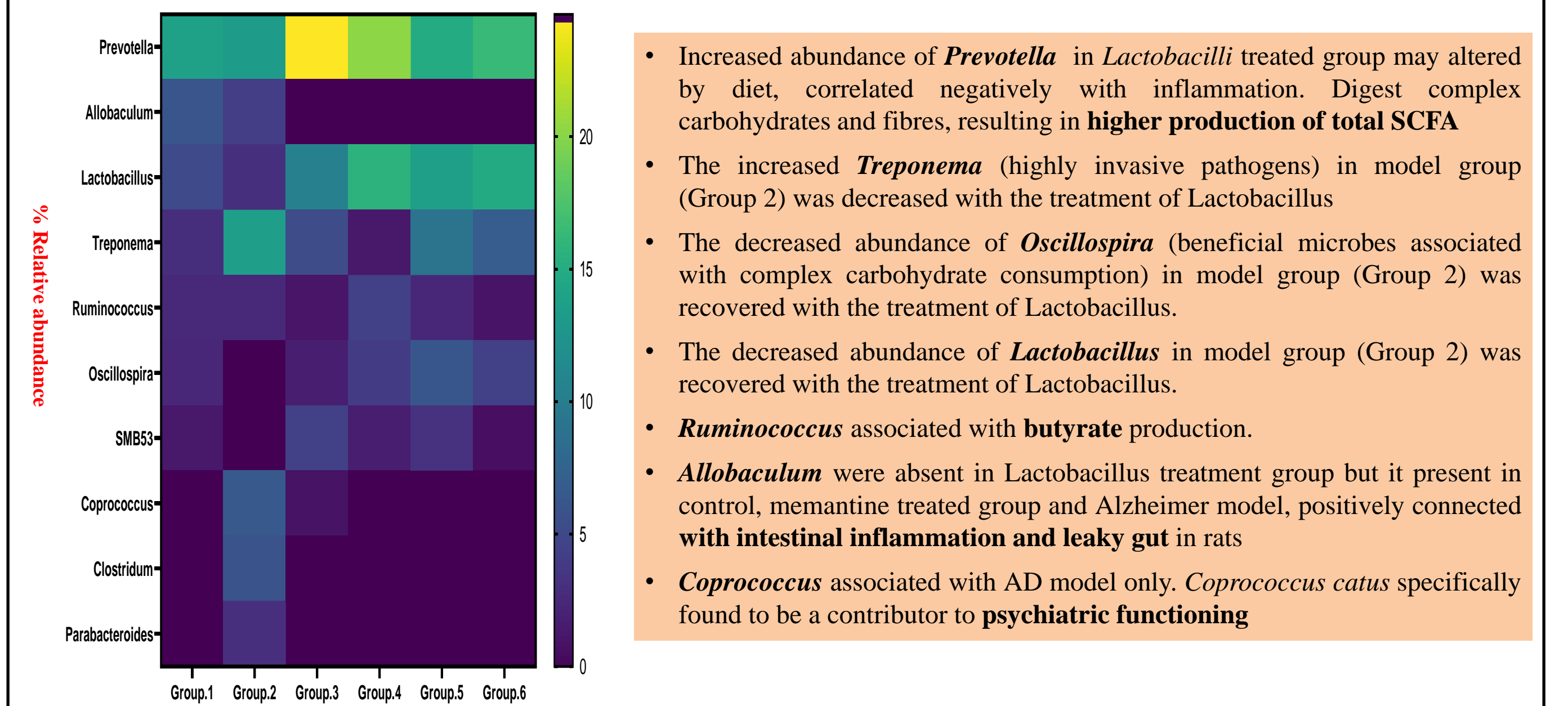
RT-PCR

Parameter	Groups	
	Alzheimer model (Group 2)	Probiotic treatment (group 4,5,6)
Tight junction		
Claudin	Decrease	Increase
ZO-1	Decrease	Increase
Occludin	Decrease	Increase
Inflammatory		
P65	Increase	Decrease
COX-2	Increase	Decrease
iNOS	Increase	Decrease

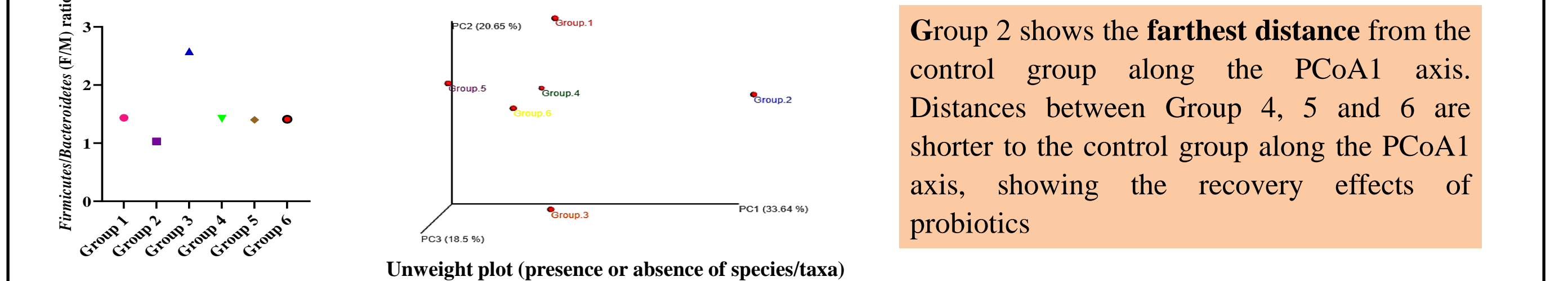
5. Determination of microbiota by 16S rRNA sequencing in fecal samples



- Firmicutes and Bacteroidetes were dominant (>68% of taxonomic units)
- Increase in Bacteroidetes (33%) (acetate and propionate producer) and a decrease in Firmicutes (Butyrate producer) (41) in Model group (Group II), compared to the control
- Increased abundance of Spirochaetes (13.69%) in Model group (Group II) compared to the control, decreased with the supplementation of Lactobacilli
- Saccharibacteria (formerly known as Candidate Division TM7) associated with both health and disease condition (Bor et al., 2019)
- Tenericutes were found higher in concentration in group 2 (5.01%) as compared to another group (control & treatment group). The proinflammatory cytokine TNF-α was strongly correlated with the number of Tenericutes (Zu et al., 2021)
- Verrucomicrobia were reduced in Alzheimer model (group 2) & recovered by administration of Lactobacillus. Low levels of Verrucomicrobia are a sign of an unstable microbial community or gut dysbiosis. It help the healthy human gut by controlling the inflammation process (Yu et al., 2019)

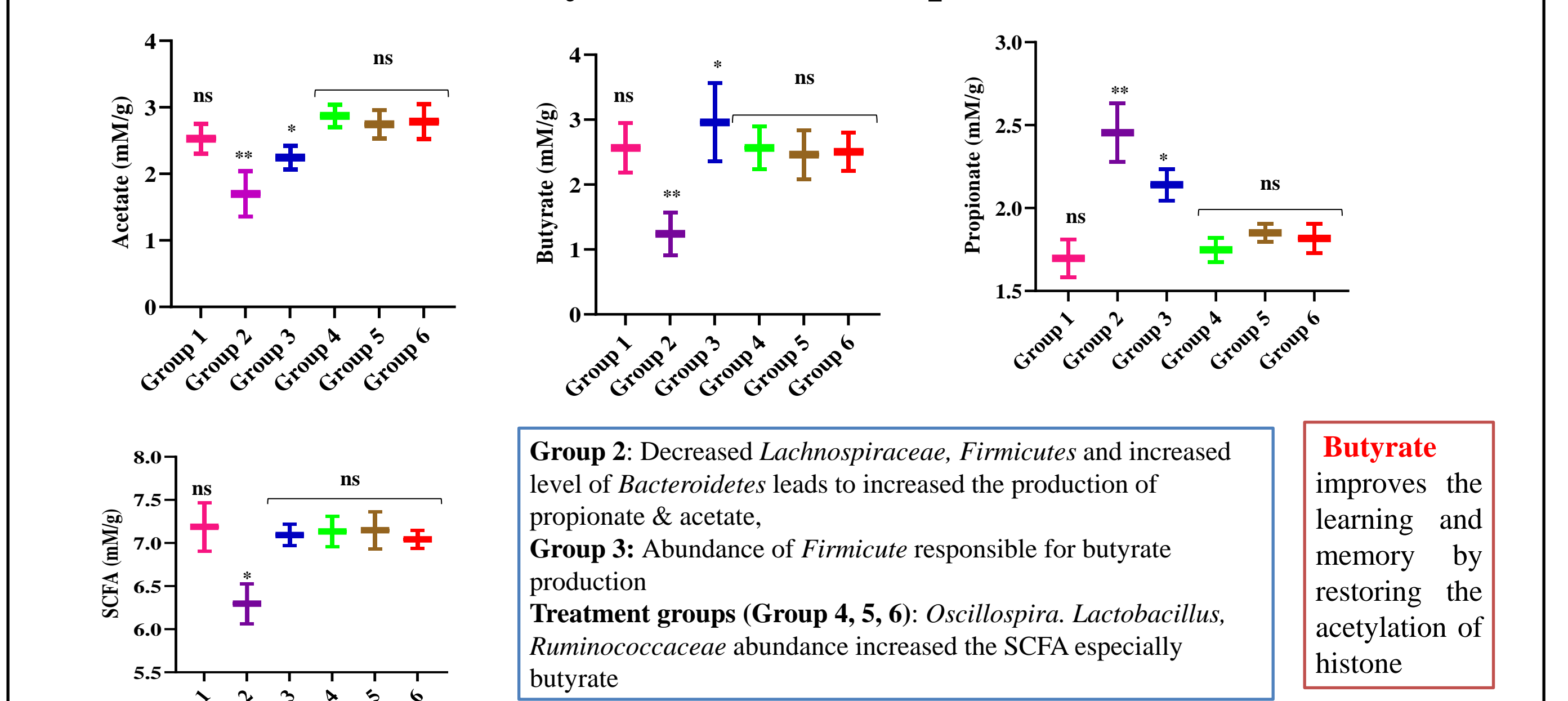


- Increased abundance of Prevotella in Lactobacilli treated group may altered by diet, correlated negatively with inflammation. Digest complex carbohydrates and fibres, resulting in higher production of total SCFA
- The increased Treponema (highly invasive pathogens) in model group (Group 2) was decreased with the treatment of Lactobacillus
- The decreased abundance of Oscillospira (beneficial microbes associated with complex carbohydrate consumption) in model group (Group 2) was recovered with the treatment of Lactobacillus.
- The decreased abundance of Lactobacillus in model group (Group 2) was recovered with the treatment of Lactobacillus.
- Ruminococcus associated with butyrate production.
- Allobaculum were absent in Lactobacillus treatment group but it present in control, memantine treated group and Alzheimer model, positively connected with intestinal inflammation and leaky gut in rats
- Coprococcus associated with AD model only. Coprococcus catus specifically found to be a contributor to psychiatric functioning



Group 2 shows the farthest distance from the control group along the PCoA1 axis. Distances between Group 4, 5 and 6 are shorter to the control group along the PCoA1 axis, showing the recovery effects of probiotics

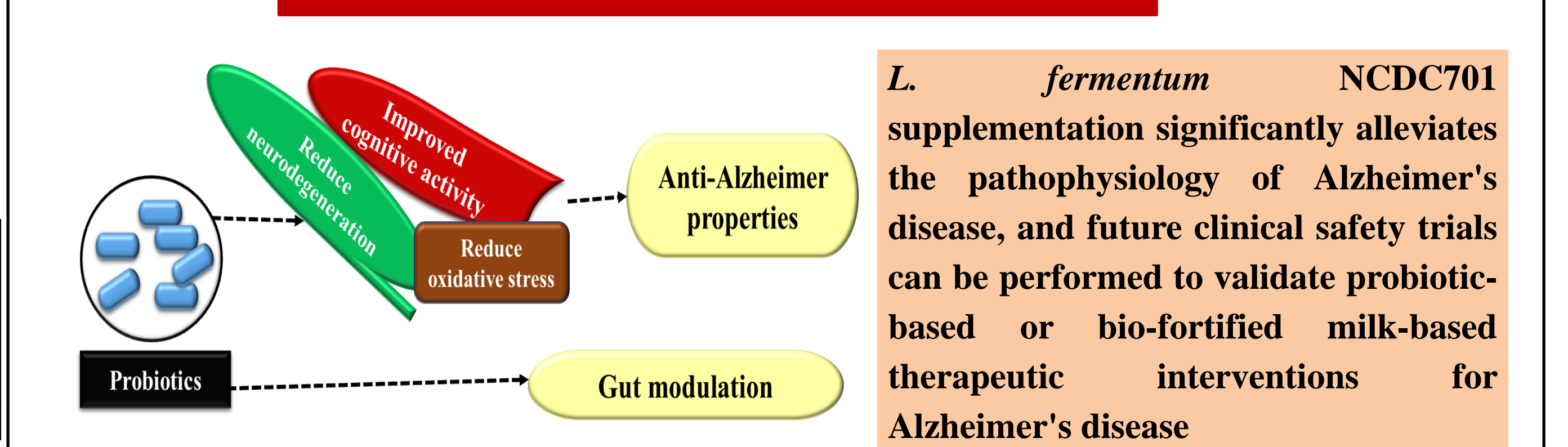
6. Estimation of SCFAs by GC in fecal samples



Group 2: Decreased Lachnospiraceae, Firmicutes and increased level of Bacteroidetes leads to increased the production of propionate & acetate, Group 3: Abundance of Firmicute responsible for butyrate production Treatment groups (Group 4, 5, 6): Oscillospira, Lactobacillus, Ruminococcaceae abundance increased the SCFA especially butyrate

Butyrate improves the learning and memory by restoring the acetylation of histone

Conclusion



L. fermentum NCDC701 supplementation significantly alleviates the pathophysiology of Alzheimer's disease, and future clinical safety trials can be performed to validate probiotic-based or bio-fortified milk-based therapeutic interventions for Alzheimer's disease