

**Table 4**

Relative distribution of yeast species in the biofilm and the soup as per D1–D2 LSU gene sequencing (% abundance of reads).

Species	Sample							
	Biofilm—day-3	Biofilm—day-7	Biofilm—day-14	Biofilm—day-21	Soup—day-3	Soup—day-7	Soup—day-14	Soup—day-21
<i>Candida stellimalicola</i>	59	72.2	71.3	65.3	61.5	17	68.5	56.5
<i>Candida tropicalis</i>	11.9	6.8	8.4	11.8	8.4	6	11.4	7.2
<i>Candida parapsilosis</i>	2	4	2.6	2.5	2.9	3.3	6.5	6.5
<i>Lachancea thermotolerans</i>	7.2	–	–	2.5	1.3	4.8	0.2	1
<i>Lachancea fermentati</i>	2.3	2.5	1.9	4.2	15.1	51.1	3.6	20
<i>Lachancea kluyveri</i>	–	0.3	–	0.1	–	1.5	–	–
<i>Eremothecium cymbalariae</i>	2.4	0.8	2.9	0.9	0.1	–	0.2	0.6
<i>Eremothecium ashbyii</i>	–	–	–	–	–	0.1	–	–
<i>Kluyveromyces marxianus</i>	2.3	2.6	2.5	2.5	2.1	4.8	2	0.9
<i>Debaryomyces hansenii</i>	0.6	2	0.9	1.5	1.5	0.6	2.2	0.6
<i>Pichia mexicana</i>	0.7	0.3	0.7	0.9	0.9	0.6	0.7	0.7
<i>Meyerozyma caribbica</i>	1.5	0.7	0.3	0.7	0.8	0.9	0.8	0.5
<i>Meyerozyma guilliermondii</i>	0.3	–	–	–	–	–	–	–
<i>Zygowilliopsis californica</i>	1	0.3	0.4	0.2	0.4	0.9	0.3	–
<i>Saccharomyces cerevisiae</i>	0.8	0.4	0.2	–	0.2	0.1	–	–
<i>Saccharomycopsis fibuligera</i>	0.7	0.6	0.4	0.6	0.6	0.7	0.6	0.7
<i>Hanseniaspora uvarum</i>	0.5	0.7	2.5	0.3	0.8	0.4	0.2	0.5
<i>Hanseniaspora meyeri</i>	–	–	–	0.1	–	–	0.8	–
<i>Hanseniaspora vineae</i>	0.1	0.1	–	0.3	0.2	1.2	–	0.5
<i>Merimbla ingelheimense</i>	–	1.2	1	–	–	–	–	–
<i>Sporopachydermia lactativora</i>	–	0.8	1	0.3	–	0.2	0.8	0.6
<i>Kazachstania telluris</i>	0.5	–	0.6	0.4	–	1.7	–	0.2
<i>Kazachstania exigua</i>	–	–	–	–	–	0.3	–	–
<i>Starmeria amethionina</i>	0.3	1.4	–	2.6	1	1.8	0.4	–
<i>Starmeria caribaea</i>	–	0.7	–	–	–	–	–	–
Unclassified	3.5	0.3	1.4	0.8	1.0	1.5	0.2	1.8
Others	2.4	1.3	1	1.5	1.2	0.5	0.6	1.2

Note: “Unclassified” indicates the percentage of reads that could not be assigned to any species. Note: ‘–’ signifies below 0.1% or absent.

The low pH is attributed to the production of various organic acids during fermentation. The major organic acids found are acetic acid and gluconic acid. The concentration of both the acids increased steadily with time and reached  $16.57 \pm 0.9$  g/L and  $7.36 \pm 0.87$  g/L respectively after 21 days of fermentation (Table 7).

### 3.5.2. Ethanol and reducing sugar content

The ethanol concentration increased initially with time to reach a maximum value of about  $0.28 \pm 0.014$  g/L at day 7 of fermentation, followed by a decrease to about  $0.073 \pm 0.003$  g/L after 21 days of fermentation (Table 7). This decrease in ethanol concentration is due to its utilization by acetic acid bacteria to produce acetic acid. We observed a period of significant production of reducing sugar ( $8.2 \pm 0.7$  g/L) in the first 7 days of fermentation when sucrose was hydrolyzed to glucose and fructose by yeast invertase. This was followed by an intensive utilization of reducing sugar as

indicated by the reduction of its concentration to  $2.25 \pm 0.3$  g/L after 21 days of fermentation.

### 3.5.3. Black tea polyphenol and caffeine content

Theaflavin was found to increase by about 88.63% whereas thearubigin was decreased by about 47.02% after 21 days of fermentation. The caffeine content was decreased by about 40% after 21 days of fermentation (Table 7).

### 3.5.4. DSL production

The fermentation of sugared black tea might result in the production of certain metabolites which were not present in unfermented black tea. In our study we have detected DSL in Kombucha tea which was not found in unfermented black tea. The DSL concentration increased gradually with fermentation time and reached a concentration of  $2.24 \pm 0.1$  g/L after 21 days of fermentation (Table 7).

**Table 5**

Relative distribution of bacterial taxa in the biofilm and soup (% abundance).

Phylum	Sample		Family	Sample		Genus	Sample	
	Biofilm	Soup		Biofilm	Soup		Biofilm	Soup
Proteobacteria	88.5	65.7	Acetobacteraceae	88.5	63.5	<i>Komagataeibacter</i>	50.3	49.9
Firmicutes	–	11.2	Oscillatoriaceae	–	5.5	<i>Gluconobacter</i>	16.8	2.5
Cyanobacteria	–	7.1	Bifidobacteriaceae	–	2.3	<i>Lyngbya</i>	–	4.4
Actinobacteria	–	4.1	Ruminococcaceae	–	2.1	<i>Bifidobacterium</i>	–	2.3
Unclassified	11.4	11.4	Peptostreptococcaceae	–	1.7	<i>Collinsella</i>	–	0.7
Others	0.1	0.5	Coriobacteriaceae	–	1.3	<i>Enterobacter</i>	–	0.6
X	X	X	Lachnospiraceae	–	1.2	<i>Weissella</i>	–	0.5
X	X	X	Enterobacteriaceae	–	1.1	<i>Lactobacillus</i>	–	0.3
X	X	X	Leuconostocaceae	–	0.5	Unclassified	32.8	34.6
X	X	X	Erysipelotrichaceae	–	0.4	Others	0.1	4.2
X	X	X	Rhodobacteraceae	–	0.4	X	X	X
X	X	X	Lactobacillaceae	–	0.3	X	X	X
X	X	X	Unclassified	11.4	17.5	X	X	X
X	X	X	Others	0.1	2.2	X	X	X

Note: ‘–’ signifies below 0.1% or absent. ‘X’ signifies not applicable.