Hypercholesterolemia is a main risk factor of cardiovascular disease. Probiotics are a safe approach to reduce elevated cholesterol without any deleterious effect to human health. *Saccharomyces boulardii* CNCM I-745 probiotic properties are well documented in a context of intestinal dysbiosis. Recent *in vitro* and preclinical studies have suggested its potential effects on dyslipidemia. This is the first controlled study investigating the effects of *S. boulardii* CNCM I-745 on lipidemic profile and gut microbiota in a hamster hypercholesterolemic model. Daily administration (3 g/kg) of *S. boulardii* for 21 or 39 days in hamsters fed a 0.3% cholesterol-diet significantly reduced total plasma cholesterol (*P*<0.001) and increased faecal total cholesterol (*P*<0.05) compared to vehicle-treated animals. *S. boulardii* significantly modified the gut microbiota composition of the hamster fed a 0.3% cholesterol-diet. These microbial abundance modifications of the microbiota were correlated to variations of lipidemic values or liver genes expressions. In particularly we found that abundance of *g._Allobaculum_, the most modified taxon after *S. boulardii* treatment (+236%; *P*<0.05), was correlated to variations in plasmatic lipoproteins level and ABCG5 hepatic gene expression. We also observed a not previously described correlation between the levels of *g._Oxalobacter_ in the gut microbiota and total cholesterol plasma concentration. In conclusion, we confirmed the cholesterol-lowering effects of *S. boulardii* intake and we demonstrated for the first time the *S. boulardii* effect on gut microbiota in the context of hypercholesterolemia in hamsters. Our results provide new insights for a beneficial and safe approach of hypercholesterolemia treatment and could be considered for clinical development, alone or in addition to conventional treatment.

**Keywords:** *Saccharomyces boulardii* CNCM I-745, probiotic, hypercholesterolemia, microbiota
risk factors, such as fat mass development, steatosis and inflammation in a db/db mice model of diabetes and obesity (Everard et al., 2014b). These effects of S. boulardii on host metabolism were thought to be associated with selective modulation of specific bacteria growth in the gut microbiota. However, this study has not assessed the S. boulardii effect on hypercholesterolemia, a main risk factor of cardiovascular disease.

Similar to humans, but unlike rat and mice, the Syrian golden hamster (Mesocricetus auratus) transports a significant proportion of plasma cholesterol in the form of low density lipoprotein (LDL); similar mechanisms are involved in the regulation of plasma LDL-cholesterol levels in response to dietary lipids (Briand et al., 2010; Sicart et al., 1984; Spady and Dietschy, 1988). In addition, the low basal rates of bile acid and cholesterol synthesis render the hamster much more sensitive than the mouse to the cholesterolemic effects of excess dietary cholesterol (Horton et al., 1995). Unlike humans, hamsters carry most of plasma cholesterol in high density lipoprotein (HDL) (Goulinet and Chapman, 1993; Yin et al., 2012) and cholesterol’s food intake increases this fraction (Weingand and Daggy, 1991). Hamsters are considered the best non-primate models for the identification of new drugs for treating dyslipidemia (Yin et al., 2012). It has been previously described that a 0.3% cholesterol-enriched diet triggered hypercholesterolemia in hamsters (Briand, 2010; Briand et al., 2010). Girard et al. (2014) showed that S. boulardii significantly reduced the cholesterol blood level increase induced by a 0.1% cholesterol-enriched diet in Golden Syrian hamsters. However, the effects of S. boulardii on the gut microbiota have not been yet investigated in this hypercholesterolemia context.

In this present study, effects of S. boulardii treatment on cholesterol metabolism parameters and gut microbiota were assessed in a hypercholesterolemic hamster model. Hamsters are hibernating animals, used to consume rich cholesterol diet before entering winter (Jefimow et al., 2014) and it has been shown than a high amount of dietary cholesterol is needed to see an increase of plasma and liver cholesterol (Cai and Carr, 1999). The model developed by Physiogenex, i.e. Golden Syrian hamsters fed a 0.3% cholesterol-enriched diet (Physiogenex, 2016), has been used by several authors (Briand, 2010; Kahlon et al., 1992; Negm and Silliman, 1995; Tréguier et al., 2011).

### 2. Materials and methods

All animal experiments were performed at the Physiogenex’s premises (Toulouse, France). Microbiota DNA extractions, purification and sequencing were conducted by Genoscreen (Lille, France).

### Animals and diets

Six-week-old male Golden Syrian hamsters (n=40). 91 to 100 g, were housed in groups of 2-3 in cages on a normal 12-h light cycle (at 8:00 pm lights off), 22±2 °C and 50±10% relative humidity. All procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals and French laws. All animal protocols were reviewed and approved by the local (Comité régional d’ethique de Midi-Pyrénées) and national (Ministère de l’Enseignement Supérieur et de la Recherche) ethics committees (protocol number CEEA-122-2014-15). Isoflurane was used as anaesthetic. At sacrifice, hamsters were anesthetised and then euthanised by cervical dislocation.

Hamsters were fed with a chow diet (diet # 105 from Safe-France) during a 5-days acclimatisation period. During the experiment, hamsters were fed ad libitum with a 0.3% cholesterol supplemented diet (diet # 105 supplemented with 0.3% cholesterol from Safe ref # U8400) until the end of the study. They were simultaneously treated by oral gavage twice daily with S. boulardii CNCM I-745 (Sb, 3 g/kg) or a vehicle (‘Control group’ received distilled water). There were two sets of experiments according to treatments duration: 21 days (set 1: HC + Sb; n = 10 and HC + veh; n=10) or 39 days (set 2: HC + Sb; n=10 and HC + veh; n=10) (Supplementary Figure S1).

### Serum collection and analysis

Blood (200 μl/EDTA) was collected at 21 or 39 days, depending on treatment duration, by retro-orbital bleeding under isoflurane anaesthesia. Plasma was used for colorimetric quantification of total plasma cholesterol and HDL-cholesterol (colorimetric assay kit ref # WCHO100 from Sobioda, Montbonnot-Saint-Martin, France). Non-HDL-cholesterol was determined from total cholesterol and HDL-cholesterol values. LDL-cholesterol levels were determined using a colorimetric assay kit from Wako Chemicals, Neuss, Germany (ref # L-type LDL-cholesterol). Fast Phase Liquid Chromatography (FPLC) total cholesterol profiles were measured at the end of both treatment periods.

### Liver collection and analysis

Hamsters were sacrificed, as described previously for tissues collection, either at day 21 or day 39 (Briand et al., 2012). Liver samples were used to measure total hepatic cholesterol (kit ref # WCHO100, Cholesterol 6×100 ml from Sobioda), triglycerides, fatty acids, and hepatic expression of genes involved in cholesterol metabolism (Supplementary Table S1): ATP-binding cassette subfamily A member A1 (ABCA1), ATP-binding cassette subfamily G member 5 (ABCG5), ATP-binding cassette subfamily G member 8 (ABCG8), hydroxymethyl glutaryl-coenzyme A reductase
(HMGCoA_R), cytochrome P450 7a1 or cholesterol 7 alpha-hydroxylase (Cyp7a1), sterol regulatory element-binding protein-2 (SREBP-2), LDL-receptor (LDLR), and acetyl-coenzyme A acetyl transferase 2 (ACAT2). RNA extraction and gene expression analysis were performed as previously described (Tréguié et al., 2011). Hepatic genes expressions were normalised to cyclophilin transcripts.

Frozen liver sections were used for oil red O and haematoxylin-eosin staining procedure as described by Briand et al. (2012). Formalin fixed liver sections were used for haematoxylin-eosin staining as described by Lai et al. (2016).

Serum, liver, faeces and gene expression data statistical analysis

Data are presented as mean (± standard deviation). Serum, liver and faeces data were analysed using 2-way ANOVA with Bonferroni correction, or unpaired two-tailed Student t-test.

Faecal collection and analysis

Faeces were collected at day 21 and day 39 from hamsters individually caged and kept under treatment for a 24-h period for the analysis of cholesterol and bile acids excretion (Briand et al., 2012); kit ref # WCHO100, Cholesterol 6×100 ml from Sobioda.

DNA extraction

DNA was extracted from faecal samples collection in treated (S. boulardii; n=20) and control (n=20) groups according to a protocol based on Qiagen QIAamp Fast DNA Stool Mini kit (Hilden, Germany). The quality of the DNA was checked by migration on 1% agarose gel and quantified by SYBR Green assay.

Sequencing and assembly

Sequencing and assembly were performed by Genoscreen (Lille, France) according to the internal validation methods. Briefly, a Metabiote® kit was used for amplicon library preparation with primers targeting the V3-V4 region of the 16S rRNA gene. The sequencing of the corresponding products was performed with paired-end Illumina MiSeq 2 × 250 bp (Illumina, San Diego, CA, USA). A total of 421,460 readings were obtained ‘single-read’, i.e. 842,290 reads ‘paired-end’. The distribution varied from 625 to 11,507 readings per sample.

On average, 8,654 full-length 16S rDNA sequences assembled at 97% nucleic identity were obtained. These full-length 16S rDNA sequences were qualitatively and quantitatively sufficient to allow their taxonomic and the profile of the bacterial populations within the different samples.

Metagenomic analysis

The data were processed according to the QIIME pipeline (Caporaso et al., 2010). USEARCH was used for Chimera filtration, and for the clusterisation in Operational Taxonomic Unit (OTU) (Edgar, 2010). The (OTU) picking by clustering was set at 97% identity level. Singleton OTUs were excluded from the analysis. The taxonomical assignment of the OTUs using GreenGenes (v. 13.8) database (DeSantis et al., 2006) and the calculations for alpha and beta diversity were executed with the QIIME pipeline.

Relative abundances of the microbiota composition between treated and control groups were analysed by Wilcoxon tests and a Benjamini-Hochberg procedure was applied for false discovery rate correction (FDR) (Benjamini and Hochberg, 1995). Phyla diversity in samples were analysed using the Shannon index; absolute species turnover was analysed using the Bray-Curtis dissimilarity and UniFrac distance, then tested between groups using an analysis of similarities (ANOSIM). Correlations between species and plasma HDL and LDL-c, faeces cholesterol and bile acids, and liver genes expressions were analysed using the CORR PLOT and LessR packages of R software® (R Core Team, 2016). The results were considered statistically significant at $P$ level of 0.05 or less.

3. Results

Effects of Saccharomyces boulardii on body, faecal and liver weight

Hamsters were fed with a 0.3% cholesterol diet and treated daily by S. boulardii (3 g/kg) as described in the materials and methods section (Supplementary Figure S1). As compared to control group, S. boulardii-treated hamsters did not display significant differences in body weight, while faeces mass was significantly 27% higher at day 21 ($P<0.05$) and 30% at day 39 ($P<0.05$); liver mass was significantly 7% lower ($P<0.05$) at day 21 but not at day 39 (Table 1).

Effects of Saccharomyces boulardii on lipid profiles in blood, liver and faeces

Total plasma cholesterol was significantly lower in S. boulardii-treated hamsters regardless of treatment duration ($P<0.001$ vs control) while the LDL-cholesterol levels were similar (Table 2). Reduction of HDL cholesterol level was significant in the S. boulardii group at day 21 (-31% vs control, $P<0.001$), but not at day 39 (-14%, $P=0.09$). By comparison with the control group, HDL/LDL ratio was significantly lower at day 21 ($P<0.05$) in the Sb group but...
not at day 39. Non-HDL cholesterol was significantly 32% lower at day 39 (\(P<0.05\)) in *S. boulardii* treated hamster. No difference was found at day 21 for non-HDL-cholesterol (Table 2).

In the liver, *S. boulardii* intake did not significantly change total cholesterol (Figure 1A) and fatty acids levels (Figure 1B) but reduced the triglycerides level significantly by 25% (\(P<0.05\)) at day 39 compared to the control (Figure 1C). Liver histology analysis with H&E and oil red O indicated a minor liver steatosis and no evidence of morphologic change of liver tissues in both groups (Supplementary Figure S3).

Faecal cholesterol mass excretion was significantly increased by 23% (\(P<0.05\)) at day 21 and by 36% (\(P<0.01\)) at day 39 in *S. boulardii*-treated hamsters compared to the control (Figure 1D). In contrast, faecal bile acid mass excretion was significantly decreased by 43% at day 21 (\(P<0.001\)) (Figure 1E) but not at day 39.

### Effects of *Saccharomyces boulardii* on gene expression in liver

*S. boulardii* significantly decreased *ABCG5* expression (\(P<0.05\) at days 21 and 39) (Figure 2A), while *ABCG8* expression significantly decreased only at day 39 (\(P<0.05\)) (Figure 2B) compared to the control. *S. boulardii* significantly increased *HMGCoA_R* gene expression (\(P<0.05\) at days 21 and 39) (Figure 2C). In contrast, *S. boulardii* treatment did not significantly alter the hepatic expression of *ABCA1, ACAT2, CYP7A1, LDLR, SREBP-2* genes regardless of study duration (Figure 2D).

### Effects of *Saccharomyces boulardii* on the gut microbiota

Rarefaction curves based on Shannon Index were used to determine the samples alpha-diversity (i.e. species richness of the microbiota ecosystem). Each curve reaches an asymptotical plateau that indicates the depth of the sequencing was sufficient to get a representative estimation of the microbial diversity present in the samples (Supplementary Figure S2). UniFrac analysis (Figure 3) revealed that the faecal microbial communities of *S. boulardii*-treated hamsters clustered separately from those of the control group. For vehicle or *S. boulardii* treatments,
the Qiime’s distance comparison tool showed no significant difference according to study duration (21-day vs 39-day treatment period). In agreement with beta-diversity analysis, no significant difference was found in control and Sb groups between 21-day and 39-day treatment period for taxa abundances. Thus, for the microbiota analysis, we compared the Sb treated groups with control groups whatever the treatment duration.

Faecal microbiota in S. boulardii- or vehicle-treated hamsters was dominated at the phylum level (Table 3) by Firmicutes (~75%), Bacteroidetes (~20%), and Proteobacteria (~2%). In comparison with control, S. boulardii-treated animals display a significantly lower proportion of Firmicutes (P<0.05), Tenericutes (P<0.005), TM7 (P=0.005) and significantly higher amount of Proteobacteria (P<0.05), Lentisphaeraceae (P<0.05) and other unknown phyla (P<0.05). In total, we identified 42 families (Figure 4A), among which 16 were significantly modified in S. boulardii-treated hamsters compared to the control (P<0.05) (Supplementary Table S2A). At genus level the microbiota was dominated by bacteria belonging to an unclassified genus and family of the Clostridiales order, the genus Oscillospira and an unclassified genus of the Ruminococcaceae family (28.53, 16.96 and 16.72% in vehicle-treated hamster and 33.74, 12.28 and 14.89% in the S. boulardii groups, respectively) (Figure 4B, Supplementary Table S2B). 20 of the 69 genera identified were significantly affected by S. boulardii. Among the most prominent changes observed, S. boulardii treatment was associated with a higher amount of g_CF231 (f_Paraprevotellaceae, p_Bacteroidetes) (+289%; P<0.001), g_Allobaculum (f_Erysipelotrichaceae, p_Firmicutes) (+236%; P<0.005) and the ‘Lachnospiraceae.Other’, (a genus of the Lachnospiraceae family that was not associated to a known genus in Greengenes) (+110%; P<0.001) and with a lower of the following genera: an unclassified genus of
Figure 2. Hepatic gene expression levels after a 21-day and 39-day treatment period with S. boulardii or vehicle. ATP binding cassette subfamily G member 5 (ABCG5), ATP binding cassette subfamily G member 8 (ABCG8), ATP-binding cassette A1 (ABCA1), hydroxymethyl glutaryl-coenzyme A reductase (HMGCoA_R), cytochrome P450 7a1 or cholesterol 7 alpha-hydroxylase (Cyp7a1), Sterol regulatory element-binding protein-2 (SREBP-2), LDL-receptor (LDLR), acetyl-coenzyme A acetyl transferase 2 (ACAT2). NS = not significant (P>0.05).

Table 3. Relative abundance of bacterial phyla and statistical significance among Saccharomyces boulardii-treated hamsters and control groups.1

<table>
<thead>
<tr>
<th>Phyla</th>
<th>Vehicle (n=20)</th>
<th>Sb (n=20)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Firmicutes</td>
<td>77.46</td>
<td>73.79</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Bacteroidetes</td>
<td>18.99</td>
<td>21.58</td>
<td>NS</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>2.10</td>
<td>3.04</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Actinobacteria</td>
<td>0.58</td>
<td>0.64</td>
<td>NS</td>
</tr>
<tr>
<td>Other</td>
<td>0.23</td>
<td>0.31</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Elusimicrobia</td>
<td>0.22</td>
<td>0.37</td>
<td>NS</td>
</tr>
<tr>
<td>Tenericutes</td>
<td>0.21</td>
<td>0.11</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Lentispharerae</td>
<td>0.04</td>
<td>0.08</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>TM7</td>
<td>0.15</td>
<td>0.06</td>
<td>0.005</td>
</tr>
<tr>
<td>Deferribacteres</td>
<td>6.08 E-04</td>
<td>6.38 E-04</td>
<td>NA</td>
</tr>
<tr>
<td>Verrumicrobia</td>
<td>1.82 E-03</td>
<td>4.47 E-03</td>
<td>NA</td>
</tr>
</tbody>
</table>

1 Results are expressed as percentage (%). The Wilcoxon statistical test was used to compare treated (Sb, Saccharomyces boulardii) and control group (Vehicle; distilled water) regardless of study duration. NS = not significant (P>0.05); NA = not applicable.
Figure 4. Gut microbiota composition at different taxonomic levels in Saccharomyces boulardii or vehicle treated hamsters. Bacterial families (A) and genera (B) from faecal samples collection of hamsters treated with the vehicle (n=20; dark grey) or S. boulardii (n=20; light grey). Each column is set at 100% to illustrate the proportion of each taxa among the two groups; the presence of only one colour indicates that the taxa was present only in this group of hamsters. Unc.: unclassified.
f_Desulfovibrionaceae (p_Proteobacteria) (-76%; P<0.05), g_Oxalobacter (f_Oxalobacteraceae, p_Proteobacteria) (-58%; P<0.001), an unclassified genus in family F16 (p_TM7) (-57%; P<0.05), an unclassified genus of the f_Mogibacteriaceae (p_Firmicutes) (-50%, P<0.005) and an unclassified genus of the Lachnospiraceae family (p_Firmicutes) (-39%; P<0.001) compared to the control.

Correlation of microbial taxa abundancy with biochemical parameters

Correlation analysis are shown in Figure 5. Total plasma cholesterol values correlated positively to HDL-cholesterol (r= +0.75) and non-HDL-cholesterol values (r= +0.66).

Hepatic triglycerides values correlated positively in a moderate way with plasmatic total cholesterol and non-HDL-cholesterol levels at day 39 (r= +0.45 and r= +0.46, respectively). Among genera abundances mostly different in S. boulardii-treated hamsters, CF231, g_Allobaculum, g_Oxalobacter and an unclassified genus of Lachnospiraceae correlated with at least one of these lipid parameters. Total plasma cholesterol negatively correlated with abundances of g_CF231 (r = -0.69) and g_Allobaculum (r = -0.4), and positively correlated with abundance of an unclassified genus of the Lachnospiraceae family (r = +0.58) and g_Oxalobacter (r = +0.44). These four genera also correlated with non-HDL-cholesterol (r= -0.4, -0.43, +0.42 and +0.44, respectively).

Figure 5. Correlation matrix between the microbiota and host markers of the lipid metabolism. Positive correlations are displayed in blue; negative correlations in red. The colour intensity is proportional to the correlation coefficients (right-hand side scale). The size of the squares is inversely proportional of the P-value. Only correlations with absolute value greater than 0.3 are shown. All the correlations shown are statistically significant (Pearson correlation, t-test P<0.05).
with abundance of g_CF231 (r= -0.57) and positively to abundance of g_Oscillospira (r= +0.64). Hepatic triglycerides values correlated with g_Oxalobacter (r = + 0.49). Faecal mass excretion of cholesterol is negatively correlated to abundance of g_Bacteroides (r = -0.5) and positively to abundance of g_Allobaculum (r = +0.51). Faecal bile acids mass excretion is negatively correlated to abundance of g_CF231 (-0.58) and positively to an unclassified genus of the Mogibacteriaceae family (r = +0.56).

**Correlation of faecal microbes with hepatic genes expression**

ABCG5 expression correlated with Oxalobacter (r = +0.46) and with an unclassified genus of Mogibacteriaceae family (r = +0.5). Expression of ABCG8 correlated with Allobaculum (r = -0.4), an unknown member of the Ruminococcaceae family (r = +0.56) and an unclassified genus of Mogibacteriaceae family (r = +0.43). Expression of ABCG5 correlated positively with an unknown Lachnospiraceae (r = +0.54), and g_CF231 (r = +0.43) and negatively with an unknown member of the Ruminococcaceae family (r = -0.51).

**4. Discussion**

Few studies have investigated *S. boulardii* potential hypocholesterolemic effect. Previous studies showed that, *S. boulardii* depletes in vitro cholesterol from laboratory culture medium by assimilation by the yeast cells. In *vivo*, *S. boulardii* also reduces seric cholesterol levels in rats fed a 0.5% cholesterol diet as in Golden Syrian hamsters fed a 0.1% cholesterol diet. Here we found that the cholesterollowering effect of *S. boulardii* was associated with changes in the gut microbiota in Golden Syrian hamsters fed a 0.3% cholesterol-diet.

**Saccharomyces boulardii** reduces lipid parameters in hamsters fed with a 0.3% cholesterol supplemented diet

Compared to the control group, Golden Syrian hamsters fed a rich cholesterol-diet and treated with *S. boulardii* had a lower total plasma cholesterol at day 21 and at day 39. At the same dosage of *S. boulardii* in Golden Syrian hamsters, *S. boulardii* treatment significantly decreased expression of ABCG5 and ABCG8 and did not alter significantly the hypocholesterolemic effect of the probiotic yeast. We also found that *S. boulardii* intake significantly reduced plasma HDL-cholesterol at day 21 and non-HDL-cholesterol at day 39 but these effects were not significant at 39-day on HDL-cholesterol and at 21-day on non-HDL-cholesterol levels. Similar transient effects on lipoproteins of hypocholesterolemic molecules or probiotics was previously described in animal models but their underlying mechanisms were not elucidated. Previous studies that examined the potential hypocholesterolemic effect of other probiotics have yielded mixed results according to probiotic species and strains, dosages and especially intervention lengths studied. The HDL-cholesterol decrease is not surprising as hamsters are well-known to carry the majority of plasma cholesterol in HDL (Goulinet and Chapman, 1993; Yin et al., 2012). Regarding non-HDL-cholesterol, the *S. boulardii*-lowering effect is similar to that of hypocholesterolemic dietary supplements in hamsters (Carr et al., 2005; Wilson et al., 2004). Interestingly, non-HDL-cholesterol is considered a biomarker and potential therapeutic target in the treatment and prevention of cardiovascular disease (Bergmann, 2010).

In the liver, *S. boulardii* treatment significantly decreased liver weight at day 21 and hepatic triglycerides level at day 39. Everard et al. (2014b) previously found similar results on liver mass and total lipid liver content in *S. boulardii*-treated-obese and -type 2 db/db mice after four weeks of treatment. We found that *S. boulardii*-treated hamsters excreted significantly more cholesterol in their faeces than the controls whatever the duration of treatment. Thus, probable causes of a decrease in cholesterolemia and lipids parameters (and an increase of cholesterol excretion) observed in *S. boulardii*-treated hamsters may be related to change at the intestinal level.

**Saccharomyces boulardii** modulates gut microbiota and expression of genes related to cholesterol metabolism in the liver in hamsters fed a high-cholesterol diet

The gut microbiota has been recognised as a key factor influencing whole-body metabolism (Clemente et al., 2012; Tremaroli and Bäckhed, 2012). It has been shown that gut microbiota play an important role in the variation of blood lipid levels (Fu et al., 2015) and that intestinal bacteria can influence cholesterol levels at least by two potential mechanisms, microbial conversion of diet cholesterol in coprostanol or metabolism of bile acids (Kriaa et al., 2018). However, metabolic pathways involved in the hypocholesterolemic effect of the probiotic yeast *S. boulardii* seems to be different from those of bacterial probiotic, as bile acids metabolism by bacteria induces liver expression or genes encoding ABCG5, ABCG8 and CYP7A1 (see reviews Jones et al., 2013 and articles cited therein) when *S. boulardii* treatment decreased expression of ABCG5 and ABCG8 and did not alter significantly.
expression of CYP7A1. S. boulardii is well established to improve gut health by modifying gut microbiota in dysbiosis context (Kabbani et al., 2017; Moré and Swidsinski, 2015; Swidsinski et al., 2008, 2010, 2016). However, the effect of S. boulardii on the gut microbiota has not yet been investigated in a hamster model of hypercholesterolemia. One previous study has shown in obese and type 2 diabetic db/db mice that S. boulardii modified the gut microbiota composition (Everard et al., 2014b). The present study, by using a high-throughput sequencing method, found that S. boulardii was associated with an increased amount of g_CF231 and g_Allobaculum and with a decreased of the following genera: an unclassified genus in family Lachnospiraceae, an unclassified genus of Desulfovibrionaceae, g_Oxalobacter and an unclassified genus in family F16 compared to the control. Among these different genera affected by S. boulardii, correlations to total plasma cholesterol was found with g_CF231, g_Allobaculum, an unclassified Lachnospiraceae and g_Oxalobacter (Figure 5). In other animal models, several studies have shown that decreased total cholesterol level due to dyslipidemia treatment occur concomitantly with increased g_CF231 and g_Allobaculum proportions in microbiota (Everard et al., 2014a,b; Lin et al., 2015; Raza et al., 2017; Zhang et al., 2015). In contrast, in the Lachnospiraceae family, the correlations with total plasma cholesterol may differ depending on the genera (Liu et al., 2015). To our knowledge, no studies showed a correlation between g_Oxalobacter and total plasma cholesterol. S. boulardii treatment decreased significantly abundance of g_Oscillospira (Figure 5) that was positively correlated to plasmatic HDL-cholesterol level in our study (Figure 5). Everard et al. (2014b) also found that S. boulardii treatment in db/db mice decrease the amount of g_Oscillospira in the same range of that we observed in our study. Our results suggest a significant role of variations in abundance of g_CF231, g_Allobaculum, an unclassified genus of the Lachnospiraceae family and g_Oxalobacter in the lowering effect of S. boulardii on hamsters’ cholesterol. However, it is still difficult to distinguish whether the gut microbiota variations were the causes of the hamsters’ lipidemic status improvement or only the consequences of the S. boulardii treatment.

By interacting with the intestinal tract, the liver plays a key role in lipid metabolism. Downregulation of genes involved in hepatic cholesterol metabolism may contribute to disrupt cholesterol homeostasis within the body. We tested the effect of S. boulardii on expression of several of these genes including ABCA1, ABCG5, ABCG8, HMGCoaA_R, Cyp7a1, SREBP-2, LDLR and ACAT2. Of the 8 genes tested, S. boulardii has altered the expression of HMGCoaA_R, ABCG5 and ABCG8 genes compared to the control. The HMGCoaA_R gene which encodes the rate-limiting enzyme for cholesterol synthesis is strongly expressed in the liver of S. boulardii-treated hamsters compared to control. The ABCG5/8 genes which encode a heterodimer transporter that promotes efficient secretion of cholesterol from hepatocytes into bile (Berge et al., 2000; Heimerl et al., 2002; Lee et al., 2001; Yu et al., 2002a,b) are repressed in S. boulardii treated hamsters. In the study of Jia et al. (2007), in hamsters, the addition of 0.7% stanol–ascorbic acid esters to the 0.25% cholesterol diet (Ch-Con diet) reduced plasma cholesterol levels and the expression of ABCG5 levels as compared with the Ch-Con diet. Valasek et al. (2008) showed that ezetimibe inhibited ABCG5 and activated HMGCoaA_R in hamsters fed lipid-rich diet, but not when fed by a normal diet. The ezetimibe effect of ABCG5 and HMGCoaA_R liver expression they observed in their dyslipidemic hamster model is greater than the effect we observed in S. boulardii treated hypercholesterolemic hamster. That difference could be explained by the difference between the 2 models (difference in the quantity of supplemented cholesterol (0.3% vs 1%) and difference in the quantity of the fat (in our model the diet did not bring additional fat supplementation)). In addition, we found that HMGCoaA_R expression correlated positively with an unknown genus of the Lachnospiraceae family and g_CF231. Faecal mass excretion of bile acid and ABCG5 and ABCG8 expressions correlated with an unclassified genus of the Mogibacteriaceae family, abundance of which was decreased in Hamster fed with S. boulardii (Supplementary Table S2). F_Mogibacteriaceae abundance has been recently correlated to bile acid excretion in the faeces in mice (Fu et al., 2018). This confirms the emerging evidence of a key role of intestinal bacteria in maintaining the entero-hepatic cycle (Tripathi et al., 2018; Visschers et al., 2013). These preliminary results establish a potential relationship between gut microbiota and cholesterol homeostasis. It can therefore be assumed that these bacterial groups which also correlated to lipid parameters and hepatic genes expressions could have a role in the observed hypcholesterolemic effect in S. boulardii-treated hamsters.

5. Conclusions

S. boulardii CNCM I-745 is a probiotic yeast currently used as a treatment of several gastrointestinal diseases in several countries for many years. Our study is the first one that has evaluated the gut microbiota modification after S. boulardii intake in hypercholesterolemic hamsters. The results demonstrate that S. boulardii CNCM I-745-treated hamsters fed a 0.3% enriched-cholesterol diet had a lower hypercholesterolemia and a modified gut microbiota profile in comparison with vehicle-treated animals. These results provide new insights for a safe approach of hypercholesterolemia treatment and could be considered for clinical development, alone or associated to conventional treatment.

Supplementary material

Supplementary material can be found online at https://doi.org/10.3920/BM2018.0134.

Figure S1. Distribution of hamsters according to treatment allocation and study duration.

Figure S2. Shannon curves – alpha diversity.

Figure S3. Representative oil red O and haematoxylin eosin (H&E) staining in hamsters treated with Saccharomyces boulardii or a vehicle.

Table S1. Hepatic genes involved in lipid metabolism.

Table S2. Gut microbiota composition at families and genera levels in hamsters treated with Saccharomyces boulardii or a vehicle.

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